Washington University Neuro-Oncology Rotation

Reading and Resources
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Adult Neuro-Oncology
Malignant brain tumors are among the most feared types of cancer, not only for their poor prognosis, but also because of the direct repercussions on quality of life and cognitive function. Prevalence studies estimate that 138,054 patients had a diagnosis of a primary malignant brain tumor in the United States in 2010.1 Malignant gliomas are the most common type of primary malignant brain tumor, accounting for 80% of patients and an annual incidence of 5.26 per 100,000 population, or 17,000 new cases diagnosed per year.2 This disease is most common in the sixth through eighth decades of life; the number of patients is expected to increase with the aging of the population.

Internists, family practitioners, and emergency physicians are likely to be the first to encounter patients with a primary brain tumor and will typically remain involved in their care throughout the entire disease course (Box 1). This review focuses on the practical aspects involved in the clinical management of malignant gliomas that such professionals may encounter.

Methods

References were identified through PubMed searches from 2000 to 2013, using the terms glioblastoma, glioma, malignant glioma, anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic oligoastrocytoma, and brain neoplasm. Articles were also identified through searches of the authors' own files. The American Heart Association classification of recommendations and levels of evidence was used to grade the quality of evidence.3
Pathology and Risk Factors

The World Health Organization (WHO) classification system groups gliomas into 4 histological grades defined by increasing degrees of undifferentiation, anaplasia, and aggressiveness. This review focuses on malignant gliomas (Table 1), by far the most common form of gliomas, which includes WHO grade IV tumors (glioblastoma and its variants) and grade III tumors (anaplastic variants of astrocytoma, oligodendroglioma, and oligoastrocytoma). WHO grade II tumors (diffuse astrocytomas, oligodendrogliomas, and oligoastrocytomas) are more differentiated neoplasms that invariably progress to a higher-grade tumor with time.

Glioblastoma accounts for 82% of cases of malignant glioma and is characterized histologically by considerable cellularity and mitotic activity, vascular proliferation, and necrosis. Because cells in these tumors vary in size and shape, ie, they are pleomorphic, glioblastomas were called glioblastoma multiforme, a term no longer in use. Glioblastoma and other malignant gliomas are highly invasive, infiltrating surrounding brain parenchyma, yet they are typically confined to the central nervous system (CNS) and do not metastasize.

Malignant gliomas arise in a multistep process involving sequential and cumulative genetic alterations resulting from intrinsic and environmental factors. Gliomas are more common in men than women and in white rather than black populations. A number of rare hereditary syndromes are associated with an increased risk of glioma, including Cowden, Turcot, Li-Fraumeni, neurofibromatosis type 1 and type 2, tuberous sclerosis, and familial schwannomatosis. A family history of glioma is rarely observed but, when present, is associated with a 2-fold increase in risk of developing glioma. Genome-wide association studies have identified a few susceptibility variants such as 20q13.33 (RTEL), 5p15.33 (TERT), 9p21.3 (CDKN2BAS), 7p11.2 (EGFR), 8q24.21 (CCDC26), and 11q23.3 (PHLD1), but these genes are only weakly associated with glioma, possibly reflecting multiple molecular subsets. Gliomas are inversely associated with the presence of atopic diseases such as asthma, eczema, and hay fever. Preventive measures, such as lifestyle changes, are ineffective in averting gliomas. Early diagnosis and treatment unfortunately do not improve outcomes, precluding the utility of screening for this disease.

Ionizing radiation is an established environmental risk factor for glioma development. This association was demonstrated in studies of children receiving cranial irradiation for cancer therapy and Tinea capitis and in individuals exposed to atomic bombs and nuclear weapons testing. Most studies of radiation used in diagnostic procedures found no increased risk of glioma except for a retrospective study in children undergoing computed tomography (CT) scans suggesting a risk excess, although the overall incidence remained low, with 1 excess brain tumor per 10 000 patients within 10 years after exposure to 1 CT scan. Glioma risk is not increased from exposure to cell phones and other types of electromagnetic fields, head injury, foods containing N-nitroso compounds, aspartame, occupational risk factors, pesticides, or season of birth. Cell phone risks have captured the public’s attention, but associations between cell phone usage and glioma are not consistent. Biological effects of radiofrequency on the brain include ipsilateral increases in cerebral blood flow and glucose metabolism during cell phone exposure, but most case-control studies have failed to demonstrate a relationship with the development of brain tumors. These studies are limited by several factors, including recall bias, variations in length of exposure and latency, and varying cell phone technologies used over time. Glioma incidence trends have not followed the explosion in cell phone use, mitigating against a glioma risk attributable to cell phones, but because trends could lag, continued surveillance is warranted, especially focusing on children who are exposed from an early age.

From a molecular standpoint, malignant gliomas are highly heterogeneous tumors. Genome-wide expression studies in glioblastomas revealed 4 transcriptional subclasses, displaying features reminiscent of distinct cell types: classical, mesenchymal, proneural, and neural. The classical glioblastoma subclass typically displays chromosome 7 amplifications, chromosome 10 deletions, EGFR amplification, EGFR mutations (point and vIII mutations), and Ink4a/ARF locus deletion. The mesenchymal subclass displays a high frequency of NFI mutation/deletion and high expression of CHI3L1, MET, and genes involved in the tumor necrosis factor and nuclear factor-κB pathways. Proneural glioblastomas are characterized by alterations of PDGFRα and mutations in IDH1 and TP53, sharing gene expression features with other glioblastomas.
lower-grade gliomas and secondary glioblastomas (ie, lower-grade gliomas that later recurred as glioblastoma). The neural subclass is characterized by the expression of neuronal markers. Many molecular abnormalities and mutations overlap across the transcriptional subclasses, for example, PTEN loss, and a large number of very rare mutations have been described in gliomas, adding to the interpatient heterogeneity.19,20

Clinical Presentation and Initial Evaluation

Headaches are relatively frequent, present in about 50% of patients at diagnosis, but usually with a nonspecific pain pattern;21 progressive severity, unilateral localization, and new-onset headache in a patient older than 50 years are some of the features that may distinguish a tumor-associated headache from a benign headache (Box 2). Papilledema is associated with significantly intracranial pressure and is now rarely seen because imaging is usually obtained at earlier disease stages. Cognitive difficulties and personality changes may develop and are often mistaken for psychiatric disorders or dementia, particularly in elderly individuals. Gait imbalance and incontinence may be present, usually in larger tumors with significant mass effect. Focal signs such as hemiparesis, sensory loss, or visual field disturbances are common and reflect tumor location. Occasionally, the development of symptoms is rapid, mimicking a stroke. Language difficulties may be mistaken for confusion or delirium. Seizures are the presenting manifestation in about 20% to 40% of patients, and usually a focal onset is reported.22

Brain magnetic resonance imaging (MRI) with and without contrast is the diagnostic modality of choice when a brain tumor is suspected (class I, level B); CT scan is reserved for patients unable to undergo MRI (eg, those with pacemakers). Malignant gliomas typically enhance with gadolinium (Figure) and may have central areas of necrosis; they are characteristically surrounded by white matter edema. Tumors are often unifocal but can be multifocal. Findings on MRI can be indistinguishable from brain metastases.

A number of nonneoplastic syndromes may mimic malignant gliomas on neuroimaging,24 including brain abscess, subacute stroke, multiple sclerosis, and other inflammatory diseases; looking for elements in the patient’s history that point to those alternative diagnoses prior to surgery is imperative (Table 2). Additional testing such as cerebral angiogram, electroencephalography, or lumbar puncture is rarely indicated.

Symptomatic Treatment

Symptomatic relief ultimately relies on the efficacy of specific antitumor therapies, but corticosteroids may temporarily alleviate neurologic symptoms caused by peritumoral edema (Figure, C). Dexamethasone is often used because of its low mineralocorticoid activity. Initial doses are typically 12 to 16 mg/d in divided doses; given the high bioavailability, oral use is comparable with intravenous. Unfortunately, corticosteroid adverse effects can be substantial, and early tapering is indicated whenever possible. The presence of primary CNS lymphoma (PCNSL) should be considered before initiating corticosteroids because these agents are lympholytic and may obscure identification of lymphoma cells on histologic examination. On MRI, PCNSL usually displays a more uniform pattern of contrast enhancement, often described as “cotton” or “snowball,” which tends to disappear rapidly with corticosteroids.25 If PCNSL is suspected, corticosteroids should not be used until after a brain biopsy has been performed, in order to avoid diagnostic delays.

In patients who present with seizures, initiation of antiepileptics is required, but there is no evidence to support prophylactic use of antiepileptics in patients without seizures (class III, level B).22 Levetiracetam is often preferred because it has a favorable toxicity profile, both oral and intravenous formulations are available, and it has no drug-to-drug interaction with most chemotherapeutic agents (class I, level B).26 Other nonenzyme liver inducers may be used, such as topiramate, lamotrigine, valproic acid, and lacosamide. If possible, potent liver enzyme inducers such as phenytoin, carbamazepine, and phenobarbital should be avoided because they may decrease the effectiveness of some chemotherapeutic agents27 and preclude participation in most clinical trials.

Neurosurgical Management

After neuroimaging, patients with suspected malignant glioma should be considered for surgical resection, aiming at relieving mass effect, achieving cytoreduction, and providing adequate tissue for histologic and molecular tumor characterization. In suspected low-grade gliomas, early surgical resection may also be indicated, providing more reliable tumor grading.28 In inoperable tumors, stereotactic biopsy may be performed for histologic diagnosis, but the limited amount of tissue acquired may preclude full molecular characterization. Whenever possible, patients should be referred for sur-
Surgery in tertiary care facilities, which provide optimized surgical tools (advanced intraoperative monitoring, awake mapping, and functional and intraoperative MRI) and allow for adequate handling, processing, and storage of the tissue, including comprehensive molecular characterization and tissue profiling that may guide subsequent treatments.

Figure. Typical Glioblastoma Features on Magnetic Resonance Imaging Studies Used in the Initial Evaluation of a Suspected Brain Tumor

Table 2. History and Physical Examination Elements to Aid in the Differential Diagnosis Between Malignant Gliomas and Tumefactive Nonneoplastic Disorders

<table>
<thead>
<tr>
<th>Clinical Elements</th>
<th>Differential Diagnosis to Consider</th>
<th>Action Prior to Biopsy or Surgical Resection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrupt symptoms onset</td>
<td>Stroke</td>
<td>Look for vascular territory distribution and gyral patterns of enhancement; DWI may have negative results</td>
</tr>
<tr>
<td>Onset in young adults</td>
<td>AIDS and other infectious or inflammatory lesions</td>
<td>HIV testing</td>
</tr>
<tr>
<td>Recent dental procedure, ears/nose/throat infection</td>
<td>Brain abscess</td>
<td>Hyperintensity on DWI</td>
</tr>
<tr>
<td>History of immunosuppression</td>
<td>Fungal and other opportunistic infections, primary CNS lymphoma</td>
<td>Homogeneous enhancement suggests lymphoma; consider LP and avoid corticosteroids until biopsy</td>
</tr>
<tr>
<td>History of autoimmune or inflammatory disease (patient or family)</td>
<td>Multiple sclerosis, sarcoidosis, Behçet syndrome</td>
<td>Look for small white matter lesions on MRI</td>
</tr>
<tr>
<td>IV drug addiction</td>
<td>Brain abscess, syphilis, AIDS</td>
<td>Obtain blood cultures</td>
</tr>
<tr>
<td>Exposure to tuberculosis, even if remote</td>
<td>Tuberculosis</td>
<td>Chest imaging, PPD test</td>
</tr>
<tr>
<td>Travel to countries with endemic infectious diseases</td>
<td>Cysticercosis, hydatidosis, and amebiasis</td>
<td>Look for calcifications on CT and scolex on MRI (cysticercosis); consider LP</td>
</tr>
<tr>
<td>History of subtle/transient neurologic deficits or visual symptoms</td>
<td>Multiple sclerosis and demyelinating diseases</td>
<td>Other lesions usually present on MRI</td>
</tr>
<tr>
<td>History or presence of oral or genital ulcers</td>
<td>Behçet syndrome</td>
<td></td>
</tr>
<tr>
<td>Rashes</td>
<td>Sarcoïdosis, AIDS, Behçet syndrome</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CNS, central nervous system; CT, computed tomography; DWI, diffusion-weighted magnetic resonance imaging; HIV, human immunodeficiency virus; IV, intravenous; LP, lumbar puncture; MRI, magnetic resonance imaging; PPD, purified protein derivative.
Specific Treatments

Glioblastoma

First-Line Adjuvant Treatment

After surgery, adjuvant radiotherapy combined with chemotherapy should be considered in all patients. The typical radiotherapy dose is 60 Gy divided in 30 fractions. The use of intensity-modulated radiotherapy has been increasingly preferred because of better targeting capability, but to date there is no evidence of superiority over other focal radiotherapy techniques. Because this is a diffusely infiltrative disease, there is currently no defined role for stereotactic radiosurgery or brachytherapy as part of first-line treatment.30

The DNA alkylating agent temozolomide is administered orally, concomitantly with radiotherapy, followed by an adjuvant course (class I, level B). The use of this regimen is supported by a randomized phase 3 study31 that found the addition of temozolomide increased the median survival to 15 months vs 12 months with radiotherapy alone (hazard ratio, 0.63; P < .001). The 2-year survival rate was 27% vs 10%, respectively. A post hoc tissue analysis suggested patients with tumors displaying promoter methylation of the DNA repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) were more likely to benefit from the addition of temozolomide to radiotherapy.32 Optimal treatments for elderly patients and patients with poor performance status remain to be established, although radiotherapy alone and temozolomide alone have both been shown effective and well tolerated, even in the older population.33-36

In addition to temozolomide, the other agent approved by the US Food and Drug Administration (FDA) for first-line treatment is biodegradable polymers containing the alkylating agent carmustine, implanted into the tumor bed after tumor resection. A phase 3 trial has suggested a modest survival benefit,37 but that study had several methodological problems, which in the setting of frequent toxicities, such as brain edema, infection, and seizures, precluded wide adoption of this treatment (class III, level B). Moreover, a direct comparison with standard chemoradiotherapy with temozolomide is lacking.

After chemoradiotherapy, many patients, especially those with methylated MGMT promoter tumors,38 present with increased tumor size and mass effect that correspond to radiotherapy effects, rather than treatment failure.39 This process, termed pseudoprogression, poses a challenging diagnostic problem, given that some patients may be experiencing real tumor progression and require a change in treatment. Magnetic resonance imaging perfusion may be helpful when it shows decreased relative cerebral blood volume (rCBV), suggesting pseudoprogression,40 but in patients with increased rCBV, the diagnostic dilemma remains. Common practice is to continue with adjuvant temozolomide with close radiographic follow-up if patients are asymptomatic and consider corticosteroids, surgery, or alternative treatments such as bevacizumab if patients are highly symptomatic.

Bevacizumab, a humanized vascular endothelial growth factor (VEGF) monoclonal antibody that targets angiogenesis, is under study in first-line glioblastoma treatment, added to chemoradiotherapy with temozolomide. Preliminary results of 2 large randomized trials demonstrated increased progression-free survival (PFS) but not overall survival with the addition of bevacizumab.41,42 Until the significance of these findings is clear, bevacizumab is usually reserved for salvage treatment, discussed later in this section.

Treatment for Recurrence

After first-line treatment, virtually all glioblastoma patients experience disease progression after a median PFS of 7 to 10 months.43 Unfortunately, none of the available salvage treatments has clearly shown improved survival and likely only benefit selected patients. Treatment choices should be individualized, and clinical trials strongly considered.

Surgical resection may be considered for mass effect relief, cytoreduction, and updating histology and molecular characteristics of the tumor, although survival benefits are unclear. Many clinical trials only enroll patients who are surgical candidates, allowing for preoperative drug exposure and then evaluation of tumor tissue to ascertain whether the new agent had the intended effect on the molecular target; such trials should be considered in all surgical candidates. However, for a majority of patients, surgery will not be indicated because of tumor location, widespread disease in the brain, or the patient’s poor physical performance status.

Salvage chemotherapy options include bevacizumab, temozolomide rechallenge, and other alkylating agents, such as nitrosoureas (carmustine and lomustine) and carboplatin (Table 3). Bevacizumab is a frequently used treatment for recurrent glioblastoma. This agent targets VEGF, a key proangiogenic factor involved in tumor progression. Vascular endothelial growth factor also promotes vascular permeability, and therefore bevacizumab often results in rapid decrease of peritumoral edema and facilitates corticosteroid taper. In a phase 2 study44 testing single-agent bevacizumab and bevacizumab combined with irinotecan, the response rates (RRs) were 28% to 39% and 6-month PFS was 42% to 50%, which compared favorably with historical controls (RR, 5%-9%; 6-month PFS, 15%-20%). However, survival benefits are less clear, with median survival of 8 months, as compared with 6 to 7 months for historical controls; randomized studies in recurrent disease are ongoing. Bevacizumab is an option for highly symptomatic patients who might benefit from decreased brain edema and tumor shrinkage, either with initial treatment or at recurrence (class IIa, level B).41-44

Metronomic temozolomide dosing schedules (ie, given at lower doses for extended periods of time)45-47 are another salvage therapy option with a favorable toxicity profile, which may be particularly helpful in early tumor progression after radiotherapy or for patients who have completed the adjuvant temozolomide and experienced recurrence while not receiving treatment. Carmustine and lomustine have traditionally been the mainstay of recurrent glioblastoma treatment,48 but hematotoxicity rates are high and efficacy is modest. Recently, the use of low-intensity alternating electric fields applied to the brain through a portable device (NovoTTF-100A; Novocure) has received FDA approval for recurrent glioblastoma. Approval was based on a phase 3 trial49 showing equivalent efficacy and a superior toxicity profile in the device group relative to a control group consisting of the treating physician’s choice of chemotherapy. However, the device’s efficacy was modest, and a noninferiority design, required for this type of comparison, was not used; the role of NovoTTF-100A in glioblastoma remains unclear (class IIb, level B). After temozolomide and bevacizumab fail, the...
Anaplastic (WHO Grade III) Astrocytoma

Because anaplastic astrocytoma is a relatively rare disease, the treatment is often based on principles established in glioblastomas. After surgery, the first line of treatment is radiotherapy. The role of chemotherapy has not been established in randomized trials. A phase 3 trial investigating the addition of temozolomide is ongoing. However, off trial, many physicians recommend a treatment similar to glioblastoma using radiotherapy and concomitant temozolomide, followed by adjuvant temozolomide (class IIa, level C). After radiotherapy, recurrence is expected, and patients usually progress to develop a secondary glioblastoma. Salvage treatments used for glioblastoma are also used for anaplastic astrocytoma, particularly cytotoxic agents. Bevacizumab may be less effective in grade III tumors than in glioblastoma and, until further studies are done, should be reserved for end-stage disease (class III, level C). With aggressive treatment, the median overall survival of grade III astrocytomas remains in the range of 2 to 3 years.

Anaplastic (WHO Grade III) Oligodendrogliomas

Tumors with oligodendroglial components have distinctive histologic features such as perinuclear clearing, giving rise to a “fried egg” appearance, and a reticular pattern of blood vessel growth and are characterized by the presence of co-deletion of 1p/19q chromosomes, resulting from an unbalanced translocation of 19p to 1q. These tumors are more responsive to therapy than their grade III astrocytoma counterparts, with a median survival of 3 to 6 years compared with 2 to 3 years for grade III astrocytoma. Addition of chemotherapy to radiotherapy prolongs progression-free and overall survival (class IIa, level A), as demonstrated in two phase 3 trials.53,54 Those studies, conducted in the 1990s, used a fairly toxic combination of procarbazine, CCNU (lomustine), and vincristine (PCV). While those trials were in progress, temozolomide became widely available,55 and ongoing clinical trials are investigating whether this agent can replace PCV.

Mixed Grade III Gliomas

These tumors are characterized by the histological coexistence of both astrocytic and oligodendroglial features. Because oligodendrogial features are typically associated with a better prognosis,
Box 3. Key Aspects in the Clinical Management and Follow-up of Patients With Malignant Gliomas

- Antiepileptics are indicated in patients with seizures, but primary prophylaxis is not required in patients who never experienced a seizure. Levetiracetam is the preferred antiepileptic, given its excellent toxicity profile and lack of interactions with most chemotherapy agents; adjustments in doses are needed in case of renal failure.
- Magnetic resonance imaging of the brain is the examination of choice for baseline and follow-up evaluations.
- Radiographic worsening shortly after radiotherapy may reflect treatment effects (pseudoprogression), rather than tumor progression.
- Minor fluctuations in symptoms are common in brain tumors, but sudden or marked neurologic changes (sudden onset of severe headaches, repeated seizures or status epilepticus, new focal deficits) should prompt urgent evaluation.
- Sudden clinical worsening may be caused by tumor progression, but other causes include tumor bleeding, nonconvalve status epilepticus, infection, corticosteroids adverse effects or withdrawal, and electrolyte and other metabolic disorders.
- A computed tomographic scan of the head without contrast is advisable to rule out tumor bleeding in patients with sudden clinical worsening. Aggressive management of chemotherapy-related thrombocytopenia is warranted to prevent tumor bleeding.
- Electroencephalography may be helpful in the evaluation of patients with unexplained symptoms worsening or confusion, to rule out nonconvulsive status epilepticus and subclinical seizures.
- Corticosteroids may be used for symptoms improvement but tapered off as soon as possible to minimize adverse effects; corticosteroid-associated hyperglycemia must be aggressively managed.
- Patients receiving chemotherapy and corticosteroids should be periodically evaluated for symptoms of oral candidiasis, mucositis, pneumocystosis, hepatitis B reactivation, skin rashes, and liver dysfunction, in addition to specific chemotherapy adverse effects (Table 3).
- Deep venous thrombosis and pulmonary embolism are frequent and require active monitoring and investigation of potential symptoms.
- Whenever required, therapeutic anticoagulation may be used; low-molecular-weight heparin compounds are preferred over warfarin.
- Patients of childbearing potential require contraception and fertility preservation discussion and counseling.
- Prior to aggressive workup for clinical complications or invasive procedures, assessment of disease stage and discussion with treating physician is warranted; if active treatment options have been exhausted, comfort care may be preferable instead.

these patients have been grouped with the anaplastic oligodendroglia.

Management of Clinical Complications of Malignant Gliomas

The key aspects in the management of malignant gliomas are summarized in Box 3. Fluctuation of neurologic symptoms is the norm throughout the disease course. Not all symptoms warrant evaluation in an emergency department setting. Minor headaches that abate with analgesics and partial seizures in a patient with known seizures may be managed in an outpatient setting. However, rapid or significant neurologic deterioration, sudden onset of severe headache, and repeated seizures or status epilepticus require emergency attention. At the emergency department, evaluation should include a noncontrast CT scan of the head to exclude acute intracranial bleeding and to characterize life-threatening tumor progression with mass effect; other treatable causes of neurologic deterioration should also be excluded.

Thromboembolic events are a frequent complication resulting from the cancer-related prothrombotic state, as well as certain treatments such as chemotherapy and bevacizumab, aggravated by neurologic deficits and immobilization. Venous thromboembolic disease is particularly frequent, occurring in 20% to 30% of patients. Therefore, anticoagulants should be used prophylactically during hospitalization in all patients and also considered in nonambulatory patients in the outpatient setting. A low threshold for investigation with a CT scan of the chest or lower extremity ultrasound ultrasound Doppler is advisable in patients with chest pain, dyspnea, increased respiratory rate or other respiratory symptoms, or lower extremity edema. The presence of the brain tumor or treatment with bevacizumab does not constitute a formal contraindication for anticoagulation. Low-molecular-weight heparin compounds are preferred because they can be reversed easily in case of CNS hemorrhage. If anticoagulation is contraindicated, such as in recent craniotomy or intracranial hemorrhage, inferior vena cava filters may be used, but unfortunately complications are frequent, including occlusion and embolism recurrence.

Corticosteroid-related hyperglycemia is common, resulting in increased morbidity and compromised tumor control. Corticosteroids increase insulin resistance, and treatment is similar to type 2 diabetes. Options include metformin, sulfonylureas, and thiazolidinediones, although an insulin regimen is sometimes necessary, individualized to address the fluctuations in glucose levels that reflect the pharmacokinetics and pharmacodynamic effects of the corticosteroid regimen used. Managing hyperglycemia is particularly challenging in the setting of the frequent fluctuations in dexamethasone doses, requiring close collaboration between the internist and neuro-oncologist. Corticosteroid-related myopathy, manifested by proximal muscle weakness with trouble taking stairs and walking, is another frequent complication that should be distinguished from tumor progression. Other corticosteroid complications that require active monitoring and prompt intervention include confusion, personality changes, insomnia, and weight gain.

Frequent chemotherapy complications are summarized in Table 3. Most glioma regimens are relatively mild, and febrile neutropenia is rare. However, aggressive management of thrombocytopenia is warranted because of the risk of CNS bleeding, especially when bevacizumab is used. Pneumocystosis prophylaxis should be considered, given frequent lymphopenia with low CD4 counts and corticosteroid use. Oral candidiasis is common and should be differentiated from chemotherapy-related mucositis, which is relatively rare. Hepatitis B screening should be considered in patients prior to initiating chemotherapy; patients previously exposed to hepatitis should receive prophylactic treatment, such as entecavir. Chemotherapy-related skin rashes may develop but are usually manageable and rarely lead to treatment discontinuation.

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Given the overall poor prognosis for glioblastoma, early discussion of palliative care and comfort care measures is recommended. It is important to establish advanced directives early in the course of disease; unlike other types of cancer, brain tumor patients may lose mental capacity unexpectedly and in the midst of active treatment, leaving family and proxies in the challenging situation of making treatment choices and defining goals of care.

Conclusions and Future Directions

Although malignant glioma remains an incurable disease, treatment options have been expanding and improving because of better understanding of the complex molecular biology of these tumors, their microenvironment, and immunologic interactions with the host. Several novel promising therapies are under evaluation, including trials of targeted agents directed at receptor tyrosine kinases and signal transduction pathways, alternative antiangiogenic agents, gene therapy, immunotherapy, reirradiation, radiolabeled drugs, and many others. Such treatments address the marked tumor heterogeneity, and many are being designed for very specific and small subgroups of patients whose tumors share distinct molecular and genetic characteristics. Participation in clinical trials and molecular screening of large numbers of patients are important to improving the care of future patients.

ARTICLE INFORMATION

Author Contributions: Dr Omuro had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Omuro, DeAngelis. Acquisition of data: Omuro. Analysis and interpretation of data: Omuro, DeAngelis. Critical revision of the manuscript for important intellectual content: Omuro, DeAngelis. Administrative, technical, or material support: Omuro, DeAngelis. Study supervision: Omuro, DeAngelis.

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Medical therapy of gliomas

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Abstract Medical therapies are an important part of adjunctive therapy for gliomas. In this chapter we will review the chemotherapeutic and targeted agents that have been evaluated in clinical trials in grade II–IV gliomas in the last decade. A number of randomized phase III trials were completed and reported. There has been a clear success in oligodendroglial tumors and low grade glioma. Although some progress has been made in glioblastoma, considerable work involving the multidisciplinary collaboration of basic science, translational and clinical investigators needs to be done to improve the outcome of patients with anaplastic astrocytoma and glioblastoma. In addition, tailoring treatment based on molecular cytogenetic characteristics is a major focus of research into precision based medicine for glioma.

Keywords Chemotherapy · Targeted treatment · Gliomas · Glioblastoma · Clinical trial · Molecular profile · Multidisciplinary

Introduction

The challenges that limit the therapeutic efficacy of chemotherapy and targeted therapies in gliomas include the blood–brain barrier (BBB), active transport mechanisms of drug efflux, and high plasma protein binding of agents [1]. In addition to the difficulty of delivery of agents across the BBB, there are other challenges that limit the efficacy of these agents. Other challenges include heterogeneity of tumors, redundancy of pathway interactions, lack of accurate and reproducible biomarkers to select patients for specific therapies, and difficulty in assessing target modulation [2–4]. Intrinsic and rapidly acquired resistance further limit the efficacy of chemotherapy or targeted therapy. Chemotherapeutic approaches have demonstrated efficacy in oligodendroglioma. For several chemotherapy-refractory tumor types including glioblastoma and anaplastic astrocytoma, new approaches continue to be explored and will be reviewed. Finally, the future directions involving precision medicine approaches to optimize the therapeutic index of drug treatments for glioma will be discussed.

Low grade gliomas (WHO grade II)

Until recently, low-grade gliomas were considered to be chemotherapy resistant and there have been limited trials evaluating the utility of chemotherapy in low-grade glioma in adults. In a small Southwest Oncology Group trial, patients with incompletely excised low-grade gliomas were randomized to radiation therapy (RT) alone or combination of RT and lomustine (CCNU). The survival in both the two arms was similar [5]. Radiation Therapy Oncology Group (RTOG) study, RTOG-9802 examined the role of adjuvant chemotherapy—procarbazine, CCNU, and vincristine (PCV) for “high-risk” adults (less than total resection, age older than 40 years) with low-grade gliomas. Two hundred and fifty-one patients were randomized to RT alone or RT followed by six cycles of PCV. Progression-free survival (PFS) but not overall survival (OS) was improved in the
RT and the PCV group compared to RT alone at the time of the initial data analysis [6]. At the time of that report however 65 % of the patients were still alive. A recent National Institute of Health press release on more mature results of this study reported significant improvement in OS in the PCV chemotherapy plus RT arm (13.3 years) compared to those assigned to RT alone (7.8 years) at a median follow-up of 12 years [7]. Correlative studies to establish the predictive role of molecular and cytogenetic characteristics [isocitrate dehydrogenase (IDH) mutations, loss of heterozygosity of 1p/19q, as well as methylation of methylguanine methyl transferase (MGMT) status] clinical outcome are pending.

The first results from the RTOG 0424 study demonstrated the improved 3-year OS of a regimen of concurrent and adjuvant temozolomide (TMZ) and radiotherapy in a high-risk low-grade glioma population compared to the 3 year OS rate of the high risk EORTC LGG patients reported by Pignatti et al. [8]. The 3 year OS rate was 73.1 % (95 % CI 65.3–80.8 %), significantly improved in comparison to the pre-specified historical control (p value <0.0001) [9]. There is an ongoing intergroup phase III trial to address the role of adjunctive TMZ for LGG.

Several studies have evaluated PCV and TMZ in recurrent low grade gliomas [10–21]. Approximately half the patients treated with either TMZ or PCV experienced imaging stability or improvement of neurologic symptoms in these studies. The limitations of these studies include small numbers and the varied imaging criteria used to assess response. Patients with low-grade oligodendroglial tumors with 1p/19q deletion or t(1p; 19q) have longer PFS and OS than those without [22] and consequently, 1p/19q determination is important in stratification in future clinical trials. A randomized phase III EORTC trial stratified patients with low-grade glioma by 1p status prior to randomization to RT versus TMZ alone [23]. In the first report of the trial, PFS was similar in both groups while median OS was not reached. This study showed 1p deletion as a positive prognostic factor irrespective of treatment at the time of this first analysis [PFS 0.0003; HR 0.59, 95 % CI (0.45–0.78); OS 0.002; HR 0.49, 95 % CI (0.32–0.77)].

Recent studies have identified alterations in the BRAF serine/threonine kinase gene as the likely causative mutation in childhood LGG and approaches to target this abnormality are being explored [24]. In addition, aberrant signaling in pathways including the phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) network [25, 26] have also been identified in LGG and clinical trials are currently ongoing to target this pathway as a therapeutic approach.

In addition to the known side effects of myelosuppression from the use of alkylating agents, there may be adverse effects on the mutational landscape of tumors following this known mutagenic treatment. Johnson et al. [27] reported on a group of patients with grade II astrocytoma for whom tumor tissue was available for genomic analysis at the time of initial diagnosis and at the time of progression. They demonstrated the potential for TMZ to induce specific driver mutations that could contribute to the malignant transformation of grade II astrocytoma to glioblastoma. It is unclear which subset of patients is at specific risk for this mutagenic effect of TMZ.

**Anaplastic oligodendrogliomas (WHO grade III)**

Several retrospective series and phase II trials suggested chemo sensitivity of oligodendrogliomas [12, 28, 29]. Two randomized prospective phase III trials evaluated the role of chemotherapy in this tumor type and patients were treated with either RT alone or RT in combination with PCV. In the RTOG-9402 trial, patients were randomized to either four cycles of intensified PCV followed by RT or immediate RT without chemotherapy. At initial report, survival in the two groups was the same and patients with 1p/19q deletions had significantly better outcomes, regardless of type of treatment [30]. A posthoc analysis showed that there was a PFS benefit from PCV that was most notable in patients with 1p/19q deletions. With over 11 years follow-up, mature data from this study showed that median survival of those with co-deleted tumors treated with PCV plus RT was twice that of patients receiving RT (14.7 vs. 7.3 years) [31]. The survival of patients with co-deleted tumors was better than those with non-co-deleted tumors regardless of treatment. The survival was not statistically significant for patients with tumors lacking 1p/19q deletion irrespective of treatment (median survival 2.6 vs. 2.7 years).

In the EORTC 26951 trial, 368 patients received immediate RT only or RT followed by six cycles of PCV [32]. Samples from 86 % of patients were available for analysis for 1p/19q codeletion. At the time of first report, the PFS was better in the PCV group, but OS was similar. Patients with 1p/19q deletion had better outcomes, irrespective of therapy. In addition, MGMT promoter methylation was of prognostic value in this cohort [33, 34]. Long-term follow up in patients with the 1p/19q codeletion showed that the addition of PCV to RT significantly increased PFS (median 157 vs. 50 months) and there was a trend toward increase in OS (OS not reached in the RT/PCV group vs. 112 months in the RT group HR 0.56; 95 % CI 0.31–1.03) [35].

Temozolomide has produced high response rates in patients with anaplastic oligodendroglioma. In 27 newly diagnosed patients treated with TMZ prior to radiotherapy the objective response rate was 33 % and the 6-month
progression rate was 10 % [36]. An international intergroup trial is being conducted in patients with newly diagnosed grade III glioma with 1p/19q status codeletion (NCT00887146). Patients are randomized to three arms, TMZ alone (phase II group); or radiotherapy with concomitant and adjuvant TMZ or radiotherapy with adjuvant procarbazine, lomustine and vincristine (PCV) (phase III).

Chemotherapy for recurrent anaplastic oligodendroglioma

Both PCV and TMZ have activity in patients that recur after radiotherapy although generally response rates are lower and the duration of disease control is shorter. The activity of TMZ was seen in a study of 48 patients with anaplastic oligodendroglioma/oligoastrocytoma who progressed on PCV [37]. The objective response rate was 44 %, including 17 % who achieved complete remission. The median PFS was 7 months and the median OS was 10 months. Although there is no direct comparison of TMZ and PCV to determine which regimen is superior in terms of efficacy, the absence of cumulative myelosuppression with TMZ makes it the preferred choice in the setting of recurrent disease.

Anaplastic astrocytoma (WHO grade III)

The role of chemotherapy in anaplastic astrocytoma is not well established. Most phase III trials have demonstrated no benefit of chemotherapy compared with radiation alone in this tumor type. Carmustine and PCV are associated with minimal improvement in survival [38]. The Glioma Meta-Analysis Trialists’ group showed a 6 % increase in 1- and 2-year survival for patients who received chemotherapy (2-year survival of 37 vs. 31 %) in a meta-analysis [39]. A large randomized trial of adjuvant PCV compared with RT alone did not show any benefit of adjuvant PCV [40]. The RTOG-9813 was a phase III study comparing radiation with BCNU or CCNU to radiation with TMZ, and the results of this study are pending.

The NOA-04 phase III trial compared the efficacy of RT followed by chemotherapy at progression, to initial chemotherapy followed by RT at progression, in newly diagnosed anaplastic gliomas [41]. Patients received conventional RT, PCV or TMZ as initial therapy. At disease progression or occurrence of unacceptable toxicity, patients in the RT arm received PCV or TMZ, whereas patients in chemotherapy arms were treated with RT. Median time to failure, PFS and OS were similar in all the treatment arms. Methylguanine DNA-methyltransferase (MGMT) promoter methylation and IDH1 mutations were included in the correlative part of the study due to their prognostic value [42–44]. Patients with hypermethylation of the MGMT promoter had prolonged PFS both in the RT and the chemotherapy arm. Hypermethylation of MGMT promoter, IDH1 mutations and oligodendroglioma histology was associated with a decreased risk of progression. The study demonstrated the prognostic value of IDH1 mutations in anaplastic gliomas, with a favorable impact that was more significant than that of 1p/19q codeletion or MGMT promoter methylation [41].

A large international trial, CATNON is being conducted in patients with newly diagnosed grade III glioma stratified by 1p/19q status. Nondeleted patients are randomized to radiation with or without TMZ; following radiotherapy there is a second randomization to adjuvant TMZ or not.

Chemotherapy for recurrent anaplastic astrocytomas

Studies of both nitrosourea-based approaches and TMZ have demonstrated efficacy in recurrent anaplastic astrocytomas. A study of TMZ in recurrent anaplastic astrocytoma demonstrated a response rate of 35 % for patients who were chemotherapy naive and 20 % for patients who had received nitrosourea-based therapy [45]. This led to accelerated approval for TMZ by the US Food and Drug Administration. Based on activity of bevacizumab in recurrent glioblastoma, it is often used in patients with recurrent anaplastic astrocytoma [46]. A retrospective study reported a 64 % radiographic response and 6-month PFS rate of 60 % in 25 patients [47].

Glioblastoma (WHO grade IV)

Over the last decade there were a considerable number of investigational studies performed and reported in patients with glioblastoma. In a landmark study of approximately 600 patients, patients were randomized to RT alone (60 Gy in daily 30 fractions) or in combination with concurrent TMZ (75 mg/m² daily up to 49 days) and followed by up to six cycles of adjuvant TMZ (150–200 mg/m² daily for 5 days, every 28 days). There was statistically significant increase in OS in the combination arm compared with RT alone [27 vs. 11 % at 1 year, hazard ratio (HR) for death 0.63] [48]. Median and 2-year survival was increased by 2.5 months and 16.1 %, respectively [48]. This study provided level 1 evidence favoring use of TMZ for patients with newly diagnosed glioblastoma (Table 1). Accompanying correlative study demonstrated that methylation of the promoter region of the MGMT gene in the tumor was associated with superior survival, regardless of treatment received, but the benefit was primarily seen in methylated patients [42]. The 2-year survival rates were 49 and 24 % with combination therapy and with RT alone, respectively.
in patients with MGMT methylation. The 2-year survival rates were 15 and 2%, respectively in those without MGMT methylation. Preclinical work suggested that different prolonged schedule of TMZ may overcome chemotherapy resistance that led to studies looking at alternative dosing of TMZ in the newly diagnosed setting and at the time of recurrence [49, 50]. A large phase III randomized international study led by the RTOG compared the standard treatment versus a 21- or 28-day adjuvant TMZ schedule [51]. Dose-dense TMZ failed to result in improved efficacy regardless of tumor methylation status but was associated with more profound lymphopenia and fatigue. Strategies to increase the therapeutic ratio of existing chemotherapies, such as the inhibition of DNA repair enzymes [i.e., poly[ADP-ribose] polymerase (PARP) or base excision repair] are being evaluated. These agents are being combined with radiation and chemotherapy to increase the cytotoxicity of the combination approach [52–54].

The evaluation of chemotherapy in the elderly glioblastoma patient has been the focus of several recent trials. More than half of all patients with GBM are aged 65 years or older at the time of diagnosis, and the incidence rate of GBM in patients aged over 65 years is increasing rapidly. In addition, age is a well-known prognostic factor in this disease and the median survival for elderly GBM patients is <6 months. The use of chemotherapy for elderly GBM patients remains controversial and several factors should be considered including age, MGMT methylation status, performance score, medical co-morbidities and patient preferences. Concurrent and adjuvant TMZ along with RT to 60 Gy have not been prospectively studied among patients aged over 70 years but should be considered for patients aged 65–70 years with excellent KPS [48]. Several approaches to shorten the duration of radiation (hypofractionated radiation) or to use chemotherapy alone have been evaluated. Based on recent randomized trials, testing for O6-methylguanine-DNA-methyltransferase (MGMT) promoter methylation should be performed routinely immediately after surgery to aid in adjuvant treatment decisions [55, 56]. For patients aged over 70 years with favorable KPS, or patients aged 60–70 years with borderline KPS, monotherapy utilizing standard TMZ dosing for patients with MGMT-methylated tumors, and hypofractionated RT (34 Gy in ten fractions or 40 Gy in 15 fractions) for patients with MGMT-unmethylated tumors should be considered. For elderly patients with poor KPS, reasonable options include best supportive care, TMZ alone or hypofractionated RT alone [55–57]. The role of concurrent TMZ with hypofractionated RT is being evaluated in an ongoing European Organization for Research and Treatment of Cancer/National Cancer Institute of Canada trial.

Targeted therapies for glioblastoma including anti-angiogenic approaches

The last decade has witnessed considerable progress being made in the understanding of the genetic and molecular pathogenesis of gliomas. This has in turn led to the identification of new potential therapeutic targets and the development of signaling pathway modulators.

Glioblastoma is a highly vascular tumor that is dependent on microvascular proliferation for survival and research into angiogenesis and its blockade have been

### Table 1 Newly diagnosed glioblastoma phase III trials—level 1 evidence

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Number of patients/treatment arm</th>
<th>Treatment arms</th>
<th>PFS</th>
<th>OS</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>EORTC/NCI [48]</td>
<td>287 versus 286</td>
<td>RT/TMZ + TMZ versus RT</td>
<td>6.9 versus 5.0 months</td>
<td>14.6 versus 12.1 months</td>
<td>RT/TMZ + TMZ is superior to RT alone</td>
</tr>
<tr>
<td>RTOG 0525 [51]</td>
<td>411 versus 422</td>
<td>Standard dose TMZ (days 1–5 every 28 days) versus dose dense TMZ (days 1–21 every 28 days)</td>
<td>5.5 versus 6.7 months</td>
<td>16.6 versus 14.9 months</td>
<td>Dose dense and standard 5 day TMZ are equivalent in efficacy regardless of methylation status</td>
</tr>
<tr>
<td>RTOG 0825 [63]</td>
<td>320 versus 317</td>
<td>RT/TMZ/Bev + TMZ/Bev versus RT/TMZ + TMZ</td>
<td>10.7 versus 7.3 months</td>
<td>15.7 versus 16.1 months</td>
<td>PFS was longer in Bev group; however there was no significant difference in OS</td>
</tr>
<tr>
<td>AVAglio [64]</td>
<td>458 versus 463</td>
<td>RT/TMZ/Bev + TMZ/Bev versus RT/TMZ + TMZ</td>
<td>10.6 versus 6.2 months</td>
<td>16.9 versus 16.8 months</td>
<td>PFS was longer in Bev group; however there was no significant difference in OS</td>
</tr>
<tr>
<td>CENTRIC [71]</td>
<td>272 versus 273</td>
<td>RT/TMZ/CIL + TMZ/CIL versus RT/TMZ + TMZ</td>
<td>13.5 versus 10.7 months</td>
<td>26.3 versus 26.3 months</td>
<td>CIL did not prolong PFS or OS in methylated MGMT gene promoter GBM</td>
</tr>
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</table>

among the top priorities in the last decade. The most important mediator of angiogenesis in glioblastoma is vascular endothelial growth factor (VEGF). Two trials, the BRAIN study and the NCI study showed that treatment with the anti-VEGF monoclonal antibody bevacizumab resulted in dramatic radiological responses and prolonged PFS relative to historical controls [58, 59]. Based on the response rates seen in the BRAIN study, which was a randomized noncomparative phase II study of 167 patients who were treated with bevacizumab alone or with irinotecan, and the NCI led single arm phase II study of bevacizumab alone, the US Food and Drug Administration granted accelerated approval to bevacizumab for recurrent glioblastoma in 2009 [46]. The PFS at 6 months was 43 and 50 % for bevacizumab alone and the combination arm in the BRAIN study respectively. The objective response rates were 28 and 38 % for the two arms and the OS was 9.2 and 8.7 months, respectively. The NCI study demonstrated a PFS at 6 months of 29 % and a radiographic response rate of 35 % with bevacizumab. The most common side effects associated with bevacizumab include fatigue, headache, and hypertension. A number of studies have examined whether additional chemotherapy or targeted therapy to bevacizumab translates into additional efficacy compared to bevacizumab alone. A Phase II trial (CABARET) evaluated the efficacy of adding carboplatin to bevacizumab in recurrent glioblastoma. The PFS at 6 months was 26 % and OS was 6.9 months for the combination versus 6-month PFS of 24 % and OS of 6.4 months for bevacizumab alone [60]. The addition of chemotherapy or targeted therapy has failed to show any added benefit in recurrent GBM trials with the exception of the BELOB study. In the BELOB study, a three-arm multicenter randomized phase II study, 148 recurrent glioblastoma patients received bevacizumab alone, lomustine alone or the combination of the two. OS at 9 months was 38, 43 and 59 % and the PFS-6 was 16, 13 and 41 % in the three arms respectively [61]. EORTC 26101 will assess the role of bevacizumab and lomustine versus lomustine alone in a randomized phase III trial in recurrent GBM.

The benefit of bevacizumab in recurrent glioblastoma prompted its evaluation in the treatment of newly diagnosed glioblastoma. There are several small single-arm phase II studies of the combination of bevacizumab with radiation and TMZ in the newly diagnosed setting [62]. Two large randomized trials evaluated the benefit of addition of bevacizumab to RT and TMZ. The first study, RTOG 0825 was a randomized, double-blinded, placebo controlled trial and was conducted primarily in the United States. In this study the addition of bevacizumab resulted in longer PFS that did not reach the preset level of significance (10.7 vs. 7.3 months, HR 0.79). There was no difference in OS between two arms (16.1 vs. 15.7 months, HR 1.13) [63]. The AVAglio study was an industry-sponsored, international, multicenter Phase III placebo-controlled randomized trial in newly diagnosed glioblastoma [64]. This study demonstrated that the addition of bevacizumab to RT and TMZ produced a clinically meaningful and statistically significant improvement in PFS (HR 0.64, \( p < 0.0001 \); median 10.6 vs. 6.2 months) as compared to RT and TMZ. However similar to the RTOG 0825 there was no difference in median survival (16.7 months for the placebo group; 16.8 months for the bevacizumab group. HR 0.88, \( p = 0.0987 \)).

The open-label GLARIUS trial was a randomized, multicenter study of MGMT-nonmethylated GBM. The patients were randomized in a 2:1 manner to receive bevacizumab during RT that was followed by maintenance bevacizumab and irinotecan compared to standard therapy of 6 weeks of concurrent RT and TMZ followed by 6 cycles of adjuvant TMZ [65]. Preliminary results of this study demonstrated a PFS-6 rate of 71.1 % in the experimental arm compared to 26.2 % in the control arm \(( p < 0.0001 \log \text{rank test})\). Final results are pending.

Despite improvement in PFS, there has been no benefit in OS with bevacizumab possibly due to resistance that can be due to intrinsic or acquired (evasive) mechanisms.
Hence a number of strategies have tested combination of bevacizumab with other targeted agents, or evaluating agents that target other antiangiogenic pathways such as platelet-derived growth factor (PDGF), integrins or hepatocyte growth factor (HGF). Despite promising results in a phase II study of cediranib (AZD2171), an orally administered pan-VEGF receptor inhibitor [66], a Phase III randomized trial (REGAL) that compared the efficacy of cediranib either as monotherapy or in combination with lomustine failed to show any improvement in PFS compared to lomustine alone in recurrent GBM [67] (Table 2). VEGF Trap (aflibercept) in a phase II study showed minimal evidence of single-agent activity in unselected patients with recurrent malignant glioma [68].

Cilengitide is a cyclic pentapeptide that selectively competitively inhibits the aVb3 and aVb5 integrins and has antiangiogenic properties [69, 70]. Cilengitide showed initial promise in recurrent GBM studies that led to its evaluation in two large newly diagnosed studies [69, 70]. The CORE study evaluated the efficacy of cilengitide in the unmethylated MGMT gene promoter in a multicenter, randomized phase II trial. The study showed a median OS of 16.3 months in the cilengitide arm compared to a median OS of 13.4 months in the control-group (HR 0.69; p = 0.033). The CENTRIC study was a phase III trial that looked at the benefit of cilengitide combined with RT and TMZ for newly diagnosed glioblastoma with MGMT promoter methylation [71]. The study failed to show any additional benefit of cilengitide in this patient population [71]. Median OS was 26.3 months in both arms and median PFS was 13.5 months in the cilengitide arm and 10.7 months in the control arm (p = 0.87). This drug is not being further developed.

The other antiangiogenic agents that have undergone investigation in recurrent glioblastoma include multi-targeted tyrosine kinase inhibitors such as sunitinib, sorafenib, cabozantinib and enzastaurin, an inhibitor of protein kinase C-beta that targets VEGF, as well as the mTOR pathway [72]. The outcomes of the studies with these agents have been similar or inferior compared to those seen with bevacizumab [72–74].

The EGFR pathway can be dysregulated in up to 40 % of glioblastoma and number of phase I and II trials of erlotinib and gefitinib for recurrent high-grade gliomas evaluated the efficacy of these agents. However, the results of most of these trials were disappointing and showed limited activity for these agents [75–78]. There were reports that tumors with the variant 3 mutant (EGFRvIII), that permits the distinction of different grades within categories of the same tumor type, such as astrocytomas, that may predict clinical outcome, it does not address the issue that distinct genetic subgroups may exist within each grade.

The future of medical treatment of gliomas

Precision medicine

As treatment for gliomas evolve in the ensuing years, studying the biological behavior of these tumors in the context of therapeutic options is increasingly important. Precision therapy that is tailor-made treatment around the molecular evolution of these tumors will require employment of high-throughput genomic technology in the clinical setting. The brain tumor centers of excellence will need to institute effective workflow that encompass tissue collection after surgery, proper as well as prompt processing, and standardization of biomaterial extraction. The tissue will require sequencing (combination of targeted capture sequencing, whole genome sequencing, and RNA sequencing), and data analysis that will lead to therapeutic recommendations for each individual will be a critical component to translate the information to the clinical management of the patient. The genomic profiling can not only inform diagnosis and but alter treatment approach as more targeted agents are available in the future.

Molecular characterization of the gliomas

The recent molecular characterization of gliomas has clarified a framework of different subtypes of these tumors and has revealed pathways that will help the development of more effective targeted therapies. The diagnosis of gliomas in the past was based on a complete clinicopathological assessment. Although this is a valuable approach that permits the distinction of different grades within categories of the same tumor type, such as astrocytomas, that may predict clinical outcome, it does not address the issue
In recent years there has been extensive work in large-scale gene expression profile studies in glioblastoma to characterize the molecular subtypes of GBM that include a report of the Cancer Genome Atlas Research Network [91]. These genomic analyses provided insights underlying tumor biology that further classify different subtypes that may inform treatment plans, impact patient outcome, and improve response to treatment [92, 93]. Verhaak et al. [93] classified glioblastoma into proneural, neural, classical, and mesenchymal subtypes based on gene expression profiles of these tumors. Aberrations and differential gene expression of \( \text{EGFR} \), \( \text{NF1} \), and \( \text{PDGFA} \) help define the various subtypes and these pathways can be targeted using novel therapies. The work in genome and transcriptome shows that glioblastoma is a heterogeneous tumor with multiple redundant pathways and distinct subtypes [94].

Considerable research in genetic alterations in WHO grade II astrocytoma in adults has shown the role of inactivation of the TP53 tumor suppressor gene, heterozygous point mutations of the IDH1, and loss of chromosome 22q in these tumors. TP53 on chromosome 17p encodes the p53 protein that has an important role in cellular processes, including apoptosis, cell cycle arrest, and response to DNA damage [95]. Somatic mutations in IDH1 are present in 50–80 % of WHO grade II and III astrocytic tumors and oligodendrogial tumors in adults and up to 5 % of the secondary glioblastomas [44, 96]. These mutations lead to conversion of \( \alpha \)-ketoglutarate into D-2-hydroxyglutarate, an oncometabolite that drives the oncogenic activity of IDH mutations [97]. Patient with tumors with IDH mutations have better outcomes than do IDH-wild-type gliomas of the same histological grade [98, 99]. Recent discoveries of pathogenic mutations in \( \text{IDH1} \) [97], \( \text{IDH2} \), \( \text{ATRX} \) [100], \( \text{CIC} \) [101], and \( \text{FUBP1} \) [101], have helped genomic characterization of low grade gliomas. These mutations form the framework of molecular pathogenesis of these tumors and offers robust markers that not only enhance classification but also guide treatment. Common cytogenetic alteration in oligodendrogial histology consists of an unbalanced t(1;19)(q10;p10) translocation that results in combined loss of chromosomal arms 1p/19q and leads to the loss of one hybrid chromosome and thus loss of heterozygosity [22]. Tumors with 1p/19q-codeletions have a better prognosis than do histologically identical tumors of the same grade that do not harbor this codeletion [102]. The key to successful treatment of these tumors will lie in the realization that these molecularly defined subsets are different disease entities and it is likely that specific targeted therapies aimed at the driver mutations will be more likely to be efficacious. In the future, the molecular classification of these tumors will be performed routinely and be defined in clinically relevant terms based on the identification of markers that define subsets and guide therapeutic options.

The next 10 years

The advances in imaging, improved targeted therapeutic options, and routine availability of molecular characterization of tumors will enhance glioma management in the next decade. A great deal of progress has been made in the last decade in the understanding of the molecular mechanisms of gliomas. The continuation of these efforts may further classify the subtypes of tumors of the same grade and warrant different therapeutic options for the patients. Accelerated developments of new drugs will likely aid improvements in therapeutic outcomes in the next 10 years. Given the complex network of pathways involved, one approach would be the use of multitargeted therapy that simultaneously aims at different constitutive pathways driving the malignancy. Further developments in drug delivery will play a key role in translating this into improved patient outcomes. While the next decade appears to be promising, considerable work involving the multidisciplinary collaboration of basic science, translational and clinical investigators will need to be done to improve the outcome of patients with gliomas.

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Low-Grade Gliomas

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Glioma • Astrocytoma • Oligodendroglioma • Surgery • Radiation

ABSTRACT

Low-grade gliomas (LGGs) are a diverse group of primary brain tumors that often arise in young, otherwise healthy patients and generally have an indolent course with longer-term survival in comparison with high-grade gliomas. Treatment options include observation, surgery, radiation, chemotherapy, or a combined approach, and management is individualized based on tumor location, histology, molecular profile, and patient characteristics. Moreover, in this type of brain tumor with a relatively good prognosis and prolonged survival, the potential benefits of treatment must be carefully weighed against potential treatment-related risks.

We review in this article current management strategies for LGG, including surgery, radiotherapy, and chemotherapy. In addition, the importance of profiling the genetic and molecular properties of LGGs in the development of targeted anticancer therapies is also reviewed. Finally, given the prevalence of these tumors in otherwise healthy young patients, the impact of treatment on neurocognitive function and quality of life is also evaluated. The Oncologist 2014;19:403–413

Implications for Practice: This review summarizes the epidemiology, presentation, diagnosis, and treatment of low-grade gliomas. The article discusses recent advances in genetic characterization of these tumors, which has become particularly important in guiding tumor identification and classification, and may in some cases offer information about prognosis and/or expected response to treatment. The major studies regarding the use of radiation, chemotherapy, and surgical approaches in the treatment of these tumors are discussed. This information will aid medical oncologists in understanding the challenges inherent in diagnosing and treating patients with low-grade gliomas, and in recognizing the important factors to consider in devising treatment plans.

INTRODUCTION

Central nervous system tumors are defined by their cell of origin and their histopathological characteristics, which predict their behavior [1]. Gliomas are neuroepithelial tumors originating from the supporting glial cells of the central nervous system (CNS). Glial tumors consist of astrocytomas, oligodendrogliomas, mixed oligo-astrocytic, and mixed gli-neuronal tumors, which arise from astrocytic, oligodendrog-lial, mixed oligoastrocytic, or neuronal-glial cells, respectively. The World Health Organization (WHO) classification system categorizes gliomas from grade 1 (lowest grade) through grade 4 (highest grade), based upon histopathological characteristics such as cytological atypia, anaplasia, mitotic activity, microvascular proliferation, and necrosis. Low-grade gliomas (LGGs) consist of grade I tumors, which contain none of the aforementioned histologic features, and grade II tumors, characterized by the presence of cytologic atypia alone [1]. Low-grade astrocytic tumors include diffuse astrocytoma, pilomyxoid astrocytoma, and pleomorphic xanthoastrocytoma (WHO grade II), as well as subependymal giant cell astrocytoma (SEGA) and pilocytic astrocytoma (WHO grade I tumors). Low-grade oligodendrogial tumors include oligoden-drogliomas and oligoastrocytomas (WHO grade II tumors) [1]. Low-grade glioneuronal tumors include the following WHO grade I tumors: ganglioglioma, desmoplastic infantile astrocytoma and ganglioglioma, dysembryoplastic neuroepithelial tumor, papillary glioneuronal tumor, and rosette-forming glioneuronal tumor of the fourth ventricle [1]. In this review, we discuss the epidemiology, clinical, and diagnostic characteristics, histopathologic and molecular features, prognosis, and treatment of LGG. For the purposes of this review, we will focus on supratentorial nonpilocytic astrocytomas, oligodendroglia-mas, and oligoastrocytomas. Selected other LGG subtypes, including subependymal giant cell astrocytoma (SEGA), pleo-morphic xanthoastrocytoma, brainstem glioma, and pilocytic astrocytoma, will be discussed briefly.

EPIDEMIOLOGY

In 2012, more than 66,000 primary CNS tumors were diagnosed in the U.S., 30% (approximately 20,000) of which were gliomas [2]. In young adults (20–34 years of age), gliomas...
account for 32% of all primary CNS tumors, 17% of which are astrocytic tumors; 28% of these are glioblastomas [2]. Available data do not separate high-grade versus low-grade tumors; thus, the annual incidence of LGG is difficult to determine. Incidence rates for oligodendrogliomas, anaplastic astrocytomas, glioblastomas, and mixed gliomas are more than two times higher in whites than in blacks [2]. The reason for this racial discrepancy is uncertain. It may represent detection bias, a genetic difference, or another as yet unidentified explanation. Various environmental risk factors have been examined for evidence of a link between environmental exposures and an increased risk of brain tumor formation. The only factor definitively shown to be correlated with an increased risk of secondary brain tumors is CNS exposure to therapeutic or high-dose radiation [3]. Other environmental exposures have been investigated, without compelling evidence to support their role in brain tumor formation. Numerous genetic mutations conferring increased glioma risk have been described, including NF1 and NF2 mutations in neurofibromatosis types 1 and 2, respectively; TSC1 and TSC2 mutations in tuberous sclerosis; TP53 mutations in the Li-Fraumeni syndrome; and a number of gene mutations associated with Turcot’s syndrome and multiple hamartomas, including APC, hMLH1, hMLH2, PMS2, and PTEN mutations [3]. However, these genetic conditions are found in only a very small percentage of patients diagnosed with LGG each year in the U.S.

**PRESENTATION**

LGGs present most commonly in the second through fourth decades of life, with peak incidence in the third and fourth decades of life. Clinical signs and symptoms vary and are largely attributed to mass effect from invasion into surrounding parenchyma or obstructive hydrocephalus [4]. Seizure is the presenting symptom in up to 80% of patients [4]. Others may present with cognitive or behavioral changes, focal neurologic deficits, or clinical signs or symptoms of increased intracranial pressure, such as headache or papilledema. However, patients may also be asymptomatic, without evident abnormalities on neurologic examination.

**DIAGNOSIS**

Diagnosis of LGGs is made through a combination of imaging, histopathology, and molecular diagnostic methods. On computed tomography scan, low-grade gliomas appear as diffuse areas of low attenuation. On conventional magnetic resonance imaging (MRI), which is currently the imaging modality of choice, LGGs are often homogeneous with low signal intensity on T1-weighted sequences and hyperintensity on T2-weighted and Fluid-Attenuated Inversion Recovery (FLAIR) sequences (Fig. 1). Calcifications may be evident as hypointensity on T1-weighted and hyperintensity on T2-weighted MR sequences, and increased relative cerebral blood volume on perfusion-weighted MRI [7, 8]. Despite characteristic radiographic findings, tumor grade cannot be determined by imaging alone. Newer imaging techniques, such as MR spectroscopy (MRS) and positron emission tomography (PET) imaging, may improve the diagnostic potential; however, at this time, histopathologic examination of tissue remains the gold standard for diagnosis and grading of LGG.

**Surgery**

The main goal of surgery is to obtain pathological diagnosis and, when feasible, to achieve gross total resection. Advances such as preoperative functional MRI and tractography, as well as intraoperative neurophysiological monitoring, allow surgeons to safely maximize resection of T2/FLAIR abnormalities on MRI often involving eloquent areas. Patients with tumors that cannot be safely resected, or who have lesions of uncertain etiology, may undergo stereotactic biopsy using preoperative or intraoperative MRI imaging to obtain tissue for histopathological analysis. Surgeons target the potentially higher grade component of the lesion (for example, contrast enhancement) for biopsy. The yield of such biopsies is as high as 90%–95%; however, because of the potential heterogeneity of these tumors, biopsy may not reflect the highest grade for diagnosis, with reported accuracy rates ranging from 51% to 83% [4].

**Histopathology**

The tissue sample is stained using hematoxylin and eosin, which allows for identification and classification of tumor type. Diffuse astrocytomas consist of well-differentiated fibrillary or gemistocytic neoplastic astrocytes on a loose matrix. Oligoastrocytomas are diffusely infiltrating tumors with a mixture of oligodendroglial and astrocytic cell types (Fig. 2) [1]. Oligodendrogliomas are infiltrating tumors containing cells with uniform-appearing nuclei and perinuclear clearing, often described as having a “fried egg” appearance.

**Molecular Pathology**

In the last decade, genetic characterization has become paramount in tumor identification and classification and is often predictive of tumor behavior, by providing information about prognosis and/or expected response to treatment. Deletion of selected regions on chromosomes 1p and 19q is of particular importance in low-grade gliomas, as it has a strong association with the oligodendroglioma tumor subtype. Loss of the 1p36 region has been noted in 18% of astrocytomas and 73% of oligodendrogliomas; loss of the 19q13.3 region is described in 38% of astrocytomas and 73% of oligodendrogliomas [9]. The 1p36 and 19q13.3 regions are coded for 11% of astrocytomas and 64% of oligodendrogliomas [9].

Isocitrate dehydrogenase 1 and 2 gene mutations (IDH1 and IDH2) have been reported in LGGs. Mutations in amino acid 132 of the IDH1 gene are observed in the majority (>70%) of WHO grade II and III astrocytomas and oligodendrogliomas, as well as in secondary glioblastomas originating from these
lesions [10, 11]. In contrast, IDH2 mutations are rare, found in only about 6% of LGG [12]. Molecular diagnostic methods are typically used to identify the presence of such mutations following tumor tissue sampling (via biopsy or resection). There are also emerging noninvasive methods of detecting such mutations. The commonly identified arginine 132 (R132) mutation in IDH1 results in excess production of the 2-hydroxyglutarate metabolite, which can be detected using optimized in vivo spectral editing and one- and two-dimensional brain MRS (Fig. 3) [11].

There has been recent interest in the role of the CIC gene, a homolog of the Drosophila gene capicua, located on chromosome 19q. Mutations in this gene have been associated with oligodendroglioma and oligoastrocytomas [13, 14]. CIC gene mutations were observed in 69% of oligodendrogliomas in one series and have been described as highly associated with isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) mutations and 1p/19q codeletion, with clinical significance as yet unclear [15]. Mutations in the FUBP1 gene on chromosome 1p have also been associated with oligodendrogliomas [13].

Mutation and overexpression of tumor protein 53 (TP53) (>60%), particularly gemistocytic astrocytomas, of which >80% carry a TP53 mutation [1, 16].

In cases in which pathologic diagnosis is difficult, such as distinguishing between gliosis and tumor, the presence of TP53 and IDH1 expression by immunohistochemistry can be very helpful in establishing the diagnosis of tumor.

**Prognosis**

Immunohistochemical markers of tumor cell proliferation, molecular and genetic characteristics of the tumor cells, and patient characteristics can all be useful in predicting tumor behavior, response to treatment, and patient prognosis.

**Proliferative Indices**

Immunohistochemical labeling of Ki-67 or MIB-1 monoclonal antibodies against the Ki-67 nuclear proliferation-related protein is used to evaluate the mitotic activity of the glioma cells. In LGG, these indices are generally low, suggesting low mitotic activity: <4% in diffuse astrocytoma, 3%–5% in oligodendrogliomas, and <6% in oligoastrocytomas [17]. Higher mitotic indices by immunohistochemistry are typically associated with more aggressive LGG behavior.
The molecular pathology of LGG is playing an increasingly important role in the prediction of tumor response to treatment and prognosis. The 1p-19q codeletion has been identified as a significant marker of prolonged survival in oligodendroglioma, regardless of tumor grade; such favorable association between 1p-19q status and prognosis was not demonstrated in patients with astrocytoma or oligoastrocytoma [18].

IDH1 and IDH2 gene mutations are also associated with prolonged survival and enhanced sensitivity to treatment. In a study of 132 LGG patients with IDH gene mutations, the mutations were associated with prolonged overall survival [12]. The authors also demonstrated a significant increase in response to the oral alkylating agent temozolomide in the patients with IDH-mutated tumors [12]. The DNA repair protein O6-methylguanine-methyltransferase (MGMT) has also been shown to play a key role in treatment-related prognosis, as this protein confers some degree of resistance to alkylating agents [19]. Methylation of the MGMT promoter, which thereby silences the gene, is associated with improved response to treatment and prolonged progression-free survival in temozolomide-treated patients [12, 19]. Combinations of two or more of these molecular aberrations also have significant prognostic power. There is longer survival in patients with combined IDH mutant/MGMT methylation status versus patients with IDH wild-type tumors and even more favorable prognosis in those patients with 1p-19q codeletion [20]. The prognostic significance of isolated TP53 mutation and overexpression is not well-established, although some studies have suggested its role as a poor prognostic marker with respect to survival [16, 17]. The combination of nuclear TP53 immunopositivity with IDH gene mutation and MGMT methylation is associated with a significant risk of malignant transformation [20]. With all of these molecular markers, some question remains as to whether they are truly prognostic indicators on their own, or are merely predictors of survival in the setting of chemotherapy and/or radiation treatment regimens [4, 21].

Additional Prognostic Factors
Several studies have identified patient and tumor characteristics that together portend poor outcomes. The European Organisation for Research and Treatment of Cancer (EORTC) conducted a multivariate analysis of prognostic factors and created a prognostic scoring system based on characteristics of...
LGG patients enrolled in the EORTC 22844 and 22845 trials. The investigators identified the following poor prognostic indicators in patients with LGG: age ≥40, astrocytic tumor type (vs. oligodendroglioma or oligo-dominant), tumor size ≥6 cm, tumor crossing the midline, and presence of neurologic deficit(s) at diagnosis (before surgery) [17, 22, 23].

TREATMENT

There are significant challenges in designing and evaluating therapeutic trials for LGG treatment. Some limitations of these studies include the incorporation of multiple histological types of LGG without distinguishing between subtypes, lack of molecular diagnostics in several studies, absence of consensus on the definition of radiographic response, failure to account for the possibility of pseudoprogression in patients treated with radiotherapy, and limited incorporation of measures regarding quality of life (QoL), neurocognitive outcomes, and neurotoxicity. A summary of LGG treatment modalities is provided in Table 1.

Surgery

Increasingly, studies have supported surgical resection rather than observation to improve overall survival [24, 25]. Additionally, some studies suggest a benefit of extent of resection on progression-free survival [26–29]. Whether gliomas are incidentally found or symptomatic, surgery has been reported to improve seizure control [30, 31].

In one review of the surgical management of LGG, the authors noted the historical arguments in favor of watchful waiting in selected patients with minimal or medically controlled symptoms, with one of the primary arguments based on data suggesting that such an approach did not worsen patients’ QoL, nor did it negatively impact overall survival, although the value of such data is limited by its retrospective nature [4]. Of nine retrospective surgical studies, six demonstrated significant overall survival benefit with extensive surgical resection. Two prospective trials evaluating resection and postoperative radiation therapy demonstrated a significant survival benefit with more aggressive resection on univariate analysis, but not on multivariate analysis. These studies are limited by unblinded assessment of resection (i.e., in many cases, the surgeon determined the extent of resection), as well as patient and treatment selection bias [4]. In another review, the authors examined all major publications since 1990 addressing the effect of extent of surgical resection on glioma outcome. They concluded that there was a trend toward improvement in survival with more extensive surgical resection. In univariate and multivariate analyses of these LGG studies, they noted that extent of resection had significant prognostic value in 7 of the 10 studies [32].

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The National Comprehensive Cancer Network guidelines for the management of low-grade infiltrative supratentorial astrocytoma/oligodendrogloma in adult patients recommend maximum safe resection of tumor tissue, if possible, with the caveat that serial observation may be appropriate for selected patients [33].

A number of surgical advances have allowed for improvement in the surgeon’s ability to maximize the degree of tumor resection, while sparing eloquent brain. The use of functional MRI and magnetic source imaging allows the surgeon to map functional brain areas such as motor and language cortices, in relation to the tumor [34]. Diffusion tensor imaging may be helpful to identify functional anatomical tracts that are as important as the functioning areas themselves; this technique allows for careful surgical planning to minimize risk of deficits and distinguish between tumor cells and peritumoral edema [7]. Intraoperative MRI and MRS may be used to evaluate the degree of tumor resection during the surgical procedure and more clearly identify residual tumor [35, 36].

Radiation

Several prospective clinical trials have examined the utility of high-dose versus low-dose radiation and the costs versus benefits of early versus delayed radiotherapy. In EORTC 22844, investigators assessed the overall effectiveness of radiotherapy and the potential of a dose-response relationship. A total of 379 adult patients with LGG was randomized to receive radiotherapy postoperatively (or postbiopsy) with 45 Gy in 5 weeks versus 59.4 Gy in 6.6 weeks. At a median follow-up of 74 months, there was no significant difference in overall survival (58% in the low-dose group and 59% in the high-dose group) or progression-free survival (47% in the low-dose group and 50% in the high-dose group), and there was no demonstrable dose-response relationship for radiotherapy in LGG [37].

Similar results were observed in the North Central Cancer Treatment Group/Radiation Therapy Oncology Group (RTOG)/Eastern Cooperative Oncology Group (ECOG) study, in which survival and toxicity were evaluated in low- and high-dose radiation arms. In this study, 203 patients with supratentorial LGG from 1986 to 1994 were randomized to either the low-dose (50.4 Gy in 28 fractions) or high-dose (64.8 Gy in 36 fractions) treatment group. There was no advantage of higher-dose radiation therapy observed in this study. In fact, there was a trend toward improved survival at 2 and 5 years with low-dose therapy (2- and 5-year survival of 94% and 72%, respectively, in comparison with 85% and 64% with high-dose therapy). However, this difference did not reach statistical significance. In addition, there was a higher incidence of radiation neurotoxicity (radiation necrosis) in the high-dose radiotherapy group (5% vs. 2.5% in the low-dose radiotherapy group) [28].

Timing of radiotherapy was addressed in the EORTC 22845 study, in which early versus delayed radiotherapy was assessed. This study, initiated in 1986, included 314 patients with LGG from 24 European centers, who were randomized to early postoperative radiotherapy versus deferred radiotherapy (postponed until the time of disease progression). In this study, there was a significant improvement in progression-free
survival in the early radiotherapy group, with a median progression-free survival of 5.3 years in the early radiotherapy group versus 3.4 years in the delayed radiotherapy group. There was no significant difference, however, in overall survival, which was 7.4 years in the early group versus 7.2 years in the delayed group ($p = .872$) [38]. In the EORTC 22845 study, it was also noted that early radiation did not increase the risk of malignant transformation of LGG to higher-grade tumors. The investigators noted that in study patients undergoing a second surgery at the time of tumor recurrence, the proportion of subjects diagnosed with high-grade glioma was no different in the early versus delayed radiotherapy groups [38]. Quality of life measures were not evaluated in the EORTC 22845 study; thus, the clinical significance of the improvement in progression-free survival in the early radiotherapy group remains uncertain [38].

Newer radiation techniques allow for more precisely directed radiation, improving the ability to target the tumor while sparing healthy surrounding brain tissue, thus minimizing radiation toxicity. Examples include intensity-modulated radiation therapy and stereotactic radiosurgery. However, at this point, neither of these techniques has demonstrated superior efficacy in the treatment of LGG. Proton radiation is another technique that allows for targeted radiation with sparing of surrounding tissues. There is an ongoing trial investigating the efficacy of proton radiation therapy on patients with LGG (ClinicalTrials.gov Identifier: NCT01358058). In this trial, the effectiveness of proton therapy with a more restricted radiation field will be compared with photon therapy, with particular attention paid to treatment-related side effects [39]. As this is a noncomparative, single arm trial, it will not directly address the efficacy and toxicity of proton radiation in comparison with conventional radiotherapy; this question could ideally be addressed by future investigational studies.

Chemotherapy

Although chemotherapy is often used in high-grade gliomas, its role, with or without radiation therapy, in the treatment of patients with LGG remains a topic of investigation. Given its demonstrated efficacy in the high-grade glioma population, temozolomide has been of particular interest. In one phase II study, there was demonstrated activity of temozolomide in LGG patients with progressive disease. In this cohort of 46 patients, 61% of subjects achieved radiographic responses—24% having achieved complete response and 37% having achieved partial response. The median progression-free survival (PFS) was 22 months, with a 6-month PFS of 98% and a 12-month PFS of 76% [40].

In a review of 7 trials evaluating postoperative temozolomide in LGG, with or without prior chemotherapy

Table 1. Treatment of low-grade gliomas

<table>
<thead>
<tr>
<th>Treatment modality</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>May be reasonable in low-risk patients with minimal or no symptoms [4]; Less favored in patients with high-risk features [17, 22, 23]: • Age $\geq 40$ • Astrocytic tumor type • Tumor size $\geq 6$ cm • Tumor crossing midline • Presence of neurologic deficits</td>
</tr>
<tr>
<td>Surgery</td>
<td>Data from retrospective studies suggest survival benefit from early maximal tumor resection [4, 32]. NCCN guidelines recommend maximum safe resection of tumor tissue, if possible, with serial observation an acceptable alternative in selected patients [33]. Improvement in seizure control with surgical resection [30, 31].</td>
</tr>
<tr>
<td>Radiation</td>
<td>No demonstrable dose-response relationship for high-dose vs. low-dose radiotherapy in LGG [37]. Improvement in progression-free survival but not overall survival in patients receiving early radiotherapy [38]. Early radiotherapy does not increase the risk of malignant transformation of LGG to higher-grade tumors [38]. There are concerns that early radiotherapy might compromise long-term neurocognitive function.</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>Limited evidence supports improved outcome in LGG patients treated with temozolomide-based chemotherapy [17, 40]. Trend toward improvement in OS in 1p-deleted tumors treated with temozolomide [45]. Investigations are underway to evaluate role of combined chemotherapy/radiotherapy approaches [46].</td>
</tr>
</tbody>
</table>

Abbreviations: LGG, low-grade glioma; NCCN, National Comprehensive Cancer Network; OS, overall survival.
and/or radiation therapy, the authors concluded that, whereas these trials demonstrated tumor “shrinkage” in response to temozolomide, it is unclear whether this shrinkage is adequate to meet formal criteria for a partial response to treatment [17].

The RTOG 9802 trial assessed treatment of LGG patients with radiation alone versus radiation followed by 6 weeks of chemotherapy with procarbazine, lomustine, and vincristine (PCV). The study enrolled 251 patients from 1998 to 2002, and there was a statistically significant improvement in progression-free survival, but not overall survival, in the radiotherapy plus PCV group. In the first 2 years of treatment, overall survival and progression-free survival were similar. The utility of this analysis was limited by the short 2-year follow-up time before data analysis. However, subsequent analysis with longer follow-up demonstrated possible delayed benefits of chemotherapy; 2-year survivors in the PCV treatment arm had a significantly increased likelihood of surviving an additional 3 years and 5 years versus nonchemotherapy recipients [41].

The RTOG 0424 trial (ClinicalTrials.gov Identifier: NCT00114140) was a phase II study of temozolomide-based chemotherapy with radiation alone versus radiation followed by 6 weeks of chemotherapy with procarbazine, lomustine, and vincristine (PCV). The study enrolled 251 patients from 1998 to 2002, and there was a statistically significant improvement in progression-free survival, but not overall survival, in the radiotherapy plus PCV group. In the first 2 years of treatment, overall survival and progression-free survival were similar. The utility of this analysis was limited by the short 2-year follow-up time before data analysis. However, subsequent analysis with longer follow-up demonstrated possible delayed benefits of chemotherapy; 2-year survivors in the PCV treatment arm had a significantly increased likelihood of surviving an additional 3 years and 5 years versus nonchemotherapy recipients [41].

The RTOG 0424 trial (ClinicalTrials.gov Identifier: NCT00114140) was a phase II study of temozolomide-based chemotherapy in high-risk low-grade glioma, which compared 3-year survival of patients with high-risk LGG treated with temozolomide alone versus those enrolled in EORTC 22844 and 22845. In this nonrandomized, multicenter study, patients received concurrent radiation and daily temozolomide for 6 weeks, followed by postradiation temozolomide for up to 12 additional months. Three-year survival was assessed and compared with patients enrolled on clinical trials EORTC 22844 and EORTC 22845. Additional primary outcome measures include PFS, toxicity, association of survival and progression-free survival with MGMT methylation status, quality of life, and neurocognitive function [42]. Patients enrolled in the RTOG 0424 trial had a 3-year overall survival rate of 73.1%, which exceeded that of historical controls [43]. This noncontrolled study is limited by its reliance upon a comparison group using data from trials that had been conducted 20 years earlier.

The EORTC 22033-26033 trial (ClinicalTrials.gov Identifier: NCT00182819) was a phase III randomized, multicenter study comparing progression-free survival of patients with LGG treated with radiotherapy versus temozolomide. In this study, patients were stratified according to participating center, chromosome 1p status (deleted vs. normal vs. undeterminable), contrast enhancement on MRI (yes vs. no), age (<40 vs. ≥40 years), and WHO performance status (0 or 1 vs. 2). Subjects were then randomized to one of two treatment arms—a radiotherapy group, which underwent radiotherapy 5 days per week for a total of 28 fractions, and a chemotherapy arm, in which patients received oral temozolomide daily for 21 days of each 28-day cycle, for up to 12 treatment cycles. Outcome measures included progression-free survival, overall survival, and quality of life [44]. In the overall study population, there was no difference in progression-free survival or overall survival between the two groups. In patients with chromosome 1p deleted who maintained who received temozolomide, there was a trend toward inferior PFS. In those patients with chromosome 1p deleted who were treated with temozolomide, there was a trend toward improvement in OS [45]. Further follow-up is required before the final results of this trial can be assessed.

There are other ongoing clinical trials that are seeking to further define the ideal treatment regimen for patients with LGG, including ECOG-E3F05 (ClinicalTrials.gov Identifier: NCT00978458), a phase III randomized study of radiotherapy with or without temozolomide in patients with symptomatic or progressive LGG. The primary objectives of this study are to determine whether the addition of temozolomide to fractionated radiotherapy improves progression-free survival and/or median overall survival. This study is currently recruiting participants [46].

Monitoring Response to Treatment

The optimal method of assessing treatment response in LGG remains an active area of investigation. Currently, MRI (T2/FLAIR sequence), with or without contrast enhancement, is used to identify tumor size and associated peritumoral edema. Some authors suggest that treatment outcomes might be more reliably evaluated using advanced imaging techniques designed to assess specific biological aspects of the tumor, including amino acid PET, MRS, and/or cerebral blood volume assessment with perfusion-weighted MRI [47]. However, none of these alternative imaging markers have been validated for use in LGG clinical trials or in clinical practice.

In addition, the challenges for assessing tumor response as described by Macdonald et al. in 1990 have been highlighted, including the use of cross-sectional rather than volumetric area to measure tumor size, failure to account for neurologic deterioration or increasing steroid usage in assessing disease status, and limitations of the imaging itself, including difficulty distinguishing between tumor borders and new lesions in gliomas, which often have satellite lesions, as well as the challenge of identifying tumor mimics such as pseudoprogression, in which increased contrast enhancement in response to treatment does not equate to true tumor progression [6, 48, 49].

The Response Assessment in Neuro-Oncology defines a set of criteria for assessing outcome in trials of diffuse LGG. This includes specific guidelines for using tumor size and appearance on T2/FLAIR MRI sequences to define complete response, partial response, and minor response to treatment, as well as stable disease and progression. The criteria take into account stability of corticosteroid dosing, clinical status, and differentiation between new T2 or FLAIR abnormalities related to tumor spread in comparison with those attributable to radiation effects [6]. These consensus guidelines await validation in future randomized studies.

Treatment-Related Complications

An important consideration in determining the optimal treatment approach in patients with LGG is weighing the potential benefits of various treatment regimens against treatment-related side effects, which may limit treatment intensity and/or duration and have a significant impact on the patient’s quality of life. For example, neurosurgeons plan surgical approaches to maximize resection (when feasible) while minimizing neurosurgical deficit.

Of particular concern in brain-directed treatment is the cognitive impact on patients, particularly related to radiation
therapy. Although radiation therapy may prolong progression-free survival, this may come at some cost of cognitive performance. This issue was addressed in a retrospective evaluation of LGG patients who had received radiotherapy in comparison with those who had not received radiotherapy. This study was a follow-up of long-term survivors (mean of 12 years) from an earlier study on cognitive outcomes with radiation therapy. The original study had assessed 195 patients with LGG at a mean of 6 years from diagnosis, and it was concluded that LGG patients had worse cognitive performance when compared with healthy controls or patients with hematologic malignancies, regardless of radiation treatment status [50]. Within the LGG patients, there was a nonsignificant trend toward inferior cognitive functioning in patients who received radiotherapy versus those who did not ($p = .145$). The authors determined that the tumor itself, not the radiation treatment, had the most significant adverse impact on cognitive functioning, although high-dose radiation ($>2$ Gy) had an additional significant impact on cognitive decline [50, 51]. In the follow-up study, 65 long-term survivors were evaluated at a mean of 12 years from the time of diagnosis; 32 of these patients received radiation therapy. The authors reported significant cognitive deficits in patients who had received prior radiation (17 patients; 53%) versus those who did not receive radiation therapy (4 patients; 27%). These deficits were evident in at least 5 of 18 neuropsychological test parameters. The authors concluded that with long-term follow-up, radiation doses less than 2 Gy caused significant long-term cognitive deficits in patients who underwent radiotherapy [50, 51]. The EORTC 22844 study included a 47-item QoL questionnaire evaluating psychological, physical, social, and symptom domains over time. The authors concluded that, whereas no major differences in QoL between the high-dose versus low-dose radiation groups were evident, significantly higher levels of fatigue/malaise and insomnia immediately after radiation therapy in the high-dose group were detected. In addition, the high-dose group experienced worse emotional functioning and reported decreased leisure time, with these effects persisting for 7–15 months after randomization [52].

Figure 4. Brainstem glioma. The diffuse pontine glioma pictured in this T2/FLAIR-weighted magnetic resonance image appears as an expansible, hyperintense lesion centered within the pons.

SPECIAL TOPICS—SELECTED LGG SUBTYPES

Brainstem Glioma

Most prevalent in children, brainstem gliomas are a heterogeneous group of tumors, which includes diffuse intrinsic pontine glioma, exophytic medullary glioma, and tectal gliomas (Fig. 4) [54]. These tumors are often diffusely infiltrative and may have cystic components. Patients often present with hydrocephalus and signs of elevated intracranial pressure (e.g., vomiting, headaches), ataxia, cranial nerve abnormalities, or other signs of brainstem dysfunction. Diffuse intrinsic pontine glioma has a median age of onset of 6.5 years and an extremely poor prognosis, with median survival of less than 1 year, although adult patients with diffuse intrinsic pontine glioma tend to have longer survival [54]. Treatment of these tumors generally involves radiation with or without chemotherapy. Surgery is not feasible in most patients because of the location of the tumors. Exophytic medullary glioma and tectal gliomas are more indolent forms of brainstem gliomas with better prognoses and may be managed conservatively with serial radiographic and clinical evaluation [54].

Pilocytic Astrocytoma

Pilocytic astrocytomas (PAs) are WHO grade I, well-circumscribed, indolent, often cystic tumors that represent 5%–6% of all gliomas...
surgery or observation. Everolimus, an inhibitor of the mammalian target of rapamycin (mTOR) complex, was studied in a nonrandomized trial of TSC patients with subependymal giant cell astrocytomas. In TSC patients, mutations in either the TSC1 or TSC2 gene lead to constitutive upregulation of mTOR complex 1, which promotes abnormal cell growth and proliferation. In this study, the administration of everolimus was associated with tumor responses and reduction in seizure frequency. The drug was subsequently approved by the Food and Drug Administration for this indication [57]. This represents an important step forward in the search for novel targeted agents in the treatment of LGG and may help guide future investigation into similarly tailored therapy in other LGG subtypes.

Pleomorphic Xanthoastrocytoma

Pleomorphic xanthoastrocytomas (PXAs) are WHO grade II tumors comprising less than 1% of all astrocytic tumors and generally occur in children and young adults, with two thirds of patients under the age of 18 years [1]. These tumors are generally adherent to the meninges and may be cystic with a mural nodule; microscopic features include nuclear and cytoplasmic pleomorphism and large xanthomatous cells containing lipid droplets [1]. BRAF V600E mutations have been described in this type of LGG [58]. PXAs are generally located superficially in the cerebral hemispheres, with 98% occurring supratentorially, and often involve the meninges, hence their description as meningoencephalic neoplasms [1]. As a result of this superficial location, patients with this type of tumor often present with seizures. Prognosis is largely favorable, with estimated 81% 5-year and 70% 10-year survival [1]. The majority of patients undergo surgical resection, which is generally possible because of the superficial location of these tumors, and there is a trend toward improved outcomes with greater extent of resection [59].

SUBEPENDYMA L GIANT CELL Astrocytoma

Subependymal giant cell astrocytomas (SEGAs) are benign, indolent, well-circumscribed, and often calcified tumors, generally arising from the wall of the lateral ventricles [1]. These tumors are associated with tuberous sclerosis complex (TSC), an autosomal dominant neurocutaneous syndrome whose characteristic features include cognitive impairment, cutaneous angiofibromas, cardiac rhabdomyomas, and renal angiomyolipomas [1]. These WHO grade I tumors often present in the first two decades of life with seizures or with signs of increased intracranial pressure [1]. Treatment may include

**REFERENCES**

For Further Reading:

Implications for Practice:
Pseudoprogression is an unsolved clinical dilemma during postchemoradiation surveillance for malignant gliomas. It has received substantial attention in the era of temozolomide-based regimens, mimicking early disease progression on imaging studies and challenging patient management and interpretation of clinical trials. This study suggests that the incidence of pseudoprogression in patients receiving antiangiogenic therapy is low and that enlarging lesions in this patient population are more likely to represent true tumor progression than transient post-treatment effects.
Meningioma

Ali-Reza Fathi · Ulrich Roelcke

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Abstract Meningiomas represent the most common primary brain tumor and comprise 3 World Health Organization (WHO) grades, the most frequent being WHO grade I (90 %). Surgery is mandatory to establish the diagnosis and to remove the tumor; however, complete resection can be achieved in only <50 % of patients. Depending on the extent of resection, tumor location and the WHO grade radiation therapy can be applied. The issue of systemic treatment such as chemotherapy or targeted therapy (eg, somatostatin receptors, antiangiogenic agents) is yet not solved, particularly as current data are derived from small uncontrolled series in patients with long-standing disease and after several pretreatments. A more thorough understanding of molecular genetics, signaling pathways and prognostic factors in meningiomas should lead to the design of studies which stratify according to these factors. These studies have to be conducted in newly diagnosed patients after incomplete resection and in tumors of WHO grade II and III.

Keywords Meningioma · Benign brain tumor · Skull base · Neurofibromatosis · Diagnosis · Imaging · Pathology · Molecular genetics · Neurosurgery · Surgery · Radiation therapy · Radiosurgery · Somatostatin receptors · Chemotherapy · Targeted therapy

Introduction

Meningiomas represent the most common intracranial extraxial neoplasia. In adults they account for approximately 30 % of central nervous system (CNS) tumors, whereas they are rare in children and adolescents (0.4 %–4.6 %) [1••]. The incidence increases with age and shows a remarkable predominance in females particularly in the 3rd to 6th decade (female:male ratio 2:1) [2]. Meningiomas are frequently detected by chance (‘incidentaloma’) and show no or only minor growth particularly when tumor calcification is present in the elderly [3]. They are found in up to 3 % of autopsy reports [4] in patients over 60 years old. Meningiomas can arise anywhere within the CNS, and multiple manifestations are not uncommon. Distant metastasis is very rare. The spontaneous rate of tumor growth varies [5]. Considering the aging population, improvements in treatment safety as well as the availability and performance of imaging, we are expecting an increased incidence in future daily practice [3]. This fact requires interdisciplinary networks in specialized centers to optimize the patient’s management. Special risk and benefit considerations are required upon diagnosis of smaller asymptomatic lesions. This article’s focus is on intracranial meningiomas (98 % of all meningiomas) which in up to 60 % are located in parasagittal regions, in the convexity, at the tuberculum sellae, and sphenoid ridge. Less common locations comprise the olfactory groove, falx, lateral ventricle, tentorium, middle fossa, and orbita [6].

Histopathology, Molecular Genetics

Meningioma cells arise from the arachnoid cap cells which form the outer layer of the arachnoid mater. According to the World Health Organization (WHO) [7] meningiomas are categorized into 3 grades. Criteria include cell type, mitotic activity, cellularity, necrosis, and brain invasion. Benign meningiomas (WHO grade I) represent approximately...
90% of all meningiomas and show several histologic variants: meningothelial, fibrous (fibroblastic), transitional (mixed), psammomatous, angiomatous, microcystic, secretory, lymphoplasmacyte-rich, metaplastic meningioma. They lack criteria of atypical or anaplastic meningioma. Atypical meningiomas (WHO grade II) represent approximately 5%–7% of all meningiomas and comprise clear-cell or chordoid variants. They show a mitotic index of ≥4 mitoses per 10 high-power fields (HPF), increased cellularity, a high nucleus:cytoplasm ratio (‘small cells’), sheet-like growth, and foci of spontaneous necrosis which are not induced by embolization or radiation therapy. Even in the absence of the cellular criteria of WHO grade II also brain invasion (Fig. 1) qualifies for WHO grade II because of recurrence and mortality rates similar to atypical meningiomas. Anaplastic meningiomas (WHO grade III, 3% of all meningiomas) include papillary and rhabdoid variants, and show a mitotic index of ≥20 mitoses per 10 HPF and larger zones of spontaneous necrosis. On microscopy they can resemble carcinoma, sarcoma, or melanoma. Immunohistochemistry eg, with staining against vimentin or epithelial membrane antigen may assist in the differentiation of meningioma from these tumors [8].

Meningiomas can originate spontaneously or be part of hereditary syndromes such as neurofibromatosis type 2 (NF2), Li-Fraumeni, Turcot, Gardener, von Hippel-Lindau, Cowden, Gorlin, and multiple endocrine neoplasia type I [9]. For most of these syndromes the exact genetic relationship to meningiomas has not been unraveled yet. The most frequent genetic abnormality in meningiomas is genetic loss of chromosome 22,q12.2. This chromosome band harbors the NF2 gene. Its product, the Merlin protein, is considered to participate in the regulation of cell-to-cell contact and motility [10]. The majority of NF2-associated meningiomas and approximately one half of sporadic meningiomas show mutations of the NF2 gene. As the frequency of NF2 mutations is similar in meningiomas of WHO grade I–III this suggests that NF2 is rather involved in meningiomas initiation than in progression. Meningioma progression is associated with a plethora of molecular and genetic alterations (eg, loss of tumor suppressor genes, hypermethylation of CpG island) as well as changes of signal transduction. The latter includes cell membrane receptors such as sex hormone and somatostatin receptors. Within autocrine loops several growth factors and their receptors are expressed, eg, epidermal growth factor (EGFR), EGF-like domain-containing protein 6 (EGFL6) [11], platelet derived growth factor (PDGFR), insulin-like growth factor II (IGF-II), transforming growth factor-α (TGF-α), which binds to EGFR) [12]. Among intracellular signaling pathways the activation of PI3K/Akt proteins reflects poor clinical course and brain invasion. Mutations in the hedgehog transmembrane receptor and activation of Notch receptors results in meningioma development and chromosomal instability. The role of all these factors (ie, whether they contribute to progression or represent secondary events) is yet unclear [12, 13].

**Prognostic Factors**

The term ‘prognostic factor’ is differently used across the literature on meningiomas and includes the ‘risk’ for incidence and events such as tumor development eg, due to radiation exposure as well as the risk for recurrence. The latter depends on factors associated with the individual tumor presentation (genetics, tumor grade) and with treatment modalities. Most studies present data on prognostic factors which show significant correlation with the WHO tumor grade. Only a few studies have addressed the variability of the clinical course or treatment response eg, within a given tumor grade. Identification of such factors would allow stratifying patients into distinct molecular or biochemical subgroups with particular regard to post-operative therapy response.

Meningiomas which present with brain or bone invasion show poorer outcomes compared with noninvasive tumors [14]. Also the status of sex hormone receptors impacts prognosis: whereas meningiomas with progesterone receptors show median recurrence rates of 5%, meningiomas with estrogen receptors or tumors lacking sex hormone receptors present with recurrence rates of up to 30% [15]. However, the extent of resection as classified by Simpson [16] and the WHO tumor grade [7] are among the most strong prognostic factors. Relapse rates in WHO grade I/II/III of 7%/40%/80% have been reported, and median survival in these studies was >10/11.5/2.7 years, respectively [17, 18]. Although many patients with completely resected grade I meningiomas can be considered
as cured, late recurrences are observed even after 20 years (11 %–56 %) [19].

The risk for ‘early’ recurrence within WHO grade I meningiomas was studied by investigating the expression of the osteopontin protein [20*]. This integrin-binding protein is involved in proliferation, adhesion, migration, and angiogenesis. In a series of 32 operated patients with WHO grade I meningiomas 28 % recurrences were observed at a mean follow up of 34 months. In patients with ‘early’ recurrences the osteopontin staining score from immunohistochemistry was approximately 6 times higher compared with nonrecurring tumors [20*]. This type of study signifies the importance to assess the biological variability within distinct histologic subgroups. Results may prompt prospective trials which address the role eg, of antiangiogenic treatment in osteopontin positive meningioma.

Diagnosis

As with other brain tumors the clinical signs of meningiomas relate to their location. Personality changes, neuropsychological deficits, sensory-motor, or visual symptoms, aphasia as well as seizures frequently occur. Skull base meningiomas present with cranial nerve dysfunction in the majority of cases. Depending on location and size hydrocephalus may evolve. Computed tomography (CT) and magnetic resonance imaging (MRI) are the tools to suspect meningioma as described below. Despite the fact that MRI has advantages over CT in assessing soft tissue characteristics, the combination with CT gives additional information regarding bone infiltration and therefore allows optimization of surgery and radiation therapy planning [21, 22].

MRI

The characteristic signal alterations in T1- and T2-weighted MRI together with anatomical extension towards adjacent structures aids to confine the differentiation from other tumors and nonmalignant lesions [23]. Due to the absence of a blood–brain barrier meningiomas usually exhibit strong enhancement of contrast medium on CT and T1-weighted MRI (Fig. 1). The extent of brain edema is well illustrated in T2-weighted and FLAIR MRI, and is thought to be particularly extensive in WHO grade I meningiomas [24, 25]. MRI can depict arachnoid layer to predict brain adhesion and facilitates to plan the surgical dissection of tumor from brain tissue. Since the prognosis highly correlates with the extent of resection of infiltrated dura, pathologic changes of the dura can be interpreted by evaluating the dural tail sign on MRI (Fig. 1a). This information helps to choose the correct size of craniotomy to achieve the maximal distance of 2.5 cm from the tumor base in convexity meningiomas [26]. Diffusion tensor imaging (DTI) helps in preoperative identification of displaced fibers, which is particularly helpful in planning surgical access to intraventricular meningiomas [27]. Assessment of tumor growth which may aid in treatment decisions was, so far, mainly based on the measurement of cross sectional tumor diameters. With the advance of automated volumetric and 3D reconstruction analysis a superior tool is provided for early detection of tumor growth [28].

CT

CT provides information on hyperostosis, bone destruction, and infiltration which is of utmost importance for planning surgical and radiation strategies [22, 29]. CT also detects tumor calcification, which is a hint for slow tumor growth particularly in elderly patients [3, 30]. In addition, CT aids to clarify the spatial relationship between skull base tumors, paranasal sinuses, and pneumatization of the anterior clinoid process.

Cerebral Angiography (CAG)

Most meningiomas harbor a very dense vascularization, which in case of large tumors carries the risk of intense intraoperative bleeding. Preoperative embolization can reduce surgical morbidity in selected cases [31]. Embolization might rarely be applied prior to radiation or as standalone therapy. Careful interdisciplinary decision making is required before the indication of embolization is made, since risks of embolization such as hemorrhage, extensive edema formation, or vascular infarctions can have negative impact on functional outcome [32]. CAG also unravels the degree of proximity between skull-base meningiomas and vascular structures with exact depiction of stenosis, occlusion, and incidental aneurysms. Vessel wall irregularities on CAG are a more sensitive hint to tumor-invasion into the vessel wall compared with MRI.

SPECT and PET

Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) provide information about cellular processes and biological characteristics of tumors. In meningiomas the clinically most relevant issue is the search for the presence of somatostatin receptors (SSR) [33]. The presence of SSR favors the diagnosis of meningioma in a patient not amenable for surgery. It aids in the target volume delineation for radiation therapy planning [34], and in the differentiation between residual or recurrent tumor from scar tissue [35]. The presence of SSR also allows targeted therapy with receptor binding ligands (see below). However, future well-designed clinical studies are
warranted to demonstrate the feasibility and value of PET for clinical routine.

Ongoing research in all imaging modalities is focusing on the prediction of histologic grade and improving differential diagnosis, eg, dural metastasis, hemangioioperticytoma vs meningioma. So far none of these modalities are reliable enough to substitute the histopathologic analysis of tumor tissue.

Treatment

Despite the lack of class I evidence based guidelines, correct decision making in patients with newly diagnosed intracranial mass suspicious for meningioma is crucial for achieving optimal clinical outcome and patient survival [36, 37]. Complete tumor resection is associated with the longest progression-free and overall survival, and Simpson grade I resection should continue to be the goal for convexity meningiomas [16]. However, total resection cannot be achieved in more than 50 % of newly diagnosed meningiomas. Therefore partial tumor removal followed by observation, radiation therapy, or in rare cases, systemic chemotherapy has to be discussed in an interdisciplinairy approach. Based on the natural history patients younger than 60 to 70 years of age and those with meningiomas characterized by surrounding brain hyperintensity on T2-weighted MRI (edema), absence of calcification, and tumor diameter > 25–30 mm exhibit a higher risk for early recurrence [3, 17, 30, 38]. Special awareness of detecting genetic disorders is crucial to respect the course of natural history and act accordingly, especially in patients with NF2 associated tumors which frequently demonstrate a saltatory growth pattern [39]. Because new tumors can develop in NF2 patients over their lifetime and because radiographic and symptomatic progression are unpredictable, resection may be best reserved for symptom-producing tumors, de novo, and brain edema-associated meningiomas in NF2 patients [39, 40].

Surgery

Indications for surgery in general include symptomatic tumors with the option for total resection, subtotal resection followed by radiation therapy of remnants, or growth of asymptomatic tumors on serial images. Surgical risks have to be discussed with the patient, and the patient’s preference has to be respected based on this discussion [41, 42]. Surgical strategies have significantly improved outcomes within the past 30 years [17, 43]. The introduction of the surgical microscope in neurosurgery was a major step in improving safety with extension of surgical tumor removal [44]. Additional technological progress enhanced surgical safety and extent of resection in the past years. This includes tools such as neuro-navigation [17], intraoperative electrophysiology, indocyanine angiography [45], 5-ALA [46, 47], intraoperative MRI [48], and intraoperative sonography [49]. With the adoption of these utilities Simpson grade I resection is achieved in the majority of convexity meningiomas with minimal morbidity [17]. In case of tumor invasion into skull, bone removal and intraoperative molded cranioplasty [29, 50] has to be considered to prevent tumor recurrence originating from skull.

Endoscopy

Advanced knowledge in the endoscopic anatomy of the skull base delivered new surgical corridors to minimize morbidity related to the surgical access and cosmetic issues. However, compared with traditional craniotomies, the major limitation of the endoscopic approach is the restricted surgical corridor, challenging cranial base repair, and limited hemostasis in hypervascularized tumors. Over the same period of time, new open surgical trans-cranial approaches have challenged the traditional operative corridors to the anterior skull base. Neither of the techniques exclude each other and the future direction will be a combined approach to provide the benefits of all techniques and to maximize resection with minimized morbidity and mortality [17, 51–54].

Radiation Therapy

Radiation therapy (RT) evolved as a standard in the treatment of meningiomas. Indications for RT depend on tumor size, stage of disease, WHO grade, and include residual or non-resectable meningioma at the time of first diagnosis, recurrent or progressive non-resectable meningioma, and meningioma of WHO grade II and III. The RT modalities comprise fractionated external beam radiation, stereotactic radiosurgery, and particle therapy (mainly proton therapy). Stereotactic radiosurgery harbors a growing list of names depending of the manufacturer’s naming (eg, linear accelerator (Linac), CyberKnife® (Accuray Inc., Sunnyvale, CA, USA), Gamma Knife® (Elekta Instruments, Stockholm, Sweden), Novalis® (BrainLAB, Heimstetten, Germany) etc.). In large tumors the side effects and limited tumor control outweigh the benefits of RT compared with surgery [55]. Complications of RT include alopecia, tooth loss, new onset of seizures, neurologic deficits, cranial nerve palsy, headache, edema, and radio-necrosis or delayed hydrocephalus. On the other hand tumor control rates for stereotactic radiosurgery are equivalent to sub-total resection with lower morbidity than surgery of skull base tumors [56, 57].

The goals of RT are prolongation of progression-free survival with preservation of neurologic function. Complete tumor eradication is not possible with RT modalities to date, however, partial tumor shrinkage has been observed in different series [58–60]. The assessment of RT effects is in general difficult because of the different RT modalities used in meningioma treatment and limited data of randomized
trials in comparison with surgery [61]. Future studies are required to elaborate the optimal radiation dosage and to prevent treatment failures [62, 63].

RT has to be considered as an alternative to surgery in elderly patients with high surgical risk. One of the largest series with gamma knife treatment of benign meningiomas (5300 treated tumors) reported on 5- and 10-year progression-free survival rates of 95.2 % and 88.6 %, respectively [64••].

### Systemic Therapy

Systemic treatment has been administered as either cytotoxic or as targeted therapy. As both strategies are not considered standard treatment at the time of first diagnosis they have been given in patients with advanced or unresectable tumors, which in many cases were heavily pretreated with various courses of surgery and radiation therapy. This led to reports on noncontrolled case studies which included small numbers of patients, and patients with various WHO grades and stages of disease. Examples are presented in the Table 1.

Targeted therapies aim at the inhibition of hormonal receptors, angiogenesis, or growth factor signaling. Overall, at best modest responses were observed yet. Somatostatin receptors (SSR) in meningiomas are of particular interest as they can be visualized with SPECT and PET [65]. SSR are present in up to 70 % of meningiomas [66]. They are categorized into 5 subtypes, where subtype 2A (sst2A) is involved in direct (eg, cytostatic, apoptosis) and indirect (eg, antiangiogenic) effects [67]. Octreotide and pasireotide represent synthetic SSR ligands which modify the receptor activity. Pasireotide exhibits higher sst2A affinity (1.0 nmol/L) compared with octreotide (0.4) and somatostatin itself (0.2) [68].

With regard to treatment the visualization of SSR using PET has 2 major applications: (1) the PET radiotracer (eg, 68Ga-DOTATOC) uptake allows delineation of the tumor volume for radiation therapy planning [34]; (2) the noninvasive detection of SSR particularly in nonoperated progressive or recurrent tumors allows meningioma treatment with either the nonradioactive labeled somatostatin (sandostatin LAR), or with octreotide charged with the therapeutic nuclide Yttrium-90 [69] or Lutetium-177 [70•] (Table 1). Two studies using pasireotide are posted on www.clinicaltrials.gov. The y are denoted as active but not recruiting (NCT00859040, NCT00813592, accessed January 2013).

### Conclusions

Long-term tumor control or even cure can be achieved in many WHO I meningiomas with modern surgical and radiotherapy techniques. In the elderly asymptomatic meningiomas

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Ref.</th>
<th>Number of patients (WHO I/II/III)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyurea</td>
<td>[71]</td>
<td>20 (16/3/1)</td>
<td>1 patient with minor response; 93 % PFS-12 in WHO I; progression within 3 to 10 months in WHO II and III</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>[72]</td>
<td>35 (0/22/13)</td>
<td>no radiographic response; 3 % PFS-6; PFS 2 months</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>[73]</td>
<td>16 (16/0/0)</td>
<td>no radiographic response; TTP 5 months; OS 7.5 months</td>
</tr>
<tr>
<td>Estrogen receptor; tamoxifen</td>
<td>[74]</td>
<td>19 (not reported)</td>
<td>3 patients with minor responses (CT or MRI), 6 patients stable for 31 months, TTP 15.1 months</td>
</tr>
<tr>
<td>Progesteron receptor; mifepristone</td>
<td>[75]</td>
<td>28 (*)</td>
<td>minor responses in 8 patients (men, premenopausal women)</td>
</tr>
<tr>
<td>SSR; octreotide</td>
<td>[76]</td>
<td>16 (8/3/5)</td>
<td>31 % partial response, 31 % stable disease; TTP 5 months</td>
</tr>
<tr>
<td>SSR; octreotide</td>
<td>[77]</td>
<td>11 (4/2/5)</td>
<td>no radiographic response; TTP 17 weeks; OS 2.7 years</td>
</tr>
<tr>
<td>SSR; 90Y-DOTATOC</td>
<td>[69]</td>
<td>29 (14/9/6)</td>
<td>33 % stable disease at 12 months; TTP WHO I 61 months, TTP WHO II,III 13 months</td>
</tr>
<tr>
<td>SSR; 177Lu-DOTATOC, -DOTATATE immediately followed by radiation therapy</td>
<td>[70]</td>
<td>9 (7/2/0)</td>
<td>each 1 patient with complete and partial remission; other patients stable at median follow-up of 13.4 months</td>
</tr>
<tr>
<td>VEGF</td>
<td>[78]</td>
<td>15 (0/6/9)</td>
<td>43.8 % PFS-6; PFS 26 weeks</td>
</tr>
<tr>
<td>VEGF alone or plus etoposide</td>
<td>[79]</td>
<td>13 (5/5/3)</td>
<td>1 partial response, 11 stable disease; PFS 15.8 months</td>
</tr>
<tr>
<td>PDGF-R; imatinib</td>
<td>[80]</td>
<td>9 (1/2/6)</td>
<td>66.7 % PFS-6; OS 16 months</td>
</tr>
<tr>
<td>EGFR; gefitinib; erlotinib</td>
<td>[81]</td>
<td>25 (8/9/8)</td>
<td>32 % stable disease; 13 % PFS-12 and 50 % OS-12 in WHO I; 18 % PFS-12 and 65 % OS-12 in WHO II and III</td>
</tr>
</tbody>
</table>

EGFR epidermal growth factor receptor, PDGF-R platelet derived growth factor receptor, PFS, OS, median progression-free or overall survival, PFS-6, PFS-12, OS-6, OS-12, percentage of patients free of progression or alive at 6 and 12 months, SSR somatostatin receptor, TTP median time to progression, VEGF vascular endothelial growth factor

*22 benign, 2 malignant meningioma, 4 patients without biopsy
"incidentalomas") can be managed with a wait-and-see strategy without surgery. Meningiomas WHO I not amenable to total resection, and meningiomas of grade II and III represent the major therapeutic challenges. Whereas surgery and radiotherapy are well established at the time of tumor diagnosis and recurrence the role for systemic therapies remains unclear. Several reasons account for the latter: (1) systemic therapies have been largely administered to patients which were heavily pretreated by surgery and radiotherapy; (2) most reports are based upon retrospective, heterogeneous, and small patient series and included meningiomas with various tumor grades; (3) molecular markers or prognostic factors linked to tumor behavior were not used for stratification or randomization; (4) response criteria are not available. Given the high frequency of meningiomas prospective studies which implement these issues are strongly requested and should be feasible.

**Disclosure** Ali-Reza Fathi declares no conflict of interest. Ulrich Roelcke declares no conflict of interest.

**References**

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance


43. Strassner C, Buhl R, Mehdon HM. Recurrence of intracranial meningiomas: did better methods of diagnosis and surgical treatment change the outcome in the last 30 years? Neurol Res. 2009;31(5):478–82. With better operating modalities and additional treatment with radiation and gamma knife, the mortality decreased significantly from 12 % to 3 % and the outcome of the patients is still improving, so that even elderly patients can undergo surgical treatment with minor risks.


Meningiomas

Ian R Whittle, Colin Smith, Parthiban Navoo, Donald Collie

Meningiomas are by far the most common tumours arising from the meninges. Progressive enlargement of the tumour leads to focal or generalised seizure disorders or neurological deficits caused by compression of adjacent neural tissue. Surgery remains the primary treatment of choice, although the use of fractionated radiotherapy or stereotactic single-dose radiosurgery is increasing for meningiomas that are incompletely excised, surgically inaccessible, or recurrent and either atypical or anaplastic. Although most meningiomas have good long-term prognosis after treatment, there are still controversies over management in a proportion of cases. We review various features of meningioma biology, diagnosis, and treatment and provide an overview of the current rationale and evidence base for the various therapeutic approaches.

Meningiomas arise from the dural coverings of the brain. They are the commonest benign intracranial tumour, accounting for 13–26% of all primary intracranial tumours. They can be classified according to their dural site of origin, the involvement of adjacent tissues (eg, venous sinuses, bone, brain, and nerves), and their histological grading. Meningiomas present clinically by causing focal or generalised seizure disorders, focal neurological deficits, or neuropsychological decline. Accurate diagnosis before treatment has been greatly facilitated by the wider availability of CT and, especially, MRI. There have been substantial advances in elucidation of the genetics, molecular biology, and neuropathology of meningiomas; this information is becoming useful in prediction of prognosis after various treatments. Surgical excision remains the preferred treatment and in many cases is preceded by endovascular embolisation. Stereotactic radiosurgery and external-beam radiotherapy are being used increasingly for surgically inaccessible, recurrent, or subtotally excised tumours, particularly if they are atypical or anaplastic.

Epidemiology

Meningiomas have an annual incidence of 6 per 100 000 population. They are twice as common in the female as in the male population, and the incidence is highest after the fifth decade of life. An estimated 2–3% of the population have an incidental asymptomatic meningioma, and in autopsy studies 8% of these are multiple. Although most meningiomas are sporadic and of unknown aetiology, there are recognised risk factors, including genetic factors (eg, neurofibromatosis type 2, in which the tumours may occur). Various architectural patterns are seen within meningiomas; this group, and the tumour cells express epithelial membrane antigen. We emphasise that the WHO grade is based on the current WHO classification. Most (about 90%) are WHO grade I, reflecting their benign nature. However, atypical meningiomas (WHO grade II), which make up 5–7%, and anaplastic variants (WHO grade III), 1–3%, are recognised by several histological characteristics (panel).

WHo grade I

Histological WHO grade I meningiomas (figure 1), generally follow a benign clinical course and have only occasional mitotic figures, although pleomorphic nuclei do occur. Various architectural patterns are seen within this group, and the tumour cells express epithelial membrane antigen. We emphasise that the WHO grade and not the architectural pattern is the important

Search strategy and selection criteria

This seminar is based mainly on papers published during the past 5 years, although some classic papers have been cited. The search strategy selected articles from MEDLINE by use of the PubMed system. The key words used were: "meningioma" cross-referenced with key words such as "epidemiology", "cytogenetics", "embolisation", "radiotherapy", "surgical resection", and "somatostatin receptor". Only papers with an abstract in English were considered.
pathological information in decisions on management of a patient. The three commonest architectural patterns are meningothelial, fibroblastic, and transitional. The characteristic histological features of cellular whorls and psammoma bodies (round calcified bodies) are seen most commonly in the transitional variant. Fibroblastic meningiomas can resemble peripheral-nerve-sheath tumours such as schwannoma, but characteristic meningothelial cells are generally present, albeit only focally. Psammomatous meningiomas contain large numbers of psammoma bodies. Angiomatous meningiomas are highly vascular and can be confused on histology with a vascular malformation, though radiologically they are clearly distinct. Foci of meningothelial cells are present in all angiomatous meningiomas, although they may be sparse. Secretory meningiomas form intracellular lumina lined by cytokeratin-immunoreactive cells. The lumina contain material that stains with periodic acid/schiff and is immunoreactive for carcinoembryonic antigen.

WHO grade II
Two subtypes of WHO grade II meningiomas are recognised on the basis of their architectural pattern: clear-cell and chordoid meningiomas. The latter (figure 1) resemble chordomas, with trabeculae of epithelioid cells in a mucinous stroma. Haematological disorders, such as Castleman’s disease, have been reported in association with chordoid meningiomas. The term atypical can be used for any architectural pattern, but specific histological features are required. Of these features, a mitotic rate of at least four mitotic figures per ten high-power fields is the most important, allowing a histological diagnosis of atypia in the absence of the other features (such as increased cellularity; small cells with a high ratio of nucleus to cytoplasm; prominent nucleoli; sheet-like growth pattern; and “geographic” necrosis). In the absence of this mitotic rate, at least three of the other five features must be present for a histological diagnosis of atypia. WHO grade II tumours have a higher rate of recurrence (29–40%) than grade I tumours (7–20%), particularly after subtotal resection.

WHO grade III
Grade III meningiomas are subclassified on the basis of their architectural pattern into papillary and rhabdoid subtypes. Papillary meningiomas are rare variants and are mostly seen in children. They are defined by a perivascular pseudopapillary pattern. Rhabdoid meningiomas contain rhabdoid cells, which have a specific microscopic appearance with eccentric nuclei, abundant globular eosinophilic cytoplasm, and paranuclear inclusions, and they show focal immunoreactivity for epithelial membrane antigen. Deletion of the INII gene (22q11.2) does not appear to be a feature of the rhabdoid cells in rhabdoid meningiomas. Anaplastic (malignant) meningiomas (figure 1) have obvious malignant cytology, a high mitotic rate (20 or more mitotic figures in ten high-power fields), or both. These tumours show a high frequency of local and brain invasion, recurrence, and metastases. Brain invasion is defined histologically as islands of neoplastic cells that have invaded through the pia to involve underlying cortical tissue, commonly producing a gliotic reaction. Brain invasion is not one of the criteria used for grading tumours in the WHO classification, but it should always be commented on in a pathological report, because brain invasion is associated with subtotal resection and a higher rate of recurrence.

Indices of biological activity
Expression of proliferation markers such as MIB-1 and Ki67 has generally shown progressive increases in...
Meningioma cytogenetics and molecular neuropathology

Mutations of the NF2 gene, which is located at chromosome 22q12, are found in meningiomas of all grades and are thought to be an early event in tumorigenesis. Up to 60% of sporadic meningiomas show a somatic mutation of NF2, resulting in a presumably non-functional merlin or schwannomin protein. There is an association between the histological variant and the frequency of NF2 mutations, with 70–80% of transitional and fibroblastic meningiomas carrying NF2 mutations, compared with only 25% of meningothelial meningiomas. The product of the DAL-1 gene (18p11.3), a member of the proto-oncogene family, which has bcl-2 homology, has also been implicated in familial meningiomas and meningioma evolution; loss of expression of this protein is an early event in tumorigenesis.

The next most common genetic mutations seen in meningiomas after loss of 22q are deletions of 1p, 3p, 6q, 9p, 10q, and 14q. A putative suppressor gene is located at 1p36.21. Deletions of 1p, 9p, 10q, and 14q are associated with increasing histological grade, and 14q deletions in benign meningiomas (WHO grade I) may reflect a propensity for recurrence. Other chromosomal losses and gains have been reported from comparative genomic hybridisation studies of atypical and anaplastic meningiomas; allelic gain and amplification of 17q occur in up to 60% of anaplastic meningiomas.

Compared with glial tumours, little is known about the molecular pathogenesis of meningiomas and their malignant progression. Gene expression profiling with microarrays enabled differentiation of WHO grade I from grade II and III tumours and confirmed altered expression of growth-hormone receptor, insulin-like growth factor II, insulin-like growth-factor binding protein 7, and endothelin receptor A. Various other genes were differentially overexpressed, including midkine (mitogenic and angiogenic regulation), EAR-2 (associated with gene hormonal regulation), and cathepsin K (expression associated with invasive phenotype). Cathepsin D expression is, however, associated with lower tumour grade, low mitotic count, and low recurrence. Other genes, including RAD (an nm23 metastasis suppressor), BCR (mediator of cell-cycle growth arrest and apoptosis), and JUN-B (represses cyclin D and cell proliferation) were downregulated in tumours of grades II and III compared with WHO grade I tumours.

Site of origin

Meningiomas can arise from the dura at any site (figure 2), most commonly the skull vault, from the skull base (the planum sphenoidal, the sphenoid wing, the petrous ridge, the cavernous sinus and perisellar region, and the clivus), and at sites of dural reflections (falk cerebri, tentorium cerebelli, and dura of the adjacent venous sinuses). Other less common intracranial sites of origin including the optic-nerve sheath and the choroid plexus (intraventricular meningioma). 10% of meningiomas arise in the spine. Very rarely, meningiomas have also arisen wholly outside the craniospinal axis, in the ear and temporal bone, mandible, foot, mediastinum, and lung.

Clinical presentation

With the wider use of CT and MRI, many meningiomas are discovered as incidental findings during investigation for unrelated symptoms. When symptomatic, intracranial meningiomas present with a wide variety of symptoms arising from compression of adjacent structures, direct invasion of or reactive changes in adjacent brain tissue, and obstruction of cerebrospinal-fluid (CSF) pathways, cortical veins, or major venous sinuses.

Meningiomas commonly present with seizure disorders (27–67%), which can be partial (37%), complex partial (8%), generalised (60%), or a combination of these. The pathogenesis of meningioma-associated seizures is poorly understood, although location, perilesional oedema, and convexity location all contribute. Symptoms and signs of raised intracranial pressure could be due to the large size of the meningioma itself or to the pronounced cerebral swelling resulting from reactive vasogenic oedema with mass effect. Meningiomas in the posterior cranial fossa can cause obstructive hydrocephalus and present with papilloedema and classic early-morning headache.
Occasionally, meningiomas mimic transient ischaemic attacks or present with intracranial haemorrhage.48,49 Focal neurological deficits caused by meningiomas generally relate to direct local brain, cranial-nerve, or spinal compression, and can be predicted from the site of origin of the tumour. Calvarial meningiomas, including those arising in the parasagittal area, can cause region-specific deficits. Language dysfunction with dominant hemispheric meningiomas is not as common as in gliomas.50 Many meningiomas arising from the anterior skull base are large at presentation, and psychomotor symptoms and behavioural disturbance are predominant, with personality disintegration (anterior falcine, olfactory groove, or orbitofrontal meningiomas). Cranial neuropathies causing visual disturbances (parasellar, medial sphenoidal wing meningiomas), ophthalmoplegia, or trigeminal dysaesthesia (cavernous sinus and petrous-ridge meningiomas) are also common. Progressive unilateral visual loss is a feature of meningiomas of the optic-nerve sheath. Such patients might have opticociliary shunting on fundoscopy and axial proptosis. The Foster-Kennedy syndrome occurs rarely with large parasellar or orbitofrontal meningiomas.

Ataxia and cranial neuropathies can occur with petroclival meningiomas (figure 2). Various spinal syndromes occur with spinal meningiomas, which are most common in the thoracic spine. They present with a slowly progressive spastic paraparesis with or without radicular or nocturnal pain. The main differential diagnosis is demyelination, and direct imaging of the spine in cases of isolated spastic paraparesis is essential to distinguish between these and identify a potentially curable lesion. The next commonest spinal sites are the cervical spine and craniovertebral junction, presenting with symptoms of spastic quadriplegia, with or without low bulbar signs. Occasional spinal meningiomas also present with a sudden spinal event, relating to minor trauma, due to acute compromise of the spinal-cord vascular supply.

Neuroradiology

Brain or spinal imaging with CT or MRI is used to diagnose meningiomas, many of which have diagnostic features. Meningiomas are well-defined, extra-axial masses, which displace the adjacent brain. They may show a characteristic peripheral CSF cleft, reflecting displacement of the brain away from the overlying dura (figure 2). However, some lesions become very large before clinical presentation, and distinction between an intra-axial and extra-axial origin is impossible in some cases.

On CT, most meningiomas are slightly hyperdense compared with normal brain, and there is strong uniform enhancement after intravenous contrast. MRI is the preferred investigation of choice because it can clearly show the dural origin of the tumour in most cases. Meningiomas are most commonly isointense or slightly hypointense to brain on T1-weighted imaging and hyperintense on T2-weighted imaging. As with CT, after gadolinium enhancement, meningiomas show strong homogeneous enhancement. Gadolinium enhancement is especially useful in delineating en-plaque meningiomas. As with other slow-growing extra-axial tumours, meningiomas can cause reactive arachnoid cysts of variable sizes in 5% of cases, particularly in meningiomas involving the basal CSF cisterns,31 and these may
Meningiomas are associated with variable oedema-like changes in the brain white matter surrounding the tumour (figure 3), reflecting a combination of vasogenic brain oedema and cerebral gliosis due to prolonged brain compression and other factors. These white-matter changes persist in some cases even after complete resection of the meningioma and do not necessarily reflect disease recurrence. The degree of white-matter change is very variable and, though more common in larger tumours, is also florid with some small lesions. The causes of oedema associated with meningiomas have been widely studied, but there is no apparent unifying factor. Venous obstruction, tumour vascularity, pial–meningeal anastomoses, capillary permeability, the presence of vascular endothelial growth factor, and tumour secretion all contribute to variable degrees.

Several other disease processes have a propensity for primary involvement of the dura mater or subdural space giving a meningioma-like appearance, including metastatic disease (lymphoma and adenocarcinoma), inflammatory lesions (sarcoidosis, Wegener’s granulomatosis), and infections (tuberculosis).

Secondary involvement of the underlying bone (reactive sclerosis, invasion, erosion) by meningiomas is not common with convexity meningiomas but occurs in nearly 50% of skull-base lesions. It reflects local bone invasion or metabolic properties of the meningioma and can be seen in neurofibromatosis type 2; invasive tumours such as these can cause problematic extracranial facial masses.

Contribute to the mass effect. Some meningiomas show central cystic degeneration or have an associated cyst that can mimic schwannomas or intra-axial tumours. In addition, most meningiomas show a characteristic marginal dural thickening that tapers peripherally (the tail sign; figure 3), accurately localising the tumour to the dural or subdural compartment. This feature is much more clearly seen on MRI than CT, because contrast enhancement on T1-weighted MRI is superior to enhancement on CT, and the subarachnoid space is more clearly distinguishable on T2-weighted images.

Meningiomas have a propensity for invasion of cerebral veins and major cerebral venous sinuses. In cases in which lesions lie close to these structures, MRI venography (time-of-flight or phase contrast) is an extremely useful non-invasive means of demonstrating patency, narrowing, or occlusion of major vessels, which helps in the selection of appropriate surgical management (figure 4).

Before CT and MRI became widely available, catheter angiography was the core investigation for diagnosis of meningiomas, showing that the tumours were fed by meningeal branches of the external carotid or vertebral systems, with a characteristic late venous “tumour blush”. However, this technique is now reserved for clarification of the diagnosis when the appearances remain ambiguous on CT or MRI, when the anatomy of feeding arteries and veins would affect the surgical approach, or in preparation for intravascular embolisation. Vascularity varies substantially, and many tumours parasite branches of the internal carotid artery.

**Management**

The management of a meningioma depends on the signs and symptoms it produces, the age of the patient, and the...
site and size of the tumour. A small incidental meningioma that is discovered in a patient who is undergoing neuroradiological investigations for other reasons can safely be managed conservatively, especially if the patient is elderly or has a medical disorder that would increase the potential morbidity of surgical excision. The wide availability and diagnostic accuracy of MRI mean that such patients can be followed up for radiological progression or reviewed on clinical progression. If the lesion is calcified on CT or hypointense on T2-weighted MRI, it is likely to remain asymptomatic. The current practice of many clinicians is to carry out MRI yearly for 2–3 years; if there is no growth, the patient can be followed up clinically only.

**Endovascular treatment**

With recent advances in design of interventional neuroradiology catheters and microvascular techniques, endovascular therapy for meningiomas has increased substantially. Selective microcatheter embolisation of the meningeal arterial supply can be achieved with several different agents, including glue and coils. These can be highly effective at devascularising the tumour, and preoperative embolisation reduces perioperative blood loss. However, other studies have suggested that the benefit is unclear. There is also uncertainty about the precise timing of the embolisation before resection. Embolisation can induce histological atypical changes associated with benign (WHO grade I) meningiomas, since tumour necrosis will follow embolisation. Although atypical features are commonly seen in embolised meningiomas, they may reflect the primary tumour grade. Endovascular embolisation as the primary treatment of meningiomas is an alternative therapy in patients unsuitable for craniotomy and surgical excision. Bendszus and colleagues have reported on a series of seven patients managed just by embolisation of the tumour, with tumour shrinkage in six patients.

**Surgical excision**

Surgical excision of the tumour and its dural base is the most common primary management. Simpson, in a seminal paper, described the recurrence rates of meningiomas after surgical excision. He proposed a grading system based on the degree of surgical excision. A grade 1 excision involved removal of the tumour bulk, its surrounding dural attachment, and any involved bone; grade 2 excision was removal of the tumour with diathermy of its dural attachment; grade 3 removal was a macroscopic tumour resection with small foci left in situ (eg, in a major venous sinus); grade 4 was an extended biopsy with macroscopic residual disease; and grade 5 was a decompression with or without biopsy. The recurrence rate at 5 years was 9% for grade 1 excision; 19% for grade 2 excision; and 29% for grade 3 excision. Although this series was retrospective and was treated before the advent of CT and MRI and microsurgery, the importance of the extent of tumour and dural resection has been confirmed in several subsequent studies. However, in all these studies the rate of meningioma recurrence increased when the follow-up period was extended. Even after Simpson grade 1 resection, recurrence rates of 20% at 10 years have been reported.

Although a total excision (Simpson grade 1) is the ideal goal, many tumours cannot be totally excised because they are enveloping vital neural or vascular structures or are en plaque. With the introduction of MRI, many of these cases were diagnosed when the tumour was small, which led to a trend for attempted total excision of these lesions by various novel skull-base and microsurgical approaches. Most of these case series had substantial methodological problems and are subject to bias. Subsequent, longer-term follow-up has suggested that successful complete excision is rare and that the morbidity associated with attempted removal is significant, particularly for meningiomas involving the cavernous sinus, the petroclival region, the posterior part of the superior sagittal sinus, and the optic-nerve sheath and for speno-orbital en-plaque tumours. Attempts at excision of these lesions can cause catastrophic vascular injury or disabling cranial neuropathies, and many falcine cases, can be excised without significant morbidity. An intermediate grade of management difficulty arises with parasagittal tumours, particularly if they involve the sinuses posterior to the coronal sutures. In these patients, debulking of the lesion is technically achievable without difficulty. However, if the sinus remains patent and is invaded by tumour, total surgical excision becomes very difficult. The options are to reconstruct the sinus during the procedure or to leave microdeposits of tumour in the parasagittal region. In a young patient, the latter is not an attractive option but over-vigorous resection and damage to the superior anastomotic vein or a patent superior sagittal sinus can result in disastrous venous infarction of the brain. The alternative approach of subtotal resection with preservation of vascular and neurological integrity is now a strategy increasingly favoured by neurosurgeons, with residual tumour either observed by serial imaging or treated with radiation.

**Radiotherapy**

Although most meningiomas grow slowly and have a low mitotic rate, clinical benefit has been reported in many case series with either tumour regression or stasis when radiotherapy has been used in the following situations: after incomplete resection; after recurrence; and when tumour histology reveals atypia or anaplasia. However, none of these studies was randomised, controlled, or prospective, and few had follow-up sufficiently long for the true efficacy of irradiation and the incidence of delayed complications to be apparent. The outcome assessments used vary widely, and many studies and reviews included patients from before the advent of CT and MRI. Local control is commonly used as an outcome measure but is defined very loosely. Most studies use radiological criteria (static or decreasing size, or lack of central enhancement on serial scanning) as evidence of local control, and few studies between 1960 and 2000 have measured changes in neurological function. Application of radiotherapy to meningiomas has evolved with the development of conformal and particularly stereotactic methods for the planning and delivery of therapy. These developments have allowed accurate and focused treatment, which not only limits the amount of radiation to normal brain but also allows larger doses of radiation to be given to the lesion, with great precision. Many meningiomas are suitable for stereotactic radiosurgery because of their shape and size. 5-year local control rates of 93% (benign) and 68% (atypical) meningiomas were reported for a series of 206 recurrent or residual meningiomas with no mortality, 8% cranial-nerve deficits, and 3% symptomatic parenchymal changes. Gamma-knife stereotactic radiosurgery for the parasagittal meningioma produced 5-year control rates of 60% for recurrent and residual disease and 93% when radiotherapy was used as a primary treatment.
Improvement and stabilisation of neurological function were noted in 65% of these patients. Symptomatic reactive brain oedema was noted in 16% of the patients but this resolved without long-term deficit. With Linac-based stereotactic radiosurgery in 127 patients, 5-year actuarial tumour control rates of 89% with 5% complications and local tumour failure in three of 180 patients with 2% treatment toxicity have been reported for benign meningiomas. Three-dimensional conformal radiotherapy for atypical and anaplastic meningiomas produced 5-year actuarial local control rates of 38% and 52%, respectively.

The success of radiotherapy in controlling meningiomas has fuelled the debate about how extensive resection should be as a primary treatment, particularly in small skull-base tumours, and indeed whether radiotherapy should be considered a primary treatment for some tumours. Meningiomas of the optic-nerve sheath seem to be well controlled with conformal external-beam or multiplet or fractionated stereotactic radiotherapy; visual improvement has been reported in 25–40% of eyes with no disease progression on radiography. Surgical resection of such lesions invariably leads to complete loss of any remaining vision. For small to moderate-sized intracranial meningiomas treated by either surgery or primary radiosurgery, the results of the latter were similar to those of Simpson grade 1 resection after mean follow-up of 5 years. In another study the actuarial tumour control rate was 93% at 5 years in a series of 219 meningiomas diagnosed on imaging criteria (with only two incorrect diagnoses) and treated with gamma-knife radiosurgery. Although a randomised controlled study comparing surgery with radiotherapy is unlikely to be undertaken, the European Organization for Research and Treatment of Cancer is planning two randomised controlled trials addressing the role of radiotherapy in atypical and anaplastic meningiomas that are either incompletely excised or have recurring after primary surgery. These trials are a welcome development because there is a startling lack of “evidence” to underpin current clinical practice. One of the reasons for this lack is the long (many years) follow-up that would be required for comprehensive evaluation of different therapies.

Despite advances in imaging, interventional neuroradiology, neuropathology, microsurgery, and radiotherapy, many meningiomas remain a challenging clinical problem that is increasingly being managed by a multidisciplinary team approach. Difficulties in decision-making arise because of the conflicts inherent in the desire to preserve optimum function and the need to treat the tumour, and the problem of longer-term control with incompletely resected, atypical, or anaplastic tumours. Such decisions require understanding of the immediate and delayed risks and benefits of surgery and radiotherapy, including long-term possible risks of a second neoplasm induced by stereotactic radiotherapy. For meningiomas that recur after surgery and radiotherapy, several experimental therapies have been assessed in small case-series, including hydroxyurea chemotherapy, interferon alfa, and a progesterone agonist. As relations between histopathology, molecular characteristics, and biological behaviour of meningiomas become clarified, and the results from randomised clinical trials become available, we hope that management approaches will become less empirically based.

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Pediatric Neuro-Oncology
Multidisciplinary management of childhood brain tumors: a review of outcomes, recent advances, and challenges

A review

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Object. Brain tumors are the most common category of childhood solid tumors. In the 1970s and 1980s, treatment protocols for benign tumors focused almost exclusively on surgery, with radiation treatment as a salvage modality, whereas the management of malignant tumors employed a combination of surgery, radiation therapy, and chemotherapy, with therapeutic approaches such as “8-in-1” chemotherapy often applied across histological tumor subsets that are now recognized to be prognostically distinct. During the ensuing years, treatment has become increasingly refined, based on clinical and, more recently, molecular factors, which have supported risk-adapted treatment stratification. The goal of this report is to provide an overview of recent progress in the field.

Methods. A review of the literature was undertaken to examine recent advances in the management of the most common childhood brain tumor subsets, and in particular to identify instances in which molecular categorization and treatment stratification offer evidence or promise for improving outcome.

Results. For both medulloblastomas and infant tumors, refinements in clinical and molecular stratification have already facilitated efforts to achieve risk-adapted treatment planning. Current treatment strategies for children with these tumors focus on improving outcomes for tumor subsets that have historically been relatively resistant to therapy and reducing treatment-related sequelae for children with therapy-responsive tumors. Recent advances in molecular categorization offer the promise of further refinements in future studies. For children with ependymomas and low-grade gliomas, clinical risk stratification has facilitated tailored approaches to therapy, with improvement of disease control and concomitant reduction in treatment sequelae, and recent discoveries have identified promising therapeutic targets for molecularly based therapy. In contrast, the prognosis remains poor for children with diffuse intrinsic pontine gliomas and other high-grade gliomas, despite recent identification of biological correlates of tumor prognosis and elucidation of molecular substrates of tumor development.

Conclusions. Advances in the clinical and molecular stratification for many types of childhood brain tumors have provided a foundation for risk-adapted treatment planning and improvements in outcome. In some instances, molecular characterization approaches have also yielded insights into new therapeutic targets. For other tumor types, outcome remains discouraging, although new information regarding the biological features critical to tumorigenesis are being translated into novel therapeutic approaches that hold promise for future improvements. (DOI: 10.3171/2011.5.PEDS1178)

Key Words • astrocytoma • brain tumor • ependymoma • medulloblastoma • molecular markers • oncology

Brain tumors are, as a group, the most common solid tumors of childhood, and are now the leading cause of childhood cancer-related deaths. Although advances in surgical and adjuvant therapy during the last two decades have produced significant improvements in the outcome for certain types of childhood brain tumors such as medulloblastoma, the outlook for other groups, such as malignant gliomas and diffuse intrinsic brainstem gliomas, has changed minimally, if at all. In addition, for patients with more prognostically favorable tumor types, there has been growing concern that “cure” often comes at a high price in terms of late sequelae, which can impair long-term quality of life. Accordingly, current management strategies for children with brain tumors strive to maintain high survival rates while reducing long-term sequelae of treatment for more favorable-risk tumors, and to improve the rate of disease.
response and long-term survival in children with prognostically high-risk tumors. This report will review how these approaches are being applied in several of the most common groups of childhood brain tumors, specifically primitive neuroectodermal tumors, low- and high-grade gliomas, ependymomas, and infant tumors, and highlight instances in which advances in clinical and biological risk-based categorization and molecularly based treatment strategies have had, or may in the near term have, an impact on therapeutic trial development and outcome.

Methods

The PubMed database was searched using the terms “medulloblastoma,” “PNET,” “ATRT,” “ependymoma,” “high-grade glioma,” “malignant glioma,” “low-grade glioma,” “astrocytoma,” and “brainstem glioma,” which are the most common groups of childhood brain tumors, and the terms “infant,” “child,” and “pediatric.” Additional searches were performed of the publications of lead or senior authors of relevant articles, and of their reference lists, as well as the abstract listing of recent international pediatric neuro-oncology meetings. An attempt was made to: 1) identify articles of critical historical significance, particularly phase III randomized trials or large centrally reviewed single-arm studies, which have had a major impact on defining current surgical and post-surgical management algorithms, and 2) to identify recent studies that have led to significant advances in the molecular categorization and treatment stratification for childhood brain tumors, and new insights for therapeutic targets. The underlying goal of this analysis was to provide an up-to-date review of those studies that have provided a foundation for the management approaches historically used in pediatric neuro-oncology as well as those that have led to recent major advancements in the field and that offer evidence or clear promise for improving outcome and reducing sequelae of therapy. No attempt was made to formally grade the evidence provided in the reviewed publications, given the rapid advances in the field, which have highlighted limitations in what were originally outstanding well-conducted clinical trials, reflecting improvements in surgical, imaging, radiological, and radiotherapeutic techniques, refinements in pathological classification based on molecular data, and evolution of chemotherapy treatment options. Instead, an emphasis was placed on discussing such studies in the context of new information that has emerged to better inform interpretations of the data generated. Results are provided for each of the major tumor subgroups that were reviewed.

Results

Medulloblastoma/Primitive Neuroectodermal Tumor (PNET)

Primitive neuroectodermal tumors are the most common group of childhood malignant brain tumors. A major controversy in the 1980s and 1990s was whether the diverse group of CNS "small round blue cell" tumors encompassed distinct, location-specific entities, such as medulloblastomas, pineoblastomas, and supratentorial PNETs, or were different manifestations of a common underlying molecular pathway of tumor development. Recent gene expression and mutational analyses support the former interpretation in that the genomic profile of cerebellar PNETs differs from that of medulloblastomas. Moreover, further studies have indicated that even among individual location-based entities such as medulloblastomas, there are diverse molecular subsets of tumors, with distinctive patterns of gene expression and genomic pathway alterations, which may have a significant impact on the biological behavior and treatment response of a given lesion.

This novel biological information may help to refine the well-known clinical risk stratification criteria that are currently in use for these tumors (Table 1). These clinical parameters are based on a series of cooperative group studies from the Children's Cancer Group (CCG), Pediatric Oncology Group (POG), and Société Internationale d’Oncologie Pédiatrique (SIOP) in the 1980s and 1990s that noted significant differences in outcome based on the extent of postoperative residual tumor, metastasis status, tumor location, and age among patients treated with approximately 3600 cGy of radiation to the craniospinal axis with a boost to a dose of 5400 cGy to the tumor bed. In these early studies, 5-year progression-free survival rates were approximately 60% to 70% for children older than 3 years of age with extensively resected, nonmetastatic (M0) posterior fossa lesions (so-called “average-risk” or “standard-risk” tumors), but less than 30% to 40% for patients younger than 3 years of age and those with extensive residual disease, metastases, or tumors originating outside of the posterior fossa (so-called “high-risk” tumors). The use of chemotherapy appeared to significantly improve survival outcome for high-risk tumors, but a comparable improvement was not apparent for standard-risk tumors, potentially because the baseline survival rate was so much higher in the latter group. It also became increasingly apparent that many of the long-term survivors suffered significant long-term sequelae from their treatment, particularly those who received standard doses of radiation (3600 cGy to the neuraxis) at a young age. These observations provided an impetus for studies that stratified therapy based on these clinical risk factors, with the goal of improving survival in the high-risk group and reducing the long-term side effects of treatment in the average-risk group.

Because initial efforts to reduce sequelae in average-risk patients by reducing the craniospinal radiation dose to 2340 cGy were associated with a decrease in progression-free survival, subsequent studies attempted to augment the efficacy of reduced-dose radiation by administering adjuvant chemotherapy. These approaches were substantially more successful with rates of long-term survival exceeding 70% and potentially lower frequencies of radiation-related cognitive and endocrine sequelae than after treatment with standard doses of radiation alone. Building upon these results, the Children’s Oncology Group (COG) initiated a randomized Phase III study (A9961) that was designed to compare 2 adjuvant chemotherapy regimens, administered with radiation, for...
Advances in childhood brain tumor management

**TABLE 1: Criteria for stratification of intracranial PNETs**

<table>
<thead>
<tr>
<th>Category</th>
<th>Prognostic Factor</th>
<th>Outcome Association</th>
<th>Application*</th>
</tr>
</thead>
<tbody>
<tr>
<td>clinical/imaging</td>
<td>age</td>
<td>adverse if age &lt; 3 yrs</td>
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</tr>
<tr>
<td></td>
<td>extent of tumor removal</td>
<td>adverse if incomplete (or &gt;1.5 cm² residual)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>tumor location</td>
<td>adverse if outside of posterior fossa</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>metastases</td>
<td>adverse</td>
<td>1</td>
</tr>
<tr>
<td>pathology</td>
<td>anaplasia</td>
<td>adverse if diffuse</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>desmoplasia</td>
<td>favorable, particularly in younger patients</td>
<td>2</td>
</tr>
<tr>
<td>molecular</td>
<td>Shh pathway alterations</td>
<td>favorable, particularly in younger patients</td>
<td>1, 3</td>
</tr>
<tr>
<td></td>
<td>Wnt pathway alterations</td>
<td>favorable, particularly in older patients</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>c-myc amplification</td>
<td>adverse</td>
<td>2</td>
</tr>
</tbody>
</table>

* 1 = used for stratification in current protocols; 2 = proposed for use in protocols under development; 3 = potential therapeutic target.

average-risk patients. This study confirmed that reducing the dosage of craniospinal radiation from 3600 cGy to 2340 cGy in conjunction with chemotherapy was not associated with an unacceptable drop in survival rates. Survival with both regimens was comparable, and was superior to results in previous studies with standard doses of radiation, possibly reflecting improved stringency in the imaging criteria used to establish tumors as average risk. These results provided a foundation for a subsequent study (ACNS0331) that is examining whether doses and volumes of radiation can be further reduced with intensification of adjuvant chemotherapy. For children between 3 and 8 years of age, one component of the study is comparing outcome in patients randomized to receive a craniospinal radiotherapy dose of 1800 cGy versus those who receive 2340 cGy, with a goal of determining whether the lower dose can maintain disease control while diminishing cognitive and endocrine sequelae, which are most severe in younger children. A second component of the study, which incorporates both the 3–8 age group as well as children between 8 and 18 years of age, will examine the safety of reducing the volume of posterior fossa irradiation using 3D imaging-based conformal delivery techniques to decrease ototoxicity.

This study also includes a battery of correlative analyses to evaluate molecular features that have been found in recent retrospective studies to identify prognostically distinct tumor subsets, as well as genome-wide screening of DNA copy number alterations and gene expression profiles to look for patterns of abnormalities that can improve upon current clinically based risk classification. The long-term objective of this effort is to identify molecular features that can highlight tumors likely to recur despite favorable clinical features, or unlikely to recur despite adverse clinical features, which would provide insights for biologically refined stratification in future studies.

The potential utility of these molecularly based risk-adapted classification schemes is supported by several recent reports. For example, Thompson et al. observed that medulloblastomas could be subdivided into 5 groups based on characteristic molecular patterns, including alterations of genes in the Wnt signaling pathway and mutations of those in the Sonic hedgehog (Shh) pathway. More recently, Kool et al. also identified 5 distinct subsets that shared similarities with the groups noted by Thompson et al., including features such as alterations in Wnt signaling, particularly mutations in the β-catenin gene, and alterations in Shh signaling associated with mutations or inactivation of PTCH1. The various molecular groups also differed significantly in their clinicopathological features in terms of disease dissemination and patient age.

Other molecular features, such as TP53 mutations and c-myc amplification have also been linked to prognostically adverse tumor subsets.

Although the above molecular subgroups of medulloblastomas may in part overlap with clinically defined subsets, the molecular data also appears to convey prognostic information that supplements clinical risk stratification. The fact that many of the above genomic and gene expression changes can be assessed on formalin-fixed paraffin-embedded samples using standardizable assays offers the possibility of tailoring therapy based on the patterns of abnormalities in the tumor, in conjunction with established clinical factors. This strategy is under consideration for implementation in future trials of the European and North American cooperative groups, particularly in average-risk patients, in whom reduction of neurotoxic therapy in the most favorable patient subgroups remains a priority.

The converse approach, specifically intensification of therapy in average-risk patients with adverse prognostic features as a way to improve outcome, is also an ongoing objective. An example in this regard is based on data from study A9961, which indicate that the subset of patients whose tumors showed anaplastic histological features had a significantly worse prognosis than those with classical histology. This has led to inclusion of anaplastic tumors with other high-risk PNETs in current COG medulloblastoma/PNET protocols, regardless of clinical features. Recent studies suggest that the subset of anaplastic tumors with large cell histology, which generally exhibit c-myc amplification, have a particularly poor prognosis, independent of clinical factors.

In contrast to the underlying therapeutic philosophy for average-risk medulloblastomas, which is directed toward reduction, where feasible, of treatment-related se-
quetae while maintaining good long-term survival rates, the focus of study designs for high-risk PNETs has been on increasing the historically low percentage of children who become long-term survivors. A variety of approaches have been pursued in an effort to achieve this goal. One strategy has involved administering intensive chemotherapy prior to radiation treatment as a way to enhance disease control, although some studies were associated with an unacceptable rate of toxicity and early disease progression.

An alternative approach, which has achieved superior rates of disease control, has focused on administering conventional chemotherapeutic agents with radiosensitizing properties during radiation therapy, followed by administration of additional postirradiation chemotherapy. In this regard, the CCG-99701 study, which involved a dose escalation study of carboplatin with vincristine during radiotherapy followed by adjuvant chemotherapy after irradiation, noted long-term survival rates that appeared substantially better than those from previous studies.

These results provided an impetus for the Phase-III ACNS0332 study, which includes a double randomization for both the type of chemotherapy administered during radiation therapy and the type administered afterward. The first randomization will determine whether administration of carboplatin and vincristine with radiation therapy will improve outcome compared with administration of vincristine alone, whereas the second randomization will examine whether adding isotretinoin to an adjuvant chemotherapy backbone will improve outcome compared with administration of adjuvant chemotherapy alone. This latter aspect of the study is based upon the observation that isotretinoin synergistically enhances the activity of platinum-based chemotherapy in preclinical models.

The ACNS0332 study also includes molecular correlative analyses to determine whether high-risk patients can be subdivided into prognostically distinct groups based on the molecular features of the tumor.

In addition to the application of molecular characterization for risk stratification of medulloblastomas, insights regarding their genomic features have also provided new opportunities for targeted therapy of these tumors. In particular, the observation that constituents of the Shh pathway are mutated or inactivated in a subgroup of medulloblastomas has provided a particularly promising target for therapy. The involvement of this pathway in medulloblastoma development was initially suggested in children with Gorlin syndrome, an inherited disorder associated with a predisposition to development of medulloblastomas. This syndrome results from mutations in the PTCH1 gene, which encodes the receptor for binding of the Shh protein. The observation that mutations of PTCH1 and other constituents of the Shh signaling pathway were present in patients with sporadic medulloblastomas provided an impetus for exploring strategies to pharmacologically block this pathway, which plays a role in developmental regulation within the nervous system. Favorable results of this approach were demonstrated in preclinical models and subsequently in patients with metastatic medulloblastoma. Currently, a trial of the Shh inhibitor GDC-0449 is ongoing in the Pediatric Brain Tumor Consortium (PBTC), and if response correlates with the status of Shh pathway activation in a given tumor, this will provide a basis for prospective molecular characterization as a criterion for therapy.

Based on recent studies that mutations and alterations in the histone deacetylase genes, which affect gene transcription, are present in a subset of medulloblastomas and that targeted inhibition of this pathway can block tumor growth in preclinical models, recent studies have also examined the activity of histone deacetylase inhibitors in children with recurrent tumors. Studies of other proteins involved in neural cell developmental regulation are currently in progress, including inhibitors of Notch signaling. Studies of antiangiogenic signaling inhibition, using agents such as bevacizumab, as well as targeted inhibition of pathways implicated in medulloblastoma growth, have also been undertaken.

**Low-Grade Glioma**

Low-grade gliomas encompass several histological subgroups of tumors, including pilocytic astrocytoma and subependymal giant cell astrocytoma, which generally are classified as Grade I lesions, and fibrillary and pilomyxoid astrocytomas, which are considered Grade II lesions. In the 1970s and early 1980s, treatment of these tumors focused almost exclusively on resection, followed by reoperation or irradiation for lesions that progressed or were believed to be at high risk for progression after initial resection. Several sizeable clinical reports based on cases treated during this era demonstrated, not unexpectedly, that deep-seated, infiltrative tumors, such as those involving the optic pathways, were much less likely than superficial lesions to be amenable to gross-total resection, and had a correspondingly worse outcome. However, the use of wide-field irradiation in these large centrally located tumors, which often occur in young children, carried a significant risk of late cognitive, endocrine, and vascular sequelae.

For superficially situated, well-circumscribed lesions in the cerebral or cerebellar cortex, resection remains the initial treatment of choice. A recent report by Wisoff et al. has strongly supported this approach, demonstrating that the factor most strongly associated with outcome in all low-grade gliomas is the extent of surgical tumor removal. In a large cooperative group natural history study (CCG9891/POG8930), 5-year progression-free survival was more than 90% in children with low-grade gliomas that had undergone gross-total resection, whereas approximately half of children with less extensive tumor removal had disease progression during that interval. In accordance with these data, a variety of surgical adjuncts are sometimes employed in an effort to enhance the likelihood of safely achieving an extensive resection. Advances in image-guided tumor localization and treatment planning, functional brain mapping, intraoperative imaging, and neurophysiological monitoring have led to theoretical improvements in the safety of extensive tumor resections, particularly for more deep-seated lesions such as thalamic tumors, although it has been difficult to design a prospective trial to prove the independent impact on outcome of any of the above approaches.
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Given the strong association between resection extent and outcome, it has also been difficult to determine conclusively whether there is a correlation between histology and prognosis. Although pilocytic astrocytomas appear to have a more favorable prognosis than fibrillary lesions, this may reflect that pilocytic tumors, particularly when superficially located, are often reasonably well circumscribed and more likely to be amenable to gross-total resection. Likewise, the contribution of tumor location to outcome is inextricably tied to the issue of the extent of resection: superficial lesions involving the cerebral and cerebellar cortices tend to have a better prognosis than deep lesions involving the basal ganglia, optic pathways, and brainstem, probably relating to the fact that they are more amenable to extensive removal without excessive morbidity.

Because patients whose tumors have undergone gross-total resection have a greater than 90% long-term survival rate, adjuvant therapy is usually not required for such lesions, which include the majority of cerebral hemisphere and cerebellar low-grade gliomas. In contrast, the adjuvant management considerations are much more complex for deep-seated lesions, particularly those involving sites such as the hypothalamus and optic pathways and those arising in the brainstem, which are usually not amenable to complete removal because of their involvement of critical surrounding structures. Management of optic pathway tumors is further complicated by the fact that they commonly arise in young children, who are at high risk of long-term sequelae from side effects of wide-field radiation therapy.

In a series of studies during the last 15 years, several chemotherapy regimens were noted to have efficacy in delaying or avoiding the need for radiotherapy in children with progressive or incompletely resected low-grade gliomas believed to be at high risk of progression. The recently completed COG A9952 study involved a Phase III randomized comparison between 2 active regimens, carboplatin and vincristine versus 6-thioguanine, procarbazine, lomustine, and vincristine, for low-grade gliomas arising in children without neurofibromatosis Type 1 (NF1) and a single-arm analysis of the results with carboplatin and vincristine in children with NF1-related low-grade gliomas. Although both regimens showed efficacy in delaying tumor progression and the need for radiation therapy, the results were slightly better with the lomustine-based regimen and significantly better in the nonrandomized cohort of patients with NF1, reflecting the often indolent growth characteristics of NF1-associated low-grade gliomas. Whereas the median time to progression in NF1 patients exceeded 8 years, most children without NF1 in both treatment arms suffered disease progression within 5 years of initial therapy, which calls attention to the importance of identifying new treatment options.

Accordingly, subsequent studies are evaluating other therapeutic approaches for these tumors. The ACNS0223 study examined the feasibility and efficacy of administering temozolomide in addition to carboplatin/vincristine. The ADVL0515 study examined the use of vinblastine, which has been observed to have independent activity for low-grade gliomas, as an alternative to vincristine in carboplatin-based regimens. Other variations on the platinum-based therapy have also been examined, including studies of cisplatin combined with etoposide, which have achieved high rates of disease control and radiation avoidance in young children with progressive low-grade gliomas. In contrast, ACNS0221 is examining the efficacy of conformal radiotherapy in children older than 10 years with progressive tumors and in younger children with chemotherapy-refractory tumors to determine whether the use of 3D treatment planning, which conforms to the shape of the tumor and minimizes the volume of surrounding normal brain that receives high doses of radiation, can achieve an acceptable level of side effects and lead to long-term disease control. More recently, a host of biological agents have been examined in these tumors, including antiangiogenic agents and growth signaling inhibitors that are directed against newly identified molecular targets in low-grade glioma.

In particular, recent studies have demonstrated that a large percentage of pilocytic astrocytomas exhibit alterations in the BRAF gene, most commonly involving translocations between BRAF and KIAA, or activating mutations, such as BRAFV600E, which induces growth signaling through mitogen-activated protein kinase (MAPK)-related pathways. Based on these findings, and data that BRAF inhibitors have preclinical activity against pilocytic astrocytoma xenografts, clinical trials of BRAF and MAPK pathway inhibitors have been launched for children with progressive tumors, such as the PBTC study of AZD6244. Because these tumors have been noted to have evidence of vascular proliferation histologically, studies of antiangiogenic agents, such as bevacizumab and nelarabine, have also been launched, and have achieved encouraging rates of response or disease control in initial Phase I and Phase II trials.

Subependymal giant cell astrocytomas are a second low-grade glioma subset for which a characteristic pattern of genomic alterations has been identified and translated therapeutically. Many of these tumors arise in the setting of tuberous sclerosis and exhibit mutations in the TSC1 and TSC2 genes, leading to dysregulated activation of mammalian target of rapamycin (mTOR) signaling. As with the BRAF anomalies that characterize pilocytic tumors, these consistent genomic alterations provide a “druggable” target for molecularly directed therapy. A recent trial of one mTOR inhibitor, everolimus, showed a high rate of tumor regression and disease control for lesions that were not amenable to resection.

In contrast to the above 2 groups, the molecular basis for childhood fibrillary low-grade astrocytomas remains less well-defined. Although in adults such tumors represent an early stage in a pathway of tumorigenesis that often ends in higher grade lesions, such a phenotype is less commonly observed in childhood lesions. At present, tumor-specific molecular therapies for these lesions are lacking, although the identification of characteristic genomic alterations that may provide a basis for targeted therapies remains a subject of intense interest in the research community.

High-Grade Glioma

Malignant (high-grade) gliomas encompass Grade
III anaplastic astrocytomas, oligodendrogliomas, and oligoastrocytomas, and Grade IV glioblastomas and gliosarcomas. As with adult malignant gliomas, the prognosis for children with these tumors has historically been poor, despite advances in surgical techniques and implementation of newer protocols for administering radiation. The addition of chemotherapy with lomustine and vincristine to postoperative radiation therapy was demonstrated in the CCG-943 study to improve survival compared with the use of radiation therapy alone, which helped to establish multimodality therapy as the standard approach for these tumors. Unfortunately, subsequent studies with the more complex “8-in-1” regimen in the CCG-945 study failed to further improve outcome, and the use of more intensive chemotherapy, administered before or after radiation therapy, also failed to increase survival and in some instances was associated with prohibitively high rates of toxicity. Moreover, with the recognition that a sizeable subset of long-term survivors in historical studies actually harbored atypical low-grade gliomas, rather than malignant gliomas, the reported survival rates for children with these tumors have actually declined in the last 20 years, reflecting that recent studies have applied more consistent entry criteria and central histological review to exclude discordant histologies.

In studies that have used central review, two clinical factors have stood out in terms of having an association with outcome, specifically histology and extent of tumor resection. In general, patients with Grade IV lesions (glioblastoma) have had a worse prognosis than those with Grade III lesions. Anaplastic (Grade III) oligodendrogial tumors, in particular, have appeared to have a better outcome than other malignant gliomas. In addition, patients with tumors that were not amenable to extensive resection have had lower rates of long-term survival than those with more resectable lesions. Molecular studies of the CCG-945 cohort have also identified several biological factors that have been associated with a worse prognosis, including overexpression and/or mutation of $TP53$, high MIB-1 proliferation index, and overexpression of methylguanine DNA methyltransferase (MGMT), which counteracts the effects of alkylating agents, such as the nitrosoureas.

Based on recent studies in adults that noted superior outcomes from administering chemotherapy with temozolomide during and after radiation therapy versus treatment with radiation therapy alone, pediatric studies of this approach were initiated. The ACNS0126 study incorporated daily administration of temozolomide during radiation therapy followed by treatment on a 5-day per 28-day (5 days in a row every 28 days) schedule thereafter. Although outcome results in this study were similar to those reported in adults, they were no better than those obtained in the CCG-945 study with CCNU and vincristine. As in the CCG-945 study, MGMT overexpression proved to be adversely associated with outcome. The 2-year event-free survival rate was $17\% \pm 5\%$ among patients without overexpression of MGMT versus $5\% \pm 4\%$ among those with overexpression (p = 0.045). A subsequent study (ACNS0423) combined both lomustine and temozolomide, which was based on the results of a pilot study that noted a comparatively better rate of 1-year survival with this combination than with temozolomide alone. Preliminary results from ACNS0423 suggest a nominal improvement in outcome in the overall cohort of patients, compared with the results from ACNS0126, although survival rates remain disappointing.

In view of the failure of conventional chemotherapy and radiation therapy to substantially improve the prognosis of children with these tumors, there has been significant interest in exploring the applicability of molecularly targeted treatment strategies. However, compared with the extensive research that has been directed at defining the molecular pathways of tumorigenesis in adult high-grade gliomas, relatively little information is available in pediatric lesions. Adult malignant gliomas have characteristically been subdivided into “primary” lesions that arise de novo as Grade IV tumors, which typically exhibit amplification and often rearrangement of the epidermal growth factor receptor (EGFR) gene and deletion of $PTEN$; “secondary” lesions that progress from low-grade fibrillary astrocytomas to Grade III and ultimately Grade IV lesions in a stepwise fashion, which typically have mutations of $TP53$ and $IDH1$ or $IDH2$ as early genetic anomalies; and oligodendrogial tumors, which often exhibit deletions of chromosomes 1p and 19q. In this regard, previous studies have noted $TP53$ mutations in approximately half of childhood malignant gliomas, similar to the rate observed in adult secondary malignant astrocytomas. However, pediatric malignant gliomas rarely arise from apparent low-grade precursors and, apart from those occurring in adolescents, infrequently exhibit mutations in the $IDH1$ or $IDH2$ genes, suggesting that despite their similarities in terms of $TP53$ alterations, most childhood high-grade gliomas arise by a mechanism that is distinct from the one seen in adult secondary malignant gliomas. Childhood lesions are also biologically distinct from adult primary malignant gliomas, because they infrequently exhibit deletions or mutations of the $PTEN$ gene or amplification of $EGFR$.

Similar to recent reports that highlight the existence of multiple pathways of tumorigenesis in adults, it is likely that pediatric lesions are not only genetically distinct from many adult lesions, but may themselves encompass several parallel pathways of tumorigenesis. Recent studies from several groups have identified amplification of $PDGFR-\alpha$ in a subset of pediatric malignant gliomas, suggesting that inhibitors of this receptor or its downstream signaling pathways may constitute a relevant targeted therapy approach. However, for most such tumors, a consistent pattern of genetic alterations has not been observed. Accordingly, molecularly directed studies to date have generally focused on targets such as EGFR signaling that are relevant in adult malignant gliomas. The results of several of these studies have been recently reported, and have unfortunately noted low rates of responses and disease control. An ongoing challenge is to identify molecular factors that may better determine prospectively which patients are likely to respond to a given approach and to identify new strategies that can achieve a higher rate of response.

In this regard, a newly opened study (ACNS0822),
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which is comparing the activity of several different agents during radiation therapy, followed by the antiangiogenic agent bevacizumab in combination with irinotecan after radiation therapy, is incorporating extensive correlative studies encompassing microarray-based genotyping and expression profiling to parallel the extensive analyses that have been recently completed in adult malignant gliomas.\textsuperscript{29,111} The goal of these correlative analyses is to define genes associated with tumor progression in pediatric malignant gliomas and to potentially identify heretofore unrecognized therapeutic targets that may be applied in future studies.

Brainstem Glioma

Brainstem gliomas are a biologically diverse group of lesions, which encompass subgroups of focal tumors that are generally benign biologically, and diffuse intrinsic tumors, which are malignant. The increasing availability of MR imaging during the 1980s and 1990s had a major impact in allowing the reliable categorization of these tumors (Fig. 1).\textsuperscript{118,94} The fact that diffuse tumors can now be identified noninvasively has diminished the role of biopsy in establishing the diagnosis,\textsuperscript{1} except in cases with atypical imaging or clinical characteristics, and has had the unintended consequence of limiting access to tumor material for molecular analysis. On the other hand, improved imaging has had undeniable benefits in terms of identifying lesions that may in selected cases be amenable to surgical intervention, such as dorsally exophytic brainstem gliomas and focal lesions of the midbrain and cervicomedullary junction, which are generally low grade histologically. Such tumors are typically managed like other low-grade gliomas in that accessible lesions, such as dorsally exophytic brainstem gliomas, are often treated using resection as the primary therapeutic modality. If an extensive resection has been achieved, adjuvant therapy is often deferred and observation alone is pursued. For more deep-seated brainstem low-grade gliomas, which may not be amenable to complete resection, the same considerations apply as noted earlier for nonbrainstem low-grade gliomas, in terms of the use of focal conformational radiation therapy or chemotherapy, depending on the age of the patient.

In stark contrast to the reasonably favorable prognosis of low-grade focal brainstem gliomas, the outcome for children with diffuse intrinsic brainstem gliomas remains exceedingly poor and has artifically worsened slightly over time, reflecting that refinements in imaging have screened out atypical low-grade lesions that may have been mistaken for diffuse tumors in early studies, leaving a more homogeneous population of prognostically adverse tumors. Historically, these tumors have been treated with radiation, which provides an interval, albeit transient, of symptomatic improvement in many patients. Cooperative group studies in the 1980s and early 1990s examined the efficacy of increasing the dose of radiation using hyperfractionated delivery approaches. Although these studies demonstrated that escalation of the radiation dose to as high as 7800 cGy was often tolerated, no improvement of progression-free or overall survival duration was observed, with 1-year progression-free survival rates clustering in the range of 15% to 20%.\textsuperscript{23,43,47,75}

Subsequently, a series of studies examined the use of pre- and/or postradiation chemotherapy for these tumors, in some cases in conjunction with hyperfractionated high-dose irradiation, but disappointing results were obtained with a variety of agents, even when administered at high doses.\textsuperscript{15,42,43,54} More recent studies have attempted to build upon the activity of radiation therapy by administering chemotherapy concurrently in an effort to add to the effects of radiotherapy (chemoradiotherapy) or synergistically enhance the activity of radiation therapy (radiosensitization). Unfortunately, results to date have been discouraging. For example, the ACNS0126 study of temozolomide with irradiation, which incorporated a stratum for patients with brainstem gliomas, noted a 1-year event-free survival rate of only 14%.\textsuperscript{10} Similarly, a recent French Society of Pediatric Oncology study of topotecan during radiotherapy failed to observe a significant survival benefit,\textsuperscript{2} and a COG study of the radiosensitizer gadolinium texaphyrin during irradiation (ACNS0222), which incorporated the maximally tolerated dose determined by the A09712 Phase I study,\textsuperscript{3} also yielded disappointing results.

Studies by the PBTC have examined several molecularly targeted treatment strategies, selected predominantly because of the known involvement of the targeted pathways in adult glial tumorigenesis, in conjunction with radiation therapy. Studies with the PDGFR inhibitor imatinib (PBTC-006), the EGFR inhibitor gefitinib (PBTC-007), and the farnesyltransferase inhibitor tipifarnib (PBTC-014) have been completed, but the results, which have recently been reported, have been disappointing.\textsuperscript{31,95,96} Studies are currently in progress using conceptually different molecularly targeted strategies in conjunction with radiation therapy. One study being conducted by the COG is examining the histone deacetylase inhibitor vorinostat, which has shown promising synergy with radiation in other tumor systems. Concurrently, the PBTC is conducting a study that uses capecitabine, a prodrug of 5-fluoro-uracil, based on the rationale that this compound may be selectively metabolized to the active agent by the increased thymidine phosphorylase activity observed in gliomas, and that this effect may be further enhanced by radiation.

Fig. 1. Sagittal T-1 images of brainstem gliomas. Left: Dorsally exophytic glioma that was found to be a pilocytic astrocytoma after resection. The patient is alive 7 years after diagnosis. Right: Diffuse intrinsic glioma. The patient died 9 months after diagnosis, having received surgery and adjuvant chemotherapy.
As noted earlier, one of the many challenges to progress in the management of diffuse intrinsic brainstem gliomas has been the lack of well-preserved tumor material to identify molecular abnormalities and potential novel therapeutic targets, reflecting that most diffuse intrinsic brainstem gliomas are now diagnosed by imaging findings alone in the context of appropriate clinical symptoms. Until recently, most biological studies involving these tumors have relied on archival biopsy specimens obtained in the era before MR imaging, cases with atypical imaging and clinical features that had undergone biopsy, and autopsy specimens. The archival nature and uncertain processing of many such specimens constrained the range of analyses that could be accomplished, which generally focused on a limited group of targets, such as EGFR and p53, which could be assayed by immunohistochemistry or DNA analysis.

More recently, a series of studies have attempted to obtain autopsy material in real time, which has allowed collection of higher-quality fresh tumor material that is amenable to whole genome microarray-based expression analysis, DNA copy number determination, and targeted gene sequencing. In addition, several groups in Europe have incorporated image-guided stereotactic biopsy of brainstem gliomas at diagnosis as a way to obtain biologically informative tumor material. The relatively low rates of morbidity reported in these studies have prompted some groups in North America to also consider the feasibility of incorporating biopsy sampling into clinical trials for brainstem gliomas. The theoretical underpinning of such studies would be to use the biopsy data to direct the selection of molecularly targeted therapeutic approaches in individual patients. Unfortunately, long-term responders have been rare in essentially all molecularly targeted studies reported to date, which has posed a quandary about which targeted agents would be included in such a clinical trial. Thus, although the rationale behind using biopsy data to direct subsequent therapy remains controversial, there is general agreement that novel approaches are critically needed to improve upon the dismal rates of response and long-term survival in children with these tumors. In this regard, pilot trials of innovative strategies, such as convection-enhanced delivery of immunotoxins and immunotherapy, are being conducted in small groups of newly diagnosed patients with these tumors in an effort to assess safety and efficacy as a basis for broader clinical trial assessments.

Ependymoma

With improvements in neuroimaging technology, there has been increasing evidence from institutional and cooperative group studies during the last two decades that the most important prognostic factor for outcome among children with ependymomas is the extent of tumor removal. Whereas children with tumors that have undergone gross-total resection have a 50%–75% chance of long-term survival after postoperative radiation therapy, less than 30% of those with subtotal resections experience prolonged survival. Recognition of this factor has been associated with a trend toward higher rates of extensive tumor resection among children enrolled in cooperative group trials, increasing from approximately 50% in the CCG-9941 study from the 1990s to approximately 75% in the ACNS0121 study completed during the last several years. Moreover, institutional studies that have incorporated a strong emphasis on achieving extensive tumor removal have reported even higher rates of gross-total resection and, coupled with administration of carefully planned 3D conformal radiation of the tumor bed, have observed 7-year overall survival rates exceeding 80%. A second factor that has more recently been associated with outcome is tumor histology: several studies have noted that anaplastic (Grade III) ependymomas have a significantly worse prognosis than Grade II lesions. A third factor that has been less convincingly associated with outcome is tumor location, which may reflect that nonanaplastic supratentorial lesions may be more likely to undergo radiologically complete removal than infratentorial tumors and in some cases may be amenable to microscopically complete removal.

The COG ACNS0121 study, which recently completed enrollment and is currently undergoing final outcome analysis, stratified therapy based on the above 3 factors. For patients who underwent gross-total or near-total resection of infratentorial ependymomas and anaplastic supratentorial lesions, and those who underwent gross-total or near-total resection of nonanaplastic supratentorial lesions with microscopic residual disease, the study will determine the efficacy of conformal radiation to the tumor bed plus a 1-cm margin for achieving long-term disease control. For children with nonanaplastic supratentorial ependymomas who underwent microscopically complete resection, the study will define the frequency of disease control without adjuvant therapy, based on favorable results with expectant management in a previous pilot study. Finally, for patients who had an incomplete initial resection, the study will determine whether a short course of chemotherapy will achieve disease regression or permit “second-look” complete resection before radiation therapy, and whether this intervention will improve long-term survival. A novel aspect of this study is the inclusion of children as young as 1 year of age. Historically, children younger than 3 years with ependymoma have had a less favorable prognosis than older children, which may reflect distinctive biological features of these tumors in terms of their invasive growth patterns around the brainstem when they arise infratentorially, and their large size and vascularity when they arise supratentorially, but may also reflect that irradiation of such tumors has often been deferred after surgery. The use of conformal radiation planning has allowed these children to be treated with tumor-directed therapy with tight margins to minimize irradiation of the surrounding brain. The sequelae of this approach, particularly in young children, are undergoing careful assessment in this cohort. A subsequent study for ependymomas (ACNS0831), which is ongoing, is a randomized Phase III trial designed to resolve a long-standing area of controversy in the management of ependymomas, specifically whether chemotherapy has any benefit when administered in addition to radiation for postsurgical therapy. Although platinum-based chemotherapy has shown efficacy in inducing
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tumor regressions, and in that context was examined in the ACNS0121 study for the ability to promote second-stage gross-total resections among children who initially had incomplete resections, the role of chemotherapy as a component of postradiation treatment has been less clear. Previous studies have had insufficient patient numbers to address this issue conclusively, particularly in view of the strong association between resection extent and outcome. The current study therefore retains the stratification approach used in ACNS0121, but then adds randomization for the use of postradiation chemotherapy in the subset of patients who have had gross-total or near-total resections, either initially or after second-look surgery. Because previous studies have indicated poor results for patients with subtotal resections that could not be converted to a minimal residual disease state by chemotherapy and second-look surgery, this subset of patients will all receive postradiation chemotherapy and their results will be compared with those from ACNS0121 without this adjuvant.

Both the ACNS0121 and ACNS0831 studies incorporate central histological review and analysis of gene expression profiles, genome-wide assessment of gene copy number alterations, and examination of telomerase activity within the tumors to determine whether histological or genomic features can identify prognostically distinct subsets of tumors, which would provide a basis for refining therapeutic stratification in subsequent studies. The potential relevance of such data is provided by recent studies that indicate the presence of several molecularly distinct subsets of ependymomas. In a recently published report, information regarding these subgroup-specific genomic alterations was then used to unravel the cellular underpinnings of ependymoma development. This work involved matching the transcriptomes of human tumors to those of mouse neural stem cells derived from different regions of the nervous system to define critical molecular substrates of tumorigenesis. These studies generated a novel mouse model of one subset of human supratentorial ependymoma, defined the molecular signaling mechanisms that contribute to tumorigenesis, and suggested a molecularly targeted therapeutic approach that could be explored to treat such tumors. Pilot studies of molecularly based approaches for these tumors are being conducted by several cooperative groups and consortia, as well as the Collaborative Ependymoma Research Network.

Brain Tumors in Young Children

The management of malignant brain tumors in children younger than 3 to 5 years of age has historically incorporated somewhat different strategies than for comparable tumors in older children, because of the particular sensitivity of the young brain to the toxic sequelae of radiation therapy. As a way to delay or avoid the need for radiation therapy, early treatment protocols in the cooperative groups examined the use of a variety of regimens for intensive postsurgical chemotherapy. Although a subset of children responded well to such treatment and did not require radiotherapy, most manifested disease progression within 1 to 2 years of diagnosis, which was generally fatal. Several of these studies used similar chemotherapy regimens for multiple distinct histologies, and not unexpectedly, there were wide differences in efficacy as a function of tumor type.

More recent studies have adopted a variety of strategies in an effort to improve on these results. One general approach, which was initially examined in the "Head Start" series of studies, involved the use of extremely intense myeloablative "consolidation" chemotherapy, often following an initial course of induction therapy. Modifications of this theme, including the use of different combinations of agents, as well as the administration of a series of submaximally intense courses of myeloablative therapy rather than a single course, have been evaluated in subsequent trials. The latter approach, examined in CCG-99703, suggested an improvement in event-free survival in the overall population of infant tumors, as well as in the subset of children with medulloblastoma, although final results from this study are pending.

A second general approach has involved the use of conformal targeting of radiation to the tumor bed for patients with localized disease, specifically nonmetastatic medulloblastomas and ependymomas. This approach has been examined in the cooperative group context for medulloblastoma in the P9934 study, in which conformal radiation was administered in conjunction with pre- and postradiation chemotherapy and was well tolerated, given that radiation-induced cognitive or functional decline was not apparent. Outcome results with this approach appear to be superior to those from the prior P9233 study that used comparable chemotherapy, but did not administer focal radiation. Conformal radiation has also been applied without chemotherapy for patients older than 1 year who underwent gross-total resection of an intracranial ependymoma and with preradiation chemotherapy in patients who underwent subtotal resection of such tumors in the ACNS0121 and 0831 studies, based on favorable results with conformal radiation in a large institutional study.

A third general approach that has been pursued in children with medulloblastoma has involved the use of high-dose systemic and intraventricular methotrexate, which has been examined in the Hirntumor-Studie SKK92 protocol of the German Pediatric Oncology Group. With all 3 approaches, outcome results have been superior to those of prior cooperative group studies that have employed less intensive therapy.

In addition to intensification of therapy and the adoption of histology-specific treatment approaches, a further advancement that has contributed to an overall improvement in infant brain tumor management has been the refinement of tumor classification, which has encompassed new insights in molecular stratification. Until recently, malignant infant "blue cell" tumors were treated on fairly homogeneous therapeutic protocols that considered them all as "embryonal tumors" or PNET variants. During the last decade, it has been recognized that this group includes a number of prognostically distinct subsets that warrant different management approaches. One group now recognized to be an entirely separate entity encompasses the atypical teratoid/rhabdoid tumors, which char-
acteristically have mutation or inactivation of the \textit{INI1} gene.\textsuperscript{16} The immunohistochemical test for \textit{INI1} expression, supplemented by mutation analysis, has now been incorporated in the screening armamentarium to facilitate rapid identification of these tumors, some of which arise in the setting of germ-line alterations in the \textit{INI1} gene.\textsuperscript{16,46} The relevance of distinguishing these tumors from infant PNETs is that atypical teratoid/rhabdoid tumors have a substantially lower survival rate than PNETs with comparable therapy, often exhibiting rapid progression during and after initial chemotherapy.\textsuperscript{27} As a result, current treatment protocols for these tumors are evaluating alternative highly intensive chemotherapy approaches and, in some cases, examining the use of radiation therapy early in the treatment regimen. The latter approach is based on the observation that long-term survivors in previous studies have often received highly intensive multiagent chemotherapy, early radiation therapy, or a combination of the two modalities.\textsuperscript{9,20,113}

Contemporary studies of the European and North American cooperative groups for infants with medulloblastomas and other PNETs are also adapting therapy based on clinical and molecular prognostic features. For example, the P9934 study specifically focused on nonmetastatic medulloblastomas, which have a much more favorable prognosis than lesions with leptomeningeal spread at diagnosis.\textsuperscript{27} Analysis of the Hirntumor-Studie SKK series, the P9934 study, and other large cohorts of medulloblastomas have highlighted the fact that, even within the well-defined group of nonmetastatic infant medulloblastomas, there are distinctive molecular subsets that would likely benefit from individualized treatment approaches. In particular, tumors with desmoplastic histological features or extensive nodularity, which commonly exhibit \textit{PTCH1} mutations, appear to have a substantially better prognosis than tumors with classical histological features, and may warrant treatment approaches that minimize the risks of late sequelae.\textsuperscript{74} Conversely, the COG ACNS0334 study focuses on infants with metastatic medulloblastoma and supratentorial PNETs, which represent a particularly high-risk subset of tumors, and uses correspondingly more intensive chemotherapy to define whether further intensification of induction therapy with the use of methotrexate as per the Hirntumor-Studie SKK regimen is tolerable and can increase the percentage of children with complete tumor regression.

\section*{Conclusions}

Advancements in imaging technology, surgical techniques, strategies for precisely targeted radiation delivery, and chemotherapy regimens have led to improvements in outcome for children with several types of brain tumors, such as medulloblastoma. In addition, refinements in risk-adapted treatment planning based on the clinical and molecular features of these tumors offer the potential for reducing the morbidity of therapy while maintaining high rates of disease control. An emerging area of study that has particular relevance to children with prognostically favorable brain tumor subgroups involves strategies for toxicity remediation that can reverse the effects of radiation on cognitive function and enhance recovery from surgical morbidities, such as the posterior fossa syndrome.\textsuperscript{86,116,120} Pilot studies of various pharmacological approaches are currently in progress, and evaluating the success of these interventions will rely heavily on the implementation of validated, widely applicable testing batteries to assess the effects of these therapies. The development of improved functional imaging approaches, such as diffusion tensor imaging,\textsuperscript{48} may enhance the safety of tumor resections by helping in the selection of surgical trajectories that spare critical structures. Such modalities may also have utility when coupled with increasingly precise techniques for radiotherapy treatment planning and delivery to reduce unintended toxicity to normal structures.\textsuperscript{63–65} Advanced metabolic imaging techniques, such as MR spectroscopy and PET, are also undergoing examination for their utility in predicting tumor histology preoperatively and assessing treatment response,\textsuperscript{49,78} although further work will be needed to determine whether these adjuncts can reliably supplement the information provided by conventional MR imaging techniques.

Despite the therapeutic advances that have been achieved for some groups of childhood brain tumors, the prognosis for children with other types of tumors, such as diffuse intrinsic brainstem gliomas and other high-grade gliomas, remains suboptimal. The increasing implementation of molecular characterization approaches for these lesions, as well as the more favorable risk tumors, has already yielded some novel targets for molecularly directed therapy, and as more refined tools for interrogation of gene expression patterns and genomic alterations within tumors are applied, it is likely that additional targeted treatment options will be identified that offer the hope of improving patient outcome and, ultimately, tailoring therapy more precisely to the distinguishing features of a given tumor.

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Advances in the Management of Paediatric High-Grade Glioma

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Abstract High-grade gliomas represent a formidable challenge to paediatric oncologists. The second most common malignant brain tumour of childhood, little progress has been made in the outcome of these tumours in the last four decades. Outcome remains dire with less than 20 % of patients surviving. Current treatment consists of maximal resection and radiotherapy, with chemotherapy both in conjunction with and adjuvant to radiotherapy being added more recently. Yet much of the evidence for the use of chemotherapy is extrapolated from adult data, and evidence for its use in the paediatric population is weak. Recent advances in the biology of high-grade glioma in children identify epigenomic subgroups distinct from those seen in adults, suggesting a separate tumourigenic mechanism and delineating a separate disease entity. The time may be to move forward with a different way of thinking about paediatric tumours. With biological insight comes the promise of more effective therapies, rationally targeted towards the biology of the tumour. This review addresses the current advances in paediatric high-grade glioma and how we can move forward to translate this into improved outcomes for these patients.

Keywords Paediatrics · High-grade glioma · Brain tumour

Introduction

The term ‘high-grade glioma’ is an umbrella term used to include all high-grade malignancies of glial origin. The WHO definition includes grade III anaplastic astrocytoma (AA), anaplastic oligodendroglioma, anaplastic oligoastrocytoma, anaplastic ganglioglioma and anaplastic pilocytic astrocytoma and grade IV glioblastoma multiforme (GBM), giant cell glioblastoma and gliosarcoma [1]. By far, the most common are glioblastoma multiforme and anaplastic astrocytoma. Diffuse intrinsic pontine gliomas (DIPGs) are also classified within this group as high-grade gliomas of the brainstem. Although most commonly the histology of DIPG is GBM, reports on histological grading can vary and yet nearly all have a fatal outcome. As DIPGs are most commonly treated and managed differently to other hemispheric high-grade gliomas, they are not included within this review. Glioblastoma multiforme is the most common high-grade glioma and occurs most often in the adult population, with the incidence increasing with age and the highest occurrence between 75 and 85 years of age [2]. By contrast, it is rare in children where it represents 3 % of paediatric brain tumours. In the USA, the incidence of high-grade gliomas is 0.8 per 100,000, making up 14 % of all paediatric brain tumours [2]. Although they can occur anywhere within the CNS, high-grade gliomas are most frequent in the cerebral hemispheres. Within the childhood population, they most commonly occur in teenagers and young adults, but can occur at any age including in neonates and infants. The current standard management is maximal surgical resection followed by high-dose focal radiation. Although there is no standard practise, chemotherapy is becoming widely accepted as an adjuvant therapy both concurrently with and following radiotherapy. Unlike in the adult population where the use of chemotherapy is based on the outcome of large randomised trials, the evidence for its benefit in the paediatric age group is weak. The current 5-year overall survival rates in children with high-grade glioma are unacceptably low at less than 20 %.
babies, the devastating long-term effects of radiation have led to chemotherapy-based treatments as radiation-sparing or radiation-delaying approaches. The results appear to be more favourable than in older children despite a proportion avoiding radiation, attesting to the idea of a different biology in this age group. Recent advances defining the biology of high-grade glioma suggest distinct subgroups, which may provide new therapeutic targets. The knowledge of divergent biology in different subsets of patients may lead to more directed therapy in the future. In this review, we discuss the current evidence for the treatment of high-grade gliomas in children, the recent advances in the biology of this disease and how to move forward with improving outcomes for these children.

Current Management of Children With High-Grade Gliomas

Clinical Behaviour

High-grade glioma usually presents as a rapidly progressing mass lesion, commonly with symptoms caused by the location of the tumour. The majority occur in the supratentorial region although they can occur anywhere in the CNS, including the spinal cord. Common presentations are with headache, nausea and vomiting and symptoms of raised intracranial pressure or with seizures or focal neurological deficits [3]. Imaging often shows a heterogeneous mass with variable contrast enhancement and evidence of local brain invasion. Imaging characteristics are often indistinguishable from other malignant brain tumours, although magnetic resonance spectroscopy (MRS) may be able to differentiate between gliomas of different grades [4]. Dissemination is rarely seen, and less than 10 % of cases are metastatic at diagnosis, with the rate of metastasis increasing to approximately 25 % cases at recurrence [5, 6].

Prognostic Factors

There has been so far no attempt to stratify the treatment of high-grade gliomas in children based on risk. Primarily, this is because they remain collectively rare with an overall poor survival for which the treatment options are limited. Therefore, differentiation based on prognostic group has not been clinically relevant. The most validated prognostic factors within high-grade gliomas are grade and extent of resection. Prognosis is primarily and heavily dependent on the extent of surgical resection. However, resection is mostly site dependent and a complete resection is rarely achieved due to the infiltrative nature of the disease. The first study to confirm the prognostic impact on the extent of resection was the study of the Children’s Cancer Group CCG-945, which randomised patients to surgery, radiation and either standard or intensified chemotherapy. Progression-free (PFS) and overall survival (OS) were significantly improved in those achieving a >90 % resection compared to those who did not (PFS 35 % versus 17 %, \( p=0.006 \)) [7]. Other studies have since confirmed the survival advantage of a complete resection [8–10]. Although most protocols treat all variants of high-grade glioma uniformly, irrespective of histology or grade, there is evidence for improved survival in grade III tumours compared to grade IV tumours [9–12]. Other significant prognostic factors identified are p53 expression and MGMT methylation status. Tumours with p53 overexpression have been found to have a significantly worse outcome [13]. Likewise, overexpression of the DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT), which increases the resistance of tumour cells to alkylating agents such as temozolomide, is known to affect prognosis [14]. MGMT overexpression in paediatric high-grade gliomas has been associated with significantly reduced PFS [15••, 16]. However, data from adult studies suggest MGMT promoter methylation, which silences the gene, may only have an impact in particular subtypes of GBM [17].

Current Treatment

Since the first descriptions characterising malignant glioma in the 1920s by Bailey and Cushing, it has been recognised that these tumours cannot be cured by surgery alone [18]. From then, the mainstay of treatment has been surgery and radiation and, with this approach, survival rates by the 1970s were 17 % at 2 years in children with GBM, with a mean survival of 10.8 months [3]. Despite advances in neurosurgery and radiation and the addition of sometimes intensive chemotherapy, no real progress has been made in the survival of children with GBM and currently reported OS ranges remain less than 20 % [15••, 19]. There is contestable evidence for a benefit of chemotherapy in children with high-grade glioma. The randomised study CCG-943 showed a clear benefit for chemotherapy [12]. This randomised high-grade glioma patients to standard therapy of surgery and radiation versus surgery and radiation plus CCNU, vincristine and prednisolone. There was a significant survival advantage to those treated with the addition of chemotherapy (5-year OS 46 versus 17 %), and despite more AA in the chemotherapy group, chemotherapy appeared to benefit essentially GBM patients. The subsequent randomised CCG-945 study failed to show an advantage for the ‘8-in-1’ chemotherapy regimen over CCNU, vincristine and prednisolone [11]. Other trials comparing different chemotherapy regimens or with historical controls have since failed to show a significant benefit for chemotherapy for the majority of patients [19, 15••]. Part of the incongruity may be contamination of the earlier studies with lower-grade lesions [20]. Some subgroups however seem to show a benefit from chemotherapy. The HIT-GBM-2 study demonstrated a
significant survival advantage for patients who received chemotherapy following a gross total resection (5-year survival of 63 versus 17 %) [19]. Despite the lack of convincing evidence for the use of chemotherapy, in the majority of centres, the standard of care includes chemotherapy. Most commonly, this is currently temozolomide, concomitant to radiotherapy and following radiotherapy as adjuvant maintenance. The evidence for the use of temozolomide is mostly extrapolated from adult data, which have shown that the use of concomitant and adjuvant temozolomide benefits both PFS and OS without a significant compromise to the quality of life [21]. In children, a phase I study of temozolomide dosed 5 out of 28 days showed response in 2 out of 5 high-grade glioma patients [22]. Subsequent studies however failed to show a clear survival benefit for temozolomide [15, 23, 24]. In particular, the results of ACNS-0126, which compared temozolomide concomitant to radiotherapy and as adjuvant therapy to CCG-945 as a historical control, showed survival was less favourable than that of CCG-945 and equivalent to other studies (3-year OS 22 %), yet this regimen has been adopted most universally as the standard of care [15, 23, 24]. Part of the acceptance of this regimen must be the acknowledged ease of administration of the oral preparation of temozolomide, the relative little impact on the quality of life, the responses seen in some patients (Fig. 1) and the lack of other treatment options.

High-grade gliomas are highly vascular tumours, and preclinical evidence suggests a role for vascular endothelial growth factor (VEGF) in the pathogenesis of this disease [25]. Addition of the VEGF inhibitor bevacizumab to the treatment of adult high-grade glioma trials is controversial at best and failed to deliver an improved overall survival in newly diagnosed glioblastoma patients (reviewed in [26]). Likewise, reports of the use of bevacizumab in childhood high-grade glioma have been disappointing with little evidence for its benefit yet clearly documented, although mild, toxicity [27–30]. The current phase II randomised HERBY trial may provide the conclusive answer to the role of bevacizumab in paediatric high-grade glioma (http://public.ukcrn.org.uk/search/StudyDetail.aspx?StudyID=10175). Again, despite a lack of evidence, bevacizumab is a widely practised addition to the treatment of paediatric high-grade glioma, particularly in the relapsed setting.

The Management of Babies With High-Grade Glioma

High-grade glioma very rarely occurs in infancy. However, as many as 20 % of gliomas in children less than 1 and 7 % in children 1–3 years are high-grade lesions [9]. The exact incidence of high-grade glioma in young children is not known, but incidence rates as high as 1.0 per 100,000 have been reported in children less than 4 years [2]. As large rapidly progressing lesions, often in the cerebral hemispheres, babies anecdotally present acutely to the neurosurgeons following a catastrophic event and consequentially receive emergency surgery. Because of the malignant appearance of the tumour, surgery is often aggressive and, as a result, associated with significant morbidity. Evidence however suggests that prognosis in babies with high-grade glioma may be significantly more favourable than in older children. Specifically, there appears to be a subset of infant high-grade gliomas, which are sensitive to chemotherapy and therefore respond well when treated on so-called baby brain protocols. Series have described OS rates of 35–66 % with radiation-delaying or radiation-sparing approaches consisting of postoperative chemotherapy (see Table 1) [31–36]. Although most patients described did receive radiation, either routinely at the end of chemotherapy or on recurrence or progression, importantly there were small subsets of patients who did not receive radiation, either as part of the protocol or at parental request. This demonstrates that some young children can be cured with chemotherapy alone, which is in stark contrast to the experience from older children. Caution must be taken when prognosticating however, as the number of patients is small and therefore it is difficult to draw firm conclusions. Diagnosis of high-grade glioma in this age group can also be challenging, and results may be confounded by other lesions. Batra showed 39 % discordance between institutional and central pathology review, with the proposed diagnosis of high-grade glioma being revised to low-grade glioma in the majority of cases [31]. As the biology of brain tumours advances, more concise molecular diagnostic markers become available, such as INI1 negativity in atypical teratoid rhabdoid tumours (ATRT) and the C19MC amplicon in a subset of highly aggressive embryonal tumours in young infants [37]. Use of such methods to refine diagnostic criteria may more accurately define high-grade gliomas in infants, re-categorising patients currently diagnosed as high-grade glioma into other diagnostic groups, and hence, the clinical behaviour may become clearer.

Although these results can be taken as an indication only that high-grade gliomas in babies may behave differently and have a different response to treatment than in older children, it should be taken into account by clinicians when treating these patients,
particularly when considering aggressive surgery or radiation. Although aggressive surgery is usually recommended in the management of high-grade gliomas, this rule may not apply to infants. In the study of the Pediatric Oncology Group, POG 8632, extent of resection was not prognostic in infants with high-grade gliomas [32]. Minimal resection followed by second-look surgery after chemotherapy may be an alternative to aggressive initial debulking. In a recent report, Kotecha et al. described two infants with congenital glioblastomas in which a complete surgical resection could be achieved after cytoeuductive chemotherapy. In both patients, complete surgical resection at diagnosis was not possible due to tumour hypervascularity and significant blood loss [38].

What is urgently required is international collaboration to investigate the clinical behaviour and biology of these tumours and establish whether the perceived differences are accurate. As a substantial proportion of patients survive, efforts should also concentrate on the long-term outcomes of these patients in terms of assessing neuro-cognition and neurological morbidity.

### Biological Subgroups of High-Grade Glioma

Although historically homogenously classified based on morphology and mitosis, profiling of the transcriptome and epigenome of high-grade gliomas has defined distinct subgroups. Initial transcriptional arrays divided adult high-grade glioma first into three subgroups: proneural, mesenchymal and proliferative [39]. As well as clustering based on transcriptional profiles, these groups had distinct clinical features. In particular, deviant survival was seen, with the proneural group having a prolonged survival compared to the aggressive course of the mesenchymal and proliferative groups. The age of onset was also different with the proneural patients being younger than the other two groups. Different cells of origin were suggested based on the different stages of stem cell differentiation in neurogenesis. Other groups then suggested refinement into four subgroups (proneural, neural, classical and mesenchymal) with subgroup-specific mutations identified (proneural; PDGFR/IDH1, neural; none identified, classical; EGFR and mesenchymal; NF1) [40]. Again, the proneural group was notable for its young age and for the presence of PDGFR and IDH1 mutations. The survival advantage of this group was found to be specific to the presence of characteristic alterations in DNA promoter methylation, termed glioma CpG island methylator phenotype (G-CIMP) [17, 41]. G-CIMP positivity was associated with young age and IDH1 mutations. Methylation arrays again identified three subgroups enriched for the proneural, classical and mesenchymal gene expression subgroups [41]. Unlike in medulloblastoma, however, where methylation and gene expression clusters correlate, in high-grade glioma, methylation subgroups are distinctly different.
from the transcriptional subgroups, raising the question of which subgrouping method would be appropriate to use.

When global methylation profiles were done on adolescent and childhood high-grade glioma, tumours rarely clustered with the adults and two separate groups were identified [42••] (reviewed by Sturm and Pfister 2014 [43•]). These methylation profiles were found to be dependent on specific mutations in the histone complex H3F3A, specifically the H3F3A K27 mutation and H3F3A G34 mutations, which result in amino acid substitutions in the histone gene [44••, 45••]. Tumours with mutations in H3F3A at K27 or G34 form mutually exclusive groups which are also clinically distinct. G34 mutant tumours show a global reduction of DNA methylation. These tumours are usually seen in older adolescents or young adults and have an ‘ALT phenotype’, meaning they activate their alternative pathway of lengthening telomeres. Tumours occur in the cerebral hemispheres and harbour mutations of TP53, ATRX or DAXX [44••]. K27-mutated tumours, on the other hand, occur in the midline in 70–80% of cases and include DIgP and thalamic GBM. Twenty percent have activation of ACVR1, and no specific cytogenetic aberrations have been found. This subgroup has a dismal prognosis with reduced survival when compared to the G34 tumours, even when the reduced gross total resection rate of the centrally located K27 tumours was taken into account.

The other methylation clusters were termed ‘IDH1’, receptor tyrosine kinase 1 (‘RTK1’), ‘mesenchymal’ and ‘RTK2’, based on identified IDH1, PDGFR (RK1 group) and EGFR (RTK2 group) alterations and enrichment for the transcriptional proneural CIMP positive, proneural CIMP negative, mesenchymal and classical groups, respectively [42••]. Correlation of epigenomic subgroups with distinct age groups and location was also shown. Analysis demonstrated the midline location of H3F3A K27-mutated tumours compared to the almost exclusively cerebral hemisphere location of the other subtypes [42••]. Interestingly, although clearly occurring in distinct age groups, with IDH1 and RTK1 patients being young adults, mesenchymal tumours spanning all age groups and the RTK2 patients occurring in older adults, all groups except the RTK2 group included some paediatric patients [42••]. This is important as, although only accounting for a small proportion and hence tiny numbers of paediatric patients, these young patients in essentially adult subgroups may have identifiable targetable mutations for which drugs are currently available (see Fig. 2).

New Therapeutic Approaches and Future Challenges

The current approach to the management of paediatric high-grade glioma is rarely successful, and little real clinical progress has been made in the last decades. Increasing insight into the biology of this disease puts hope on the horizon and the prospect of novel therapies. However, this knowledge is currently in the realm of the scientists and needs to move to...
clinicians if it is to influence the outcome of patients. The challenge facing us is how to translate these molecular advances into meaningful results.

Defining the New Landscape of High-Grade Glioma

The first challenge is to prospectively validate high-grade glioma subgroups and characterise the new landscape of this disease. Firstly, biological subgrouping must become a clinical tool and patients categorised based on their tumour’s biological signature in real time. This calls for clinicians to set a high priority to obtaining tissue for diagnosis and molecular characterisation, irrespective of tumour location, and for scientists to come to a consensus on the definitions of subgroups, the best methods to use and how to make them universally accessible. Secondly, current knowledge should be interpreted in light of biology. Grade is currently felt to be of prognostic value yet it is unclear how to fit histological diagnosis and molecular diagnosis together and the relevance of each. Histone mutations appear to be relatively specific to GBM; however, the profiles of anaplastic astrocytoma are not as well defined. H3F3A K27 mutations have also been identified in some pilocytic

Table 2 Current targets of interest in paediatric HGG

<table>
<thead>
<tr>
<th>Target</th>
<th>Rational for use</th>
<th>Status in clinical investigation</th>
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<tbody>
<tr>
<td>EGFR</td>
<td>Infrequent mutations identified in paediatric population but frequent high expression of EGFR [46, 47]. Co-existing PDGFR mutations may be mechanism of resistance and co-inhibition may be required [46]</td>
<td>Erlotinib: no improvement in survival in phase II trial (unselected patients) [48]</td>
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<td></td>
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<td>Cetuximab: currently in phase II trial (NCT01012609)</td>
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<td></td>
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<td>Nimotuzumab: prolonged PFS and OS compared to historical controls [49, 50]</td>
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<td></td>
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<td>Gefitinib: phase I halted due to concerns regarding intratumoural bleeding [51]</td>
</tr>
<tr>
<td>PDGFR</td>
<td>PDGFRα amplification is the most frequent focal event in paediatric high-grade gliomas [52]</td>
<td>Imatinib: phase I trials completed, concern of intratumoural hemorrhage [53]. Crenolanib is currently in phase I trial (NCT01393912)</td>
</tr>
<tr>
<td>PTEN/Akt</td>
<td>Although PTEN mutations are infrequent in paediatric high-grade gliomas, Akt activation is a common occurrence and may be associated with a poor prognosis [54]</td>
<td>MK-2206: in phase I trials in children (NCT01231919)</td>
</tr>
<tr>
<td>BRAF^{V600E}</td>
<td>Mutations identified in 14 % paediatric high-grade glioma (11 of 78) [55]</td>
<td>Dabrafenib currently in phase I trial (NCT01677741)</td>
</tr>
<tr>
<td>IDH1</td>
<td>Mutations rare in children, more common in young adults, but do affect approximately 5 % paediatric GBMs [42••]</td>
<td>Inhibitors of mutated IDH1 are in pre-clinical development and induce differentiation of glioma cells [56]</td>
</tr>
<tr>
<td>ACVR1</td>
<td>20 % of H3F3A K27-mutated tumours have ACVR1 activation</td>
<td>Pathway may be targeted by TGFβ or BMP inhibitors which are in very early clinical development</td>
</tr>
<tr>
<td>TP53</td>
<td>33 % paediatric high-grade glioma have a p53 mutation which is associated with a poor prognosis [13]</td>
<td>No current clinically applicable agent available but hopes for future development</td>
</tr>
<tr>
<td>PARP</td>
<td>Potentiation of radiation and chemotherapy through inhibition of DNA repair mechanisms</td>
<td>ABT-888: phase I in combination with radiation and temozolomide completed (NCT00946335)</td>
</tr>
<tr>
<td>Histone deacetylase</td>
<td>Histone acetylases (HDAC) are known to modify histones and affect transcription and are upregulated in paediatric glioma [57]</td>
<td>Valproic acid: safety as anti-epileptic agent, part of HIT-GBM-2 trial which showed no survival benefit, currently in phase II trial with bevacizumab (NCT00879437) and with sorafenib and sildenafl (NCT01817751) [19, 58]</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>High vascularity, increased expression of VEGF</td>
<td>Verinostat: phase I in combination with temozolomide showed tolerability, currently in phase II trials [59], ongoing trial in combination with bevacizumab and temozolomide (NCT01236560)</td>
</tr>
<tr>
<td>Dentritic cells</td>
<td>Autologous dentritic cells primed with tumour protein to induce an immune response show promising survival in initial trials [60]</td>
<td>Bevacizumab: previous trials little efficacy. HERBY trial ongoing (EudraCT Number: 2010-022189-28)</td>
</tr>
<tr>
<td>Alternative method of telomerase lengthening</td>
<td>High-grade gliomas display a alternative lengthening of telomere (ALT) phenotype in Schwartzentruber [44••]</td>
<td>Currently in clinical trials (NCT01808820)</td>
</tr>
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</table>

Potential future target

Current status of open trials can be found at www.clinicaltrials.gov, clinicaltrials.gov identifier provided, accessed July 2014
astrocytomas, which may reflect diagnostic uncertainty [44••]. The relative importance of biology over histology needs to be clarified. By better defining our patient groups, we may be able to better tailor the treatment we already have. For instance, removing C19MC amplified INI1 negative tumours from the cohort of infant high-grade gliomas may show the chemo-sensitivity of these patients. Likewise, by subgrouping patients, it may be possible to determine which patients derive benefit from chemotherapy. In adults, the efficacy of treatment may be dependent on subgroup with the proneural group gaining the least benefit from intensive chemotherapy regimens [40] and MGMT DNA methylation status only predicting response to treatment in the classical subgroup of GBMs [17]. Outlining patients who do not have any benefit from chemotherapy would at least allow us to avoid toxicity and define which patients could be directed up-front to experimental treatments.

Implementing New Agents Into Clinical Trials

With the lack of efficacy of conventional chemotherapy agents, a number of targeted agents or new treatment approaches have been or are currently in trial for the treatment of high-grade glioma in children (see Table 2.). Some are promising and, although results only provisional and in the early phases of development, long-term survivors are seen [46] (reviewed in [47] and [48•]). Many however are based on biologically rational targets yet have failed to significantly impact on survival. Most agents have been tested on all patients, without specific target identification. The recent knowledge that distinct molecular subgroups exist confirm that ‘targets’ will only present in a proportion of patients. Trials treating all patients then are predetermined to failure as, even if drugs are 100 % successful in their targeted population, this proportion remains small. Such blind testing of drugs without biological correlation then is no longer acceptable if potentially useful and efficacious drugs are not to be discarded because of poor trial design. New models of trial design should be based on the ‘personalised medicine’ approach. Adult trials are already based on this approach where drugs are allocated based on the biology of that specific tumour [49•]. Prototype paediatric trials are also in the early phases where targeted agents are based on the biology of individual tumours (www.clinicaltrials.gov NCT02015728, NCT01182350, BIOMEDE trial [43•]). Irrespective of finding the right drug for the right target, other challenges face the clinical trialist. Subdividing already small patient numbers into specific narrow subgroups will require international collaboration for clinical trials to acquire sufficient numbers to illicit treatment differences. One target per subgroup may also not be enough as intratumoural heterogeneity and resistance mechanisms may require inhibition of more than one pathway, raising the challenges of effective scheduling and cumulative toxicities [50, 43•, 51].

Summary

After decades of despair for clinicians treating patients with high-grade glioma, recent advances into the epigenetic subgrouping of these tumors gives hope that there may be effective treatment in the future. The biology of this disease is still evolving however, and there is much to learn by putting the biology into the context of the clinical disease. To keep pace with advances in science and be ready to implement new treatments, clinicians should start thinking differently about high-grade glioma now. Biology needs to become part of our routine practise. We need to learn from our patients now, in the context of biology, even if it may be many steps before treatment is impacted. International collaboration and organisation is needed in order to provide the structure for future trials that will either have multiple arms, accounting for stratification based on biology, or be multi-national as the high-grade glioma population is fractionated into multiple subgroups. When new agents arrive, they should be tested in intelligently designed trials based on robust biological data. By acting now, we can lessen the gap between the science and the clinic and be prepared to bring these exciting advances to our patients sooner.

Compliance with Ethics Guidelines

Conflict of Interest Dr. Jenny Adamski, Dr. Uri Tabori and Dr. Eric Bouffet declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

** Of major importance


Molecular Insights into Pediatric Brain Tumors Have the Potential to Transform Therapy

Amar Gajjar1, Stefan M. Pfister2, Michael D. Taylor3, and Richard J. Gilbertson1,4

Abstract

High-throughput genomic technologies have shed light on the biologic heterogeneity of several pediatric brain tumors. The biology of the four common pediatric brain tumors—namely medulloblastoma; ependymoma; high-grade glioma (HGG), including diffuse intrinsic pontine glioma; and low-grade glioma—is highlighted in this CCR Focus article. The discovery that medulloblastoma consists of four different subgroups, namely WNT, SHH, Group 3, and Group 4, each with distinct clinical and molecular features, has affected the treatment of children with medulloblastoma. Prospective studies have documented the efficacy of SMO inhibitors in a subgroup of patients with SHH medulloblastoma. Efforts are ongoing to develop specific therapies for each of the subgroups of medulloblastoma. Similar efforts are being pursued for ependymoma, HGG, and diffuse intrinsic pontine glioma where the disease outcome for the latter two tumors has not changed over the past three decades despite several prospective clinical trials. Developing and testing targeted therapies based on this new understanding remains a major challenge to the pediatric neuro-oncology community. The focus of this review is to summarize the rapidly evolving understanding of the common pediatric brain tumors based on genome-wide analysis. These novel insights will add impetus to translating these laboratory-based discoveries to newer therapies for children diagnosed with these tumors.

See all articles in this CCR Focus section, "Discoveries, Challenges, and Progress in Primary Brain Tumors."

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Introduction

The ability to analyze tissues on a genome-wide scale has transformed our understanding of childhood brain tumors. Tumors once regarded as single entities have been shown to include multiple subgroups, each with distinct patterns of gene mutation and expression, clinical behavior, and in some cases, cellular origin (1–4). These advances have been made possible by technologies capable of cataloging the sequence, copy number, and expression of all genes. In addition to advancing understanding of the molecular basis of pediatric brain tumors, the use of these technologies across species has pinpointed cells in the developing nervous system that generate brain tumors (5). These studies have unmasked a common theme in pediatric brain tumors in which molecular discrete subtypes of each tumor likely arise from topographically discrete neural progenitor cells that are selectively susceptible to specific transforming mutations (2). This article highlights how these new approaches and concepts are informing understanding and treatment of the common types of pediatric brain tumors, which differ from therapeutic approaches in adult gliomas discussed in the other CCR Focus articles (6–8).

Medulloblastoma

The term “medulloblastoma” was originally used to describe all small round blue cell tumors of the cerebellum. Although histologic variants of medulloblastoma were recognized, for example, classic and nodular desmoplastic, the molecular basis of these variants was not known and all patients with the disease received the same treatment (9). The discovery of hSNF5/INI1 mutations in atypical teratoid/rhabdoid tumors (ATRT), provided the first firm evidence that not all tumors treated as medulloblastoma were the same disease (10). Indeed, infants with ATRT emerged as those with especially poor prognosis among large cohorts of patients previously regarded as having medulloblastoma (11). The development of high-quality antibodies to detect ATRTs by routine IHC equipped clinicians with a tool to reliably segregate ATRTs from medulloblastoma in routine clinical practice (12). Similar analyses have separated other small round blue cell tumors from medulloblastoma, including ETANTR (embryonal...
tumor with abnormal neuropil and true rosettes), medul-loepithelioma, and ependymoblastoma. ETANTR has been shown to harbor a recurrent amplified fusion between the embryonal gene \textit{TTHY1} and a primate-specific microRNA cluster on chromosome 19 (C19MC; ref. 13).

In addition to resolving medulloblastoma from other small round blue cell tumors, studies conducted over the past 10 years have identified distinct subtypes of medulloblastoma. These discoveries were made possible by genomic technologies, particularly those that measure gene expression. These studies have identified four main subgroups of medulloblastoma: WNT, SHH, Group 3, and Group 4 (Table 1; Fig. 1; ref. 14). Alternative approaches to genomics to diagnose medulloblastoma subgroups are in development, and are likely to prove increasingly important to clinical management in light of the significant prognostic and treatment differences among subgroups (15).

WNT medulloblastomas are typically diagnosed in older children and teenagers, rarely metastasize, and have an excellent prognosis with >95% event-free survival rates at 10 years in recent clinical trials (16, 17). About 80% of WNT medulloblastomas have mutations in the gene encoding $\beta$-catenin, and about 80% have a deletion of one copy of chromosome 6 (monosomy 6; ref. 1). The first mouse model of WNT medulloblastoma together with analyses of human MRI demonstrated that these tumors arise outside of the cerebellum from the embryonic lower rhombic lip (5). Subsequent studies of large numbers of human MRIs have confirmed this finding. Thus, WNT medulloblastoma represents a distinct disease form with excellent prognosis. Therefore, clinical trials enrolling these patients

| Table 1. Subgroups of common pediatric brain tumors with distinct molecular and clinical features |
|---------------------------------|---------------------------------|
| **Tumor** | **Molecular subgroup** | **Molecular and clinical features** |
| Medulloblastoma | WNT | Monosomy 6; nuclear $\beta$-catenin staining; CTNNB1 mutations in exon 3; 10% of patients; older age group; midline tumor location; excellent clinical outcome |
| | SHH | Heterogeneous molecular features depending on age of presentation; PTCH1, SMO, and SUFU mutations, GLI2 and MYCN amplification; germline TPS3 mutations; 30% of patients; cerebellar hemispheric location; intermediate prognosis except in infants who have a good prognosis |
| | Group 3 | MYC amplification; high incidence of metastatic disease; 25% of patients; male predominance; younger age group; poor prognosis |
| | Group 4 | i(17)q; MYCN amplification; 35% of patients; male predominance; intermediate prognosis |
| Ependymoma | C11orf95-RELA + RELA fusion transcripts; 70% supratentorial tumors |
| | CIMP + RELA fusion negative | 30% of supratentorial tumors |
| | CIMP + CIMP | CPG island methylator phenotype; chr 1 q gain; 80% of posterior fossa tumors; younger age group; male predominance; anaplastic histology; intermediate prognosis |
| | CIMP | Multiple chromosomal abnormalities; older age group; good prognosis |
| | Spinal cord tumors | <10% of pediatric tumors; NF2 mutation; myxopapillary histology; good prognosis |
| HGG | K27 | H3.1 and H3.3 K27 mutation; PDGFRA focal amplification; TP53 mutation; high proportion of pediatric patients; midline location; poor outcome |
| | G34 | H3.3 G34 mutation; TP53 mutation; hemispheric location; poor outcome |
| | IDH1 | IDH1 and TP53 mutation; frontal lobe location; rare in pediatric patients; intermediate outcome |
| | RTK1 | PDGFRA and EGFR focal amplification; CDKN2A and CDKN2B homozygous deletion; rare in pediatric patients; poor outcome |
| | Mesenchymal | NFI mutation; PDGFRA and EGFR focal amplification; CDKN2A and CDKN2B homozygous deletion |
| LGG | BRAF V600E | ~70% of pleomorphic xanthoastrocytoma (PXA); ganglioglioma (GG); diffuse astrocytoma |
| | KIAA–BRAF fusion; BRAF duplication | ~90% of pilocytic astrocytoma (PA) |
| | MYBL1 rearrangement; FGF11 duplication | High proportion of angiogenic glioma |
| | FGFR1 duplication | PA; diffuse astrocytoma; dysembryoplastic neuroepithelial tumor (DNET) |

Abbreviations: HGG, high-grade glioma; LGG, low-grade glioma.
are focused on deescalating radiotherapy and/or chemotherapy with the goal of maintaining excellent survival while diminishing long-term side effects. Ongoing efforts in the laboratory are seeking to generate additional new, relatively nontoxic treatments for these patients.

SHH medulloblastomas are emerging as an especially heterogeneous subgroup of medulloblastomas that affects patients from infancy to adulthood (18). The availability of drugs that target Smoothened, the main upstream activator of the SHH pathway, positioned this subtype as the first in which targeted therapies have been translated to the clinic (19, 20). Some forms of SHH medulloblastoma are especially challenging to treat, for example, teenagers with SHH tumors have a particularly poor prognosis. SHH medulloblastomas also appear to arise in patients with germline mutations in TP53 and evidence of shattering of the chromosomes (chromothripsis) in their tumors (21, 22).

Group 3 medulloblastoma arises exclusively in children, is frequently metastatic, and has the worst prognosis of all the subgroups (14). Consequently, there is considerable interest in developing new curative treatments for this disease. The development of a new mouse model of the disease has enabled the first high-throughput drug screens (HTDS) to identify new treatments of this subgroup (23). The FDA-approved drugs gemcitabine and pemetrexed are among the most promising first candidates and are now being tested prospectively in the SJMB12 clinical trial (NCT01878617; ref. 24). Developing molecular-based therapies of Group 3 medulloblastoma will be more challenging because these tumors contain genetic alterations that are difficult to target, for example, amplification of MYC, or the associated fusion gene PVT1–MYC (25). Group 4 medulloblastomas are the most common, but perhaps the least understood of the subgroups. The only subgroup-specific genetic event identified to date in this subgroup is tandem duplications of the Parkinson disease–associated gene SNCAIP on chromosome 5 (26–28). Mouse models of Group 4 medulloblastoma have not yet been developed, further hindering understanding of this common subtype.

Beyond the broad divisions provided by gene expression profiles, further studies have unmasked additional heterogeneity within subgroups, particularly high-risk SHH patients and an interesting group of relatively low-risk Group 3 patients. Next-generation sequencing and copy number studies of large numbers of patients have identified a disappointing number of highly recurrent events that could serve as therapeutic targets. However, patterns of gene mutation have emerged that may inform new treatment approaches, and in particular a convergence of mutations on genes controlling epigenetic processes (29). As epigenetic events are by definition reversible, this may offer a therapeutic window for the treatment of patients with medulloblastoma.

In addition to inter-tumoral heterogeneity, medulloblastomas display considerable intra-tumoral heterogeneity that may present even more of a challenge to those seeking to develop new treatments. Medulloblastoma metastases are genetically and biologically very different from their parent primary tumors (30). This observation has important implications for treatment because the vast majority of research and preclinical development focuses on the primary tumor. The fact that most Group 3 and Group 4 medulloblastomas recur metastatically rather than at the primary site strongly suggests that our drug
development strategies need to take into account differences between primary and secondary disease. If metastases are different from the primary tumor, novel therapies against the primary tumor may not increase survival rates.

**Ependymoma**

Ependymomas are tumors of the brain and spinal cord. Surgery and irradiation remain the mainstay of treatment of this disease because chemotherapy is ineffective in most patients (31). Consequently, ependymoma is incurable in up to 40% of cases. Histologic similarities among ependymomas have led investigators to treat these tumors as a single entity; however, recent genomic studies of gene expression and DNA copy number alterations have shown that ependymomas from different regions of the central nervous system (CNS) include discrete subtypes that display disparate prognoses, transcriptional profiles, and genetic

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**Figure 2.** Schematic presentation showing the role of epigenetic changes to the histone tails that influence gene expression. A, addition or removal of methyl group by methyltransferases leads to hyper- or hypomethylation of DNA, leading to gene repression or activation, respectively. B, a chromosome segment composed of compact DNA wrapped around octamers of core histones (nucleosomes) in which the DNA is inaccessible. C, epigenetic modification of the histone tails leads to unwinding of the DNA, rendering genes accessible for transcription. D, common posttranslational modifications occurring in the histone H3.3 tail that regulates gene transcription. In pediatric high-grade glioma, K27 and G34 are often mutated.
alterations, suggesting that they are different diseases. Recent data have documented that ependymoma comprises five distinct tumor subtypes—C11orf95-RELA–positive and –negative disease in the supratentorial compartment; CIMP (CG island methylator phenotype)-positive and CIMP-negative disease in the posterior fossa; and spinal cord tumors (Table 1; Fig. 2; refs. 32, 33). Spinal cord ependymomas in patients with NF2 are clinically distinct as they often have an indolent course following surgical resection alone and can be observed following resection of the tumor (34, 35). Thus, contemporary efforts to cure all patients with ependymoma must be concerned with understanding the biologic basis of these disease subtypes, and where necessary, developing subtype-specific therapies.

Initial cross-species genomic studies showed that ependymomas from different regions of the CNS share the gene expression profiles of neural stem cells (NSC) in the corresponding region of the developing brain and spine (2). These data provided the first explanation for the regional heterogeneity of ependymoma and identified regionally discrete NSCs as candidate cells of origin of the different disease subtypes. Support for this notion was provided by subsequent studies that demonstrated that EPHB2, a putative oncogene of supratentorial ependymoma, transformed forebrain, but not hindbrain or spinal NSCs, to generate ependymomas in mice. In addition to providing new insights into the origin and biology of ependymoma, this work established a new paradigm for understanding the molecular and cellular origins of cancer subtypes.

Building on the understanding that ependymoma comprises regionally discrete subtypes, investigators performed whole-genome sequencing of supratentorial and posterior fossa ependymomas with the aim of identifying driver mutations of these tumors (32, 33). Both studies found very few single nucleotide variations, insertion/deletions, or focal (<5 genes) copy number variations in ependymomas. However, Parker and colleagues noted that structural variations (SV) occurred significantly more frequently in supratentorial than other forms of the disease (32). Further analysis showed that these SVs clustered within a highly focal region of chromothripsis on chromosome 11q12.1-q13.3. This genomic disruption resulted in a novel translocation that fused a poorly characterized gene, C11orf95, to RELA, the principal effector of canonical NF-κB signaling in 70% of supratentorial, but no posterior fossa or spinal ependymomas, making it the most recurrent genetic alteration in ependymoma. Whole-genome sequencing demonstrated that splicing is required to generate the mature C11orf95-RELA transcript of which there are seven distinct variants. The most frequent includes exons 1–2 of C11orf95 and, except of the first two codons, the entire open reading frame of RELA. C11orf95-RELA–positive ependymomas also expressed high-levels of CCND1, a direct transcriptional target of NF-κB signaling, and L1CAM, which is associated with aberrant cell–cell adhesion, invasion, and NF-κB activation in tumors. Because IHC detected strong CCND1 and L1CAM expression only in C11orf95-RELA–positive formalin-fixed paraffin-embedded supratentorial ependymomas, these markers also afford a potential diagnostic test for translocation positive disease. Functional studies showed that the C11orf95-RELA fusion drives an aberrant NF-κB transcriptional program in mouse NSCs as well as highly penetrant brain tumors that recapitulated the “clear cell” and finely branched vasculature characteristic of “vascular-variant” human supratentorial ependymoma.

In light of the paucity of SNVs in ependymoma, Mack and colleagues studied the concept that posterior fossa ependymomas are driven by a dysregulated epigenome (33). Studying DNA methylation patterns in this group identified two distinct groups of posterior ependymomas: group A posterior fossa ependymomas, which have a much higher extent of CpG island methylation and exhibit a “CpG island methylator” or “CIMP” phenotype (PFA-CIMP⁺) ependymomas, and PFB CIMP-negative tumors. Interestingly, genes CpG methylated in PFA-CIMP⁺ ependymoma showed a remarkable convergence on genes documented as silenced in embryonic stem cells by the Polycomb repressive complex 2 (PRC2).

These data suggest that drugs that target DNA CpG methylation, PRC2/EZH2, and/or histone deacetylase inhibitors could represent the first rational strategies for therapy of PFA-CIMP⁺ ependymoma. Indeed, treatment of PFA-CIMP⁺ ependymoma, but not supratentorial ependymoma, with the tool compound 3-deazaneplanocin A (DZNep) that targets the PRC2 complex decreased expression of EZH2, decreased trimethylation of H3K27, and increased cleavage of PARP. Furthermore, in vivo treatment of human PFA-CIMP⁺ ependymoma xenografts with DZNep decreased tumor volume and improved survival.

In an effort to develop new treatments, investigators have performed HTDS of different ependymoma subtypes. One study developed an HTDS campaign that detects compound toxicity in dose response, against ependymoma cells, and NSC with high reproducibility and sensitiviy. The screening of the following was conducted: 3,161 bioactive compounds, 1,648 “orphan-kinase inhibitor scaffolds,” 367 inhibitors of specific kinases in Glaxo-SmithKline’s Published Kinase Inhibitor Set (GSK-PKIS), and 275 FDA-approved drugs. Of these compounds, 2.6% (n = 140/5,303) displayed anti-ependymoma activity, and pinpointed specific cell functions and pathways that are vulnerable to attack. Of particular note, C11orf95–RELA fusion–negative tumors were shown to be highly sensitive to 5-fluourouracil treatment, significantly prolonging the survival of mice with these tumors relative to control or conventionally treated (i.e., carboplatin, topotecan, or irinotecan) mice (36).

High-Grade Glioma, Including Diffuse Pontine Glioma

Glioblastoma, anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), gliomatosis cerebri, and diffuse intrinsic pontine glioma (DIPG) in children have...
historically comprised high-grade glioma (HGG) for the purpose of clinical studies. Biopsy results from DIPG and gliomatosis often document the tumor as being a grade 2 astrocytoma, but the clinical behavior of these tumors is similar to aggressive HGGs. As a group, these diseases are the least common malignant brain tumors in children. With a long-term survival rate of less than 10%, HGG remains one of very few incurable pediatric cancers (37). Current treatment protocols include maximal-safe surgical resection while maintaining functional integrity, radiotherapy, and a variety of chemotherapy options. Radiotherapy typically has a temporary effect on HGGs, and no chemotherapy has proven effective against it. The heterogeneity seen, in terms of survival of pediatric patients with HGG, has yet to be explained. However, some ongoing molecular studies suggest that tumors from long-term survivors might not actually be HGGs.

Mutations in the IDH1 gene are a hallmark genetic lesion in adult AA and AO, but they are exceedingly rare in children (38). The same holds true for 1p/19q deletion, the quasidiagnostic cytogenetic lesion in adult oligodendroglioma. In adult neuro-oncology, it has become evident that HGGs lacking either of these two hallmark aberrations have the same aggressive clinical behavior as glioblastoma, even without some morphologic features usually required for this diagnosis (e.g., necrosis and microvascular proliferation; ref. 39). Thus, we must now question whether AA and AO occur at all in the pediatric population. If so, they are so rare that their (presumably different) genetic makeup remains elusive. Gliomatosis cerebri is a particularly invasive and disseminating phenotype of HGG, and whether this HGG has a distinct underlying molecular cause is unknown. We focus here on glioblastoma and DIPG, which comprise the vast majority of pediatric HGGs.

In addition to the absence of hallmark alterations seen in adult AA and AO, common changes in adult glioblastoma (i.e., loss of chromosome 10, gain of chromosome 7, CDKN2A/B deletion, and EGFR amplification) are extremely rare in childhood HGG. However, some genetic alterations, such as PDGFRA or MET amplification and TP53 mutation, are found in both pediatric and adult HGGs. PDGFRA amplification, a potentially druggable lesion, is more common in pediatric HGG cohorts, and it seems to be more frequently observed in secondary glioblastoma after CNS radiotherapy for another indication (or in posttreatment primary HGG samples obtained at postmortem; ref. 40).

Next-generation sequencing studies published over the past 2 years have started to elucidate the unique mutational landscape of pediatric HGG. The most intriguing finding, so far, has been highly recurrent hotspot mutations affecting two distinct residues (K27 and G34) of the noncanonical histone gene H3F3A, as well as the canonical histone genes HIST1H3B and HIST1H3C (3, 41, 42). These studies have demonstrated a striking pattern of spatial heterogeneity, with K27M substitutions almost exclusively occurring in midline tumors (i.e., those in the thalamus, brainstem, or spine) and G34R/V substitutions restricted to hemispheric glioblastomas. Although G34 mutations typically co-occur with ATRX mutations and alternative lengthening of telomeres (a potential point of targeted interference), those mutations are less frequently coexpressed in tumors carrying K27M substitutions (43).

Several groups have shown that K27M mutations functionally inactivate PRC2 by entrapping EZH2, the main enzyme required to establish the repressive chromatin mark H3K27me3, thus conferring a dominant effect at the level of H3K27me on histones H3 and H3.3 (44). Both histone mutations are thought to arrest the respective cells of origin in a primitive progenitor state and may require additional genetic hits (e.g., TP53 mutation) to drive tumorigenesis and proliferation (Fig. 2). How to molecularly target these constellations of H3, TP53, and ATRX mutations remains to be determined and is the focus of several ongoing HTDS. Four recent DIPG studies have independently identified driver mutations in the ACVR1 gene, mostly in combination with HIST1H3B/C mutations (45–48). The mutated residues are similar to those in the developmental disorder fibrodysplasia ossificans progressiva, and targeted compounds are currently in preclinical development. In a distinct subgroup of midline tumors that mostly occur in the thalamus, investigators have identified recurrent FGFR1 hotspot mutations for which targeted compounds are in clinical trials in other disease entities. Finally, recurrent fusions involving NTRK1, NTRK2, or NTRK3 described in one recent study in a relevant subset of infant (non-brainstem) HGGs might also provide a promising drug target (46).

Attempts to classify HGGs into meaningful molecular subgroups based on gene-expression profiling have proven challenging. More recent work exploiting DNA-methylation fingerprints to guide subgroup affiliations across ages has shown more consistent results. In the pediatric setting, the four major methylation subgroups are highly enriched for the following: H3K27 mutation, H3G34 mutation, PDGFRA amplification (receptor tyrosine kinase subgroup 1), or none of the previous (mesenchymal subgroup; Table 1; Fig. 1). Methylation-based subgrouping can be performed using standard formalin-fixed, paraffin-embedded tumor samples; thus, it is also a feasible test for routine clinical application (49).

In summary, the genetic landscape of pediatric HGG differs greatly from that of the adult disease. Pediatric HGG is also very heterogeneous; the gross split between hemispheric tumors and midline tumors most likely indicates different cells of origin. It remains to be elucidated whether most long-term survivors of childhood HGG simply have a different molecular disease (e.g., low-grade glioma; LGG) and therefore may not require intensive radiotherapy and chemotherapy. The most important translational lesson learned from recent retrospective studies, however, is the value of routine biopsy of all HGGs, including DIPG (for patients enrolled on clinical protocols), the diagnosis of which has been typically based on radiologic findings alone. Without the molecular information gained from analyzing biopsy or autopsy samples (50), these discoveries would not
have been possible and, more importantly for the patients, decisions about novel therapeutic options (conventional as well as molecularly targeted) would remain a trial-and-error approach.

Although genetic mosaicism (i.e., different amplifications in different cells within the same tumor) and intratumoral heterogeneity in general seem to be more common in pediatric HGGs than in other pediatric malignancies, molecularly targeted therapies are worth further exploration. High-quality diagnostic specimens will be required as a basis for treatment stratification, regardless of whether the patient will receive a combination of novel drugs or one of targeted drugs and conventional chemotherapeutic agents. *H3F3A* and *HIST1H3B/C*-mutation status and molecular subgrouping should be considered standard diagnostic assays for pediatric HGG; *PDGFRA* and *MET* amplifications, *ATRX* status (by IHC analysis), *FGFR1* and *ACVR1* mutations, and *NTRK2* fusions would be the first candidates to be routinely assessed as potential molecular drug targets. Further work is required to elucidate the molecular features of the remaining proportion of cases of HGG that lack all of the alterations presented here, to establish predictive biomarkers or signatures for the rational use of available targeted drugs, and to understand how to target the epigenetic phenotype conferred by the histone mutations.

Low-Grade Glioma

Among the myriad of tumors that can arise in the developing brains of children, LGGs are the most common type. The World Health Organization has recognized two subtypes of childhood LGG, grade 1 and grade 2 tumors. However, using various approaches, independent laboratories have identified three major histologic classes, each comprising several subtypes of the disease: (i) astrocytic tumors, which consist of diffuse astrocytoma, fibrillary astrocytoma, pilocytic astrocytoma, and pleomorphic xanthoastrocytoma; (ii) oligodendroglial tumors, which include oligoastrocytoma and oligodendroglioma; and (iii) neuronal and mixed neuroglial tumors, which consist of ganglioglioma, angiocentric glioma, desmoplastic infantile tumors, and dysembryoplastic neuroepithelial tumors (51).

LGGs that arise in the cerebral hemispheres or the posterior fossa typically are not aggressive and are often cured via gross total resection. LGGs in the hypothalamus pose a greater clinical challenge because of disease and treatment-related morbidities (52). Chemotherapy is the main approach to treating young children with hypothalamic LGGs, and radiotherapy is reserved for those who experience treatment failure following chemotherapy (53–55). Although few, less-toxic approaches to treating LGG have been developed, next-generation sequencing technologies are enabling investigators to identify the genetic lesions expressed in these tumor cells, which hold promise as potential targets of novel therapies (Table 1; Fig. 3; ref. 56).

The most frequent genetic lesion in LGG is the gain of chromosome 7. This aberration has been identified by several laboratories and is especially common in pilocytic astrocytoma. In pediatric LGGs, the most common alteration is seen in the oncogene *BRAF*, specifically, a gain of the 7q34 region, which includes the *BRAF* locus. Duplication of *BRAF* is a common copy-number variation that occurs in tumors that originate in the cerebellum, hypothalamus, or optic chiasm (57–60). The 7q34 gain has been characterized as a *BRAF* duplication with a tandem insertion in the *KIAA1549* gene (61, 62). Fusion genes containing *BRAF* variants activate the MAPK signaling pathway; therefore, this pathway holds promise as a potential therapeutic option for pediatric LGGs. The *BRAF* V600E mutation, which also disrupts the MAPK pathway, is a common alteration seen in pediatric LGGs and several other cancers (e.g., leukemia, melanoma, and HGGs). The *BRAF* V600E mutation occurs most commonly in pleomorphic xanthoastrocytomas, gangliomas, diffuse astrocytomas, and pilomyxoid astrocytomas and is only rarely detected in pilocytic astrocytomas (63).

Tuberous sclerosis, a hereditary disorder in which benign tumors form in many organs, including the brain, is caused by mutations in two tumor-suppressor genes, *TSC1* and *TSC2*. Patients with tuberous sclerosis are at increased risk of LGG, which develops in 5% to 14% of patients. *TSC1* and *TSC2* are negative regulators of the mTOR pathway, which mediates cell proliferation; thus, mutations in *TSC1* and *TSC2* activate mTOR, thereby increasing a child’s predisposition to LGGs. mTOR inhibitors have demonstrated efficacy in subependymal giant cell astrocytomas and are now the standard of care for these tumors (64–67).

MYB gene mutations also occur in multiple types of LGG. *MYB* amplification occurs in diffuse astrocytomas, and focal deletions occur in angiocentric glioma. *MYB* expression is upregulated in the majority (60%) of diffuse LGGs and in a large portion (41%) of pilocytic astrocytomas. *MYBL1*, another member of the MYB family of proteins, is mutated in diffuse astrocytomas and angiocentric gliomas (4, 67, 68).

Whole-genome sequencing of large cohorts of pediatric LGGs has identified recurrent alterations in the fibroblast growth factor receptor type 1 gene, *FGFR1*. *FGFR1* N546K and K656E mutations occur in 5% of the supratentorial pilocytic astrocytomas (4, 69). Gene-expression analysis has revealed that *FGF2*, a ligand of *FGFR1*, is overexpressed in pilocytic astrocytomas, compared with the level expressed in other astrocytic tumors. This finding suggests that the FGF/FGFR pathway has a crucial function in tumorigenesis of LGGs in children. In addition, *FGFR1* mutations and duplication of its tyrosine kinase domain have been described in pilocytic astrocytomas, diffuse astrocytomas, and dysembryoplastic neuroepithelial tumors (4). Alterations of other MAPK members have also been described in pediatric LGGs. These include genomic alterations affecting the kinase domain of neurotrophic tyrosine kinase type 2 (NTRK2), which have been described in pediatric pilocytic astrocytomas (69). Finally, *KRAS*-activating mutations have been described in 3% to 5% of sporadic pilocytic astrocytomas (70, 71).
Current efforts to advance treatments for children with LGGs include testing of drugs that target mTOR, BRAF, or the MAPK pathway. The PI3K/Akt/mTOR intracellular signaling pathway has been implicated as an important promoter of tumor growth for many LGGs. Activation of the PI3K/Akt/mTOR pathway seems to play a central role in pediatric LGG pathogenesis. Several published studies demonstrate the PI3K/Akt/mTOR pathway activation in approximately 50% of pediatric LGGs based on phosphorylation of the mTORC1 targets S6 and 4E-BP1 and provide the preclinical rationale to test inhibitors of PI3K/Akt/mTOR for the treatment of pediatric LGGs. mTOR, lying downstream of PI3K, is an ideal target for pediatric LGG therapy.

Summary and Future Directions

Biologic insights have trigged a fundamental transformation in the landscape of pediatric brain tumors. This surge of new knowledge has the promise to radically transform the field over the next decade. A recent consensus meeting of neuropathologists has suggested a way to integrate the rapidly emerging molecular information into the diagnostic work up of brain tumors (72). Treatment strategies directed toward specific subtypes of brain tumors defined by...
histopathology and molecular diagnostics are being integrated in first-line clinical protocols. With the availability of genetically engineered mouse models and orthotopic xenografts being generated and widely available to research laboratories, there is an added impetus to find newer more effective and less toxic therapies, using high-throughput screening for some of the clinically aggressive tumors. The level of optimism in the pediatric neuro-oncology community is unprecedented, in anticipation that these recent discoveries will lead to a paradigm shift in the diagnosis and treatment of pediatric brain tumors.

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A. Gajjar is a consultant/advisory board member for AstraZeneca and Celgene. No potential conflicts of interest were disclosed by the other authors.

References


Medulloblastoma—translating discoveries from the bench to the bedside

Amar J. Gajjar and Giles W. Robinson

Abstract | Medulloblastoma is a form of brain cancer that mainly arises during infancy and childhood. Our understanding of this disease has transitioned rapidly; what was once thought of as a single disease entity is now known to be a compendium comprising at least four distinct subtypes of tumour (Wnt, sonic hedgehog [SHH], group 3, and group 4 medulloblastomas) that have characteristic molecular signatures, distinctive clinical features, and are associated with different outcomes. Importantly, medulloblastomas occurring in infants (aged up to 3 years) and adults have unique characteristics, which distinguish the disease from that seen in children aged >3 years. Accordingly, modern treatment approaches in medulloblastoma integrate the molecular and clinical features of the disease to enable provision of the most-effective therapies for each patient, and to reduce long-term sequelae. This Review discusses our current knowledge of medulloblastoma. In particular, we present the genetic and histological features, patient demographics, prognosis, and therapeutic options for each of the four molecular tumour subtypes that comprise this disease entity. In addition, the unique features of medulloblastoma in infants and in adults, as compared with childhood and/or adolescent forms, are described.

Introduction

Medulloblastoma is a disease that predominantly occurs in infants and children, and is the most-common type of paediatric malignant brain tumour, accounting for about 20% of all childhood brain cancers. Contemporary therapy for this disease consists of surgical resection, craniospinal irradiation, and chemotherapy. The use of these modalities in modern therapeutic protocols has resulted in a cure rate of approximately 70–75% among children aged ≥3 years. Current treatment protocols stratify patients into high-risk and average-risk groups according to the presence or absence of metastasis at diagnosis or of postoperative residual disease. This approach has effectively improved cure rates for patients with high-risk disease, and enabled treatment exposure (for example, craniospinal radiation doses) to be reduced in patients with average-risk disease, resulting in decreased toxicity. However, medulloblastoma survivors continue to pay a high price, in terms of long-term adverse sequelae, for cure. Deficits in neurocognitive and neuroendocrine function, hearing, fertility, cardiopulmonary fitness, and physical performance are some of the common effects of therapy. Furthermore, among survivors, rates of academic failure and unemployment are high, and quality of life is reduced.

A major drawback of the current risk-stratification scheme is that it fails to recognize the heterogeneity of medulloblastoma. Detection and categorization of this heterogeneity is of fundamental importance because a multitude of studies have shown that intrinsic differences in the molecular profiles of medulloblastomas result in substantial differences in disease manifestation and clinical outcome; therefore, to improve cure rates among patients with molecularly aggressive medulloblastoma and reduce treatment-related morbidities, a therapeutic strategy in which treatment is tailored to match these distinctions is needed. The intensity of therapy could be reduced in subpopulations of patients predicted to have good outcomes, resulting in a low prevalence of treatment-related morbidities, whereas patients with adverse prognostic features that were not apparent at clinical presentation could be allocated more-intensive therapy, which might improve overall survival. Patient groups for whom current maximal therapy is not sufficient could be treated with novel therapies, and patients harbouring highly specific genetic abnormalities could be treated with targeted agents specific to the aberrations detected.

The aim of this Review is to discuss our current knowledge of medulloblastoma. We outline the contemporary understanding of the molecular subtypes of medulloblastoma, with regard to genetic and histological features, patient demographics, probabilities of survival, and treatment. We also outline the unique features of medulloblastoma in infants (aged <3 years) and those in adults, versus the phenotype of medulloblastomas presenting in children and adolescents.

Medulloblastoma subtypes

Histological classification

Pathologists have long described medulloblastoma as a heterogeneous disease, on the basis of multiple consistently identified histological variants. The WHO
Armed with this knowledge, paediatric oncologists find themselves at an opportune moment to capitalize on these newly elucidated characteristics to improve survival and reduce morbidity by tailoring therapy towards the individual subtypes.

Classification of nervous system tumours lists ‘classic’ medulloblastoma and four histological variants of the disease: desmoplastic nodular (D/N), medulloblastoma with extensive nodularity (MBEN), anaplastic, and large cell. The four histological medulloblastoma variants can be grouped into two pairs with overlapping morphologies: desmoplastic tumours comprising D/N and MBEN variants; and large cell and anaplastic (LCA) tumours (Figure 1). The identification of medulloblastoma variants has clinical utility, as desmoplastic tumours are associated with a better outcome than classic or LCA medulloblastomas in infants, and LCA tumours are associated with a poorer outcome than classic or desmoplastic tumours in children aged ≥3 years. On the basis of these findings, two ongoing clinical trials are assigning therapy according to histological variants, in addition to clinical risk. This strategy recognizes the importance of thoroughly characterizing each tumour before initiating treatment, owing to intertumour variability, but it does not reflect the differences that exist within the same histological variants of medulloblastoma.

The current molecular classification

Our understanding of medulloblastoma biology has been substantially enhanced by high-throughput genomic and proteomic methods, such as transcriptomic and methylomic analyses. An early genomics study from 2006 demonstrated that medulloblastoma might consist of biologically distinct subgroups of tumours. The groups, which were distinguished by transcriptomic differences, exhibited intragroup similarities in mutation profiles, structural chromosomal alterations, histology, demographics, and clinical outcome. These initial data have been validated in several laboratories worldwide using large cohorts of patients with tumours and technologically more-advanced transcriptomic techniques than those used in the 2006 study. The result of these efforts is a consensus that medulloblastoma consists of four clinical and molecular subtypes of disease: the Wnt subtype, in which canonical Wnt signalling is upregulated; the sonic hedgehog (SHH) subtype, with hallmark activation of the SHH-signalling cascade; and ‘group 3’ and ‘group 4’ medulloblastomas.

The demographic, transcriptional, genetic, and clinical differences among these four disease subtypes have important clinical implications. The most clinically relevant finding is that prognosis differs markedly across tumour subgroups. The Wnt subtype has an amazingly high 5-year overall survival rate that can exceed 90% with the current standard therapy, which consists of maximal safe surgical resection of tumour, risk-adapted radiation therapy, and adjuvant chemotherapy. By contrast, group 3 tumours have a substantially worse prognosis, with 5-year overall survival ranging from 40–60%. The other two subgroups of medulloblastoma, the SHH subtype and group 4 tumours, have an intermediate overall survival rate at 5 years after treatment of around 75%, which varies according to the presence or absence of metastatic disease, molecular abnormalities, and histological category. A potential caveat of these data, is that the outcomes were derived from study protocols in which the patients were treated mainly according to clinical-risk features, rather than on the basis of molecular characteristics; indeed, these findings have led to the obvious conclusion that molecular subtyping should be used clinically to tailor therapy.

Wnt-subtype medulloblastoma

The rarest subtype of medulloblastoma is the Wnt subtype, which makes up only about 10% of all medulloblastoma diagnoses. Patients with this tumour subtype have the best prognosis of all the subtypes. Wnt-subtype tumours are typically uniform in their genetic aberrations, histological pattern, and clinical presentation.

Genetics

All medulloblastomas with nuclear accumulation of β-catenin are categorized as Wnt-subtype tumours. Nuclear β-catenin interacts with members of the transcription factor/lymphoid enhancer-binding factor (TCF/LEF) family of transcription factors to activate the canonical Wnt-signalling pathway. More than 90% of the Wnt-subtype medulloblastomas harbour mutations in CTNNB1, the gene that encodes β-catenin (Box 1). The resulting mutant β-catenin protein is resistant to degradation, leading to its accumulation in the cell nucleus. Wnt-subtype medulloblastomas also frequently have deletions of one copy of chromosome 6 (monosomy 6; Box 1), although some Wnt tumours retain two copies of this chromosome. Other than monosomy 6, Wnt-subtype medulloblastoma is associated with limited occurrence of gains and/or losses of chromosomal regions across the genome. Thus, monosomy 6, in conjunction with nuclear β-catenin accumulation, serves as a sensitive and highly specific marker for this subtype of disease.

Whole-genome sequencing (WGS) studies have identified recurrent mutations specific to this medulloblastoma subgroup. The most prevalently mutated genes, in addition to CTNNB1, are DDX3X, SMARCA4, TP53, KMT2D, CSNK2B, and CREBBP (Box 1). Many of these genes (that is, CREBBP, SMARCA4, and KMT2D) encode proteins that interact with nuclear β-catenin and remodel chromatin, suggesting that cooperative mutations occur in the development of this tumour subtype.
Histology
Most Wnt-subtype tumours have classic histological features of medulloblastoma. However, rare examples of Wnt-subtype medulloblastoma with LCA-variant histology have been reported (Box 1).

Patient demographics and outcomes
The male to female ratio for Wnt-subtype medulloblastoma is almost 1:1, with a slight female predominance. These tumours are most commonly found in older children and teenagers, and are rarely observed in infants (Figure 2; Box 1).

The tumours are typically located in the midline of the brain, occupying the fourth ventricle and infiltrating the brain stem. A mouse model that mimics the human disease has demonstrated that the cell of origin for Wnt medulloblastoma is located in the lower rhombic lip and fails to migrate normally after accumulating an oncogenic mutation. As introduced previously, Wnt-subtype medulloblastomas have a highly favourable outcome, with 5-year overall survival exceeding 90% in most studies in patients with average-risk disease (gross total resection and no metastatic disease).

Therapeutic options
On the basis of the good prognosis of Wnt-subtype medulloblastoma, this form of the disease clearly lends itself to a judicious reduction in therapy. Nevertheless, reduced-dose craniospinal radiation and/or reduced-intensity chemotherapy is warranted only for patients without metastatic disease, which accounts for around 90–95% of Wnt-subtype tumour diagnoses (Box 1). Furthermore, testing of these alterations to standard treatment is recommended only in patients who have been carefully subtyped in a central reference laboratory and enrolled on approved clinical protocols, with close monitoring to document treatment outcome.

SHH-subtype medulloblastoma
SHH-subtype medulloblastomas constitute about 30% of all medulloblastoma diagnoses. Patients with this tumour type have a 5-year overall survival rate of approximately 75% when treated with current standard therapy. Unlike Wnt-subtype medulloblastomas, tumours characterized by activation of SHH signalling are associated with a variety of genetic aberrations, histological features, and clinical presentations (Box 2). The heterogeneity within the SHH medulloblastoma subtype is related to patient age at diagnosis and the underlying genetic alterations. Consequently, risk classification of these tumours is essential to determining prognosis and, therefore, treatment strategies.

Genetics
Activation of the SHH-signalling pathway was first linked with medulloblastoma as a result of the finding that patients with Gorlin syndrome were found to have a strong predisposition to the disease. These patients have a high prevalence of basal-cell carcinoma and developmental anomalies that are caused by germline mutations in the PTCH1 tumour-suppressor gene. This gene encodes protein patched homologue 1 (PTC1), which is a receptor for SHH and other hedgehog homologues. In the absence of ligand occupancy, PTC1 interacts with and prevents smoothened homologue (SMO) function, thus acting as a negative regulator of the SHH-signalling cascade, a pathway that has many functions in normal development. Normal cerebellar development is highly reliant on SHH signalling; however, unrestrained SHH activity can lead to neoplasia. Indeed, the integral role of this signalling pathway in cerebellar development became apparent when engineered...
Box 1 | Clinical and genomic features of Wnt-subtype medulloblastoma*

**Clinical features**
- Proportion of all medulloblastomas: ~10%
- Gender ratio (male:female): ~1:1
- Incidence: most common in older children and adolescents (median age ~10 years); rare in infants (aged <3 years)
- Histology: classic; very rare cases of large cell/anaplastic
- Proposed cell of origin: lower rhombic lip progenitor cells
- MRI location: 4th ventricular; infiltrating brainstem
- Prevalence of metastasis at diagnosis: ~5–10%
- 5-year overall survival rate: ~95%

**Genomic features**
- Expression signature: Wnt signalling
- Chromosomal gains and losses: monosomy of chromosome 6
- Driver genes*: CTNNB1 (90.6%); DDX3X (50%); SMARCA4 (26.3%); TP53 (13.5%); KMT2D (12.4%)

*Subgroup frequency, demographics, clinical features and cytogenetic aberrations were derived from a cohort of 827 medulloblastomas distributed into subgroups described by Northcott et al.26

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**Figure 2** | Schematic distribution of the prevalence of the molecular medulloblastoma subtypes among different age groups. The approximate frequency of each of the four molecular subtypes of medulloblastoma (Wnt, SHH, group 3 and group 4) according to age at diagnosis is shown schematically as a proportion of total medulloblastoma occurrence in each age group, based on data from 827 medulloblastomas that were distributed into these molecular subgroups by Northcott et al.26 The data are stratified into medulloblastomas occurring in infants (aged <3 years), children (aged >3 years) and adults (aged >16 years). Wnt-subtype tumours are rarely observed in infants, but make up a similar proportion of medulloblastoma cases in children and adults. SHH-subtype tumours have a unique bimodal distribution, and occur most commonly in infants and adults, whereas group 4 medulloblastomas are most frequently seen in children—and are the predominant form of medulloblastoma in this age group. The peak age of onset for group 3 tumours is in late infancy/early childhood; this medulloblastoma subtype is almost never observed in adults. **Abbreviation: SHH, sonic hedgehog (protein).**

**PTCH1** deletions in mice were demonstrated to result in medulloblastoma;41 this model represents a potentially valuable tool for investigating multiple facets of SHH-subtype medulloblastoma.

**PTCH1** mutations have been reported in 25–30% of SHH-subtype medulloblastomas, making it the most-prevalent mutation of the subgroup (Box 2).31

Interestingly, most of these mutations occur sporadically in patients without Gorlin syndrome. Other genetic anomalies in the SHH pathway that have been detected in this medulloblastoma subtype include mutations in *SMO* and *SUFU* (encoding suppressor of fused homologue, another suppressor of SHH signalling), as well as amplification of *SHH*, and the transcription factors *GLI2* and *MYCN*,34–36 confirming the link between this type of medulloblastoma and the SHH pathway. The prevalence of these genetic anomalies is associated with age at diagnosis. Most infant medulloblastomas carry *PTCH1* or *SUFU* mutations, which are present in the patient’s germine in a number of cases.32 Adult SHH-subtype medulloblastomas are characterized by *PTCH1* and *SMO* mutations.42 In children, however, SHH-subtype tumours display broader molecular heterogeneity, with amplifications of *SHH*, *GLI2*, and *MYCN*, as well as somatic and germine *TP53* mutations being observed together with *PTCH1* mutations.42 This pattern of genetic anomalies in the SHH pathway is important as it might predict disease response to treatment with SMO inhibitors.36,42,43

In addition, WGS studies of SHH-subtype medulloblastoma have reported recurrent somatic mutations in genes that are seemingly unrelated to the SHH pathway. These include KMT2D, *TP53*, *BCOR*, *DDX3X*, *LDB1*, and *GABRG1*.34–36 Thus, similar to Wnt-subtype medulloblastoma, recurrent mutation of genes encoding chromatin remodelling proteins—KMT2D and BCOR—features strongly in the SHH medulloblastoma subtype (Box 2); this finding warrants further investigation.

Copy-number variation studies have described some intriguing chromosomal structure alterations, as well as focal aberrations, that are characteristic of SHH-subtype medulloblastoma. In particular, broad chromosomal losses of 9q, 10q, and 17p have been described (Box 2).44,45 Focal events that include loss of *PTEN* and amplification of genes involved in insulin-like growth factor signalling implicate increased PI3K activity in development of some tumours within this subtype.34,44

**Histology**

Several case series have shown that D/N medulloblastomas belong predominantly to the SHH-subtype of disease; similarly, MBENs are almost exclusive classified as this subtype.17,30 However, not all SHH-subtype medulloblastomas have D/N or MBEN histology; the remaining tumours have either classic medulloblastoma or LCA histological features. Indeed, the SHH subtype is the only subgroup of medulloblastoma with a considerable representation of all medulloblastoma histological phenotypes (classic, D/N, MBEN and LCA; Box 2).28,32

**Patient demographics and outcomes**

SHH and Wnt subtypes of medulloblastoma are similar in that the disease is observed at comparably frequency in males and females, although SHH tumours have a slight male predominance among infants (Box 2).28 Cerebellar hemispheric tumour location is almost exclusively predominant to the SHH subtype, although some SHH-type tumours do arise in the
**Box 2 | Clinical and genomic features of SHH-subtype medulloblastoma***

**Clinical features**

- Proportion of all medulloblastomas: ~30%
- Gender ratio (male:female): ~1.5:1
- Incidence: bimodal—first peak in infants and young children (~5 years of age); second peak in older adolescents and adults (aged >16 years); less common in children aged 5–16 years
- Histology: classic > desmoplastic nodular > LCA > medulloblastoma with extensive nodularity
- Proposed cell of origin: cerebellar granule-neuron precursor cells of the external granule-cell layer and cochlear nucleus; neural stem cells of the subventricular zone
- MRI location: cerebellar hemispheres and rarely midline
- Prevalence of metastasis at diagnosis: ~15%–20%
- 5-year overall survival rate: ~75%

**Genomic features**

- Expression signature: SHH signalling
- Chromosomal gains and losses: frequent loss of 9q, 10q, and 17p; gain of 3q and 9p
- Driver genes*: PTCH1 (28%); TP53 (13.6%); KMT2D (12.9%); DDX3X (11.7%); MYCN amplification (8.2%); BCOR (8%); LDB1 (6.9%); TCF4 (5.5%); GLI2 amplification (5.2%)

*Subgroup frequency, demographics, clinical features and cytogenetic aberrations were derived from a cohort of 827 medulloblastomas distributed into subgroups described by Northcott et al.26 Driver genes are determined by the relative frequency of mutations or significant copy-number aberrations in medulloblastomas that were distributed into either Wnt, SHH, group 3 or group 4 molecular subtypes, as described in recent medulloblastoma sequencing and copy number studies.25,26,28,31,34–36 Abbreviations: LCA, large cell/anaplastic; SHH, sonic hedgehog (protein).

Midline of the brain. Metastatic disease at diagnosis is also relatively uncommon, occurring in less than 25% of cases.17,31,32 In contrast with other medulloblastoma subtypes, SHH-subtype tumours have a unique bimodal pattern in incidence, with most cases involving either infants younger than 3 years of age or older adolescents and adults (Figure 2; Box 2). In general, the 5-year overall survival rate in patients with SHH medulloblastoma is about 75%. However, this outcome probably reflects the heterogeneity within the subgroup; more-detailed analyses of risk factors have documented that outcomes differ according to patient age, histological subtype, presence of metastasis at diagnosis, and underlying molecular abnormalities.17,30,47

**Therapeutic options**

Although a good outcome is achieved for most patients with SHH-subtype medulloblastoma using current therapies, improved and refined therapeutic strategies are needed for this subtype. In this regard, the challenge lies in our ability to identify diversity within this subgroup of patients and tailor therapy accordingly. Treatment of infants with D/N disease has demonstrated that certain SHH tumours can be cured without radiotherapy, whereas other forms of the disease, such as tumours with MYCN and GLI2 amplifications, carry such a dismal prognosis that treatment even with high-dose craniospinal radiation and adjuvant chemotherapy is rarely curative.45

SMO inhibitor therapy (with agents such as vismodegib and erismodegib) have been associated with a marked but relatively short-lived response in patients with recurrent SHH-subtype medulloblastoma.48,49 Prolonged exposure to these drugs induces a mutation in SMO that changes the structure of the protein and prevents binding of the drug.50 Unfortunately, only tumours with either PTCH1 or certain SMO mutations are predicted to respond to this form of intervention, and although these mutations have been documented in the majority of adult patients with medulloblastoma, they occur in only about half of paediatric patients with the disease.52 Trials are currently underway to evaluate the use of SMO inhibitors after surgery, irradiation, and modified adjuvant chemotherapy.51

Owing to the limited applicability of SMO inhibitors in the treatment of medulloblastomas with activation of the SHH pathway, novel strategies are needed for patients who have SUFU mutations, deletion of PTEN, and amplifications of GLI2 and MYCN; these strategies could potentially include epigenetic modifiers and PI3K–AKT inhibitors. A small group of patients with SHH-subtype medulloblastoma have large cell histology, MYCN amplification, and germline TP53 mutations. Such patients need to be identified and potentially offered novel therapeutic strategies, as they have been documented to have poor prognosis and often have Li–Fraumeni syndrome.62 International collaboration will be imperative to driving therapeutic advances through well-planned clinical protocols.

**Group 3 medulloblastoma**

Group 3 medulloblastoma accounts for around 25% of all medulloblastoma diagnoses. Patients with group 3 disease have the worst outcome among all the medulloblastoma subgroups due to the high frequency of adverse prognostic features, namely younger age at diagnosis, high prevalence of metastatic disease at diagnosis, LCA histology, and MYC amplification.38

**Genetics**

No germline mutations that predispose children to group 3 medulloblastoma have been described, and recurrent somatic genomic aberrations have only recently been reported.22,24–36,41 These aberrations include amplifications of MYC and OTX2 (Box 3); mutations in the chromatin remodelling proteins encoded by SMARCA4, KMT2D, and CHD7; and a variety of mutations in the lysine-specific demethylase (KDM) gene family (KDM6A, KDM3A, KDM4C, KDM5B, and KDM7A).31,34 MYC amplicons are highly prognostic and mutually exclusive from OTX2 amplicons, suggesting similar but prognostically distinct pathways to neoplasia in group 3 medulloblastoma.44 Frustratingly, >50% of group 3 tumours do not harbour any of these genetic aberrations, but rather have widespread chromosomal structural alterations (such as copy-number gains in 1q and 7, and losses in 10q and 16q).31 Although the role of these structural variations in the pathogenesis of medulloblastoma remains poorly understood, a study published in 2014 provided a potential solution to this conundrum. Therein, recurrent structural variations common to both group 3 and group 4 tumours were identified to reposition active
enhancer regions in close proximity to the known oncogenes \textit{GFI1} or \textit{GFI1B}, thus serving to aberrantly activate oncogene expression within these tumours.\textsuperscript{35}

**Histology**

Only two histological subclasses of medulloblastoma are seen in group 3 tumours: classic and LCA (Box 3). Group 3 tumours harbour the highest prevalence of the LCA histology of all medulloblastoma subtypes—as high as 40%.\textsuperscript{17,31} D/N histology, on the other hand, is almost never seen in group 3 tumours.\textsuperscript{17,31}

**Patient demographics and outcomes**

Group 3 tumours have a male predominance, being generally twice as common in males than in females (Box 3). Group 3 tumours present predominantly in infants and children, rarely in teenagers and not in adults (Figure 2).\textsuperscript{25} Metastatic disease is present at diagnosis in approximately 40–45% of patients. The higher prevalence of \textit{MYC} amplification, metastatic disease, and LCA histology impart the extremely poor prognosis in group 3 medulloblastoma. In fact, in a retrospective meta-analysis published in 2012,\textsuperscript{17} group 3 tumours were associated with the worst outcomes of all the medulloblastoma subtypes among all patient age groups: the 5-year and 10-year overall survival rates in infants with group 3 tumours were 45% and 39%, respectively; in children, the rates were 58% and 50%, respectively.\textsuperscript{17,31}

**Therapeutic options**

Group 3 medulloblastoma is the most challenging form of the disease to treat, and as is evident from the outcomes presented, the current therapeutic strategies are often ineffective. Several groups are conducting preclinical studies in mouse models that mimic the human disease in order to discover effective therapies for this aggressive subtype.\textsuperscript{33,55} Two FDA-approved compounds, gemcitabine and pemetrexed, were identified by a high-throughput screen as potentially having efficacy against \textit{MYC}-overexpressing or \textit{MYC}-amplified medulloblastoma.\textsuperscript{55} Another therapeutic strategy with promise in group 3 medulloblastoma is the use of BET bromodomain inhibitors, which interfere with \textit{MYC}-associated transcriptional activity.\textsuperscript{56} Carefully planned prospective clinical studies will be needed to determine if these or other agents identified in preclinical models in the future improve the outcome of patients with group 3 medulloblastoma.

**Group 4 medulloblastoma**

Group 4 medulloblastoma is the most-prevalent subtype of this disease and accounts for as many as 35% of medulloblastoma diagnoses (Box 4).\textsuperscript{17} Group 4 tumours are seen in all age groups and chromosome 17 abnormalities, although not exclusive to group 4 tumours, are the molecular hallmark of this subgroup. Overall, patients with group 4 medulloblastoma have an intermediate prognosis among the medulloblastoma subtypes when treated with standard therapy.\textsuperscript{28}

**Genetics**

Although group 4 medulloblastoma is the most common of the medulloblastoma subgroups, the underlying biology of this form of the disease is not well understood.\textsuperscript{28} No familial syndromes predispose an individual to group 4 medulloblastoma, and no murine model of the disease has been generated. Genes that are recurrently mutated or altered in copy number overlap with those associated with group 3 medulloblastomas. These are mutations affecting the KDM family members, \textit{OTX2} ampiclons, \textit{DDX31} deletions, \textit{CHD7} mutations, activation of \textit{GFI1}/\textit{GFI1B} expression, and \textit{KMT2D} and \textit{KMT2C} mutations. Unlike group 3 tumours, however, preferential amplification of \textit{MYCN} rather than \textit{MYC} is observed in group 4 tumours.\textsuperscript{31,34–36,44}
The most frequent focal somatic copy-number aberration, which occurs in 10% of patients with group 4 tumours, is a single-copy gain on chromosome 5q23.2, focused on the SNCAIP gene (Box 4).31,44 SNCAIP encodes synphilin-1, which binds to α-synuclein to promote the formation of Lewy bodies in the brains of patients with Parkinson disease; however, a connection between SNCAIP function and medulloblastoma has not been established.31,44

The most frequent mutation seen in group 4 medulloblastomas occurs in the KDM6A gene (Box 4).31,34–36 KDM6A is a demethylase enzyme that regulates the methylation of lysine-27 of histone H3 (H3K27). H3K27 represents a histone lysine residue that characteristically retains a trimethylated state in stem cells. Therefore, by preventing H3K27 demethylation, this mutation might preserve or initiate a stem-cell-like state in tumour cells. All the more intriguing is that KDM6A is found on the X chromosome and, although a homologue exists on the Y chromosome, KDM6A seems to be more-frequently mutated in boys with medulloblastoma, compared with girls with the disease. These mutations and others on the X chromosome (such as ZMYM3) might explain the observed male predominance of this medulloblastoma subtype.34

Another commonly identified aberration in group 4 tumours is isochromosome 17q, which is formed when the p-arm of chromosome 17 is lost and is replaced by the q-arm of the same chromosome—generating a chromosome with two 17q arms. Although some group 3 tumours also exhibit this genetic abnormality, isochromosome 17q occurs at a much higher frequency in group 4 medulloblastomas, and is rarely seen in tumours of the Wnt or SHH subtypes. Similar to group 3 tumours, many group 4 tumours display none of the aforementioned mutations or focal copy-number alterations, and continued studies are needed to elucidate the pathogenesis of this medulloblastoma subtype.17,26,31,34,44

Histology
As in group 3 tumours, the classic and LCA histology are the only histological subclasses regularly seen in group 4 medulloblastoma.17 Furthermore, the frequency of LCA is substantially lower in group 4 tumours than among group 3 medulloblastomas.17 A D/N histology has been documented for some group 4 tumours;17 however, this finding probably reflects incorrect categorization rather than actual histological features associated with the group 4 subtype of medulloblastoma.

Patient demographics and outcomes
Group 4 medulloblastomas have the most skewed distribution, according to gender, with the disease occurring three times more often in males than in females. This ratio is seen across all age groups, although group 4 tumours rarely arise in infants (Figure 2; Box 4).17,28,32 This subgroup accounts for 40–45% of childhood medulloblastomas and 25% of adult medulloblastomas.17,26,47 Metastatic disease at diagnosis is reported in approximately 35–40% of patients with group 4 tumours.17,31

Patients with average-risk group 4 medulloblastoma, as determined by gross-total surgical resection of the tumour and the absence of metastatic disease, have a 5-year overall survival rate that exceeds 80%.17,45 Patients with high-risk disease (that is, metastatic disease or tumours with a LCA histology) have an inferior prognosis: 5-year overall survival is observed in approximately 60% of these patients. A retrospective study identified a molecularly defined subgroup of patients within the average-risk group 4 population in whom a cure rate >90% might be achievable; however, these data await confirmation in a prospectively treated patient cohort.

Therapeutic options
As described, current standard therapy cures a high proportion of patients with average-risk group 4 medulloblastoma. Whether there is an opportunity to decrease the intensity of therapy for a subgroup of these patients who might have a particular high cure rate will emerge as findings from the next generation of clinical trials are published. As the stratification of patients according to molecular characteristics in the newest clinical cohorts will more-clearly identify the tumour subtypes and their intricacies, we anticipate that outcomes among these subgroups will be better determined and thereby enable rational reductions in therapy in the future.

The absence of a preclinical murine model that mimics the human group 4 medulloblastoma has hampered the development of novel approaches to treat patients with high-risk disease who experience poor outcomes with standard therapy. The molecular overlap between group 3 and group 4 medulloblastomas implies that drugs that are effective in group 3 medulloblastoma might also be effective in group 4 tumours that have adverse clinical and molecular features.

Unique aspects of medulloblastoma
Infant medulloblastomas
The SHH subgroup is the most-common form of the disease in infants, accounting for more than half of all medulloblastomas observed in this age group;17 group 3 tumours, which constitute around 30–40% of infantile medulloblastoma, are the next most-frequent form.17 Group 4 tumours comprise the small remaining fraction of cases, as Wnt-subtype medulloblastomas are virtually nonexistent in infants.17 Most infants with SHH-subtype medulloblastoma have tumours with a D/N or MBEN histology and favourable molecular characteristics (that is, somatic or germline mutations in PTCH1), suggesting that the disease might be curable with chemotherapy alone. Very few infantile medulloblastomas harbour the MYCN or GLI2 amplification or germline TP53 mutations that are characteristic of more-aggressive SHH tumours and are observed more frequently in SHH-subtype medulloblastomas in children aged >3 years.42 Indeed, an international meta-analysis of prognostic factors in 270 patients younger than 5 years of age demonstrated that infant medulloblastoma with desmoplastic (D/N or MBEN) histology has an excellent prognosis and can be usually cured with postoperative
chemotherapy alone. Moreover, several prospective studies have reported superior survival for infants with this subtype of medulloblastoma.

To protect infants from the toxicity of whole-brain irradiation, investigators have used several treatment modalities in attempts to improve the survival of infants with adverse prognostic features, such as tumours with classic or LCA histology, metastatic disease at diagnosis, and non-SHH-subtype medulloblastoma. These approaches include using focal radiation therapy, and high-dose myeloablative chemotherapy with stem-cell rescue, but the cure rate among these infants remains vastly inferior to that achieved in infants with SHH tumours. Nevertheless, biological-risk features (such as histology and molecular phenotypes) were not integrated into the staging criteria used in the previous generation of clinical trial protocols; hence, clinically average-risk patients with high-risk biological (histological or molecular) characteristics might have been undertreated and account for the inferior outcomes observed in these studies. Introduction of biologically and clinically staged patients in study protocols that include novel chemotherapeutic strategies that are not toxic to the developing brain and organs will be necessary to improve the outcome of infants with medulloblastoma.

Adult medulloblastomas
The lack of prospective clinical trials in adult patients with medulloblastoma has limited the documentation of the clinical features, molecular characteristics, and outcome of the disease in this age group. However, the available literature indicates that SHH-subtype tumours account for 57% of adult medulloblastoma diagnoses. The remaining adult medulloblastomas consist of the Wnt-subtype (13%) and group 4 (28%) tumours; group 3 medulloblastoma is particularly rare in adults (comprising around 2% of adult medulloblastoma diagnoses). Both Wnt-subtype and group 4 tumours in adults are associated with worse outcomes than their paediatric counterparts, whereas the SHH-subgroup adult tumours have a similar outcome. Interestingly, the molecular characteristics of SHH-subtype medulloblastoma in adults are transcriptionally distinct from those in children (a group in which this form of medulloblastoma is less common; Figure 2), but have similar molecular characteristics to those seen in infant SHH-subtype tumours—the exception being tumours with SUFU mutations, which are comparatively rare in adult medulloblastoma when compared with the infant disease. On the basis of these molecular findings, SMO antagonists should be an effective therapeutic option, at least in the short term, in a large subset of adults with SHH-subtype medulloblastoma. Initiating prospective clinical trials that direct therapy according to the molecular subgroups will increase our knowledge of the outcomes and unique toxicities of such agents in adults with medulloblastoma.

The next generation of clinical trials
Translating the newly acquired knowledge of the molecular underpinnings of medulloblastoma to tailor allocation of therapy according to prognosis has already been implemented in ongoing clinical trials: by the National Cancer Institute (NCI)-funded paediatric clinical consortium (Children’s Oncology Group ACNS012214) and the St Jude Children’s Research Hospital (SJMB122 and SJYC0736) in the USA; and by the International Society of Paediatric Oncology (PNET 54) in Europe. The study designs for each of these trials vary from pilot phase II to single-arm phase III studies. Larger randomized phase III studies will be considered at a later stage, after the data from current studies have matured.

Conclusions
The rapid progress in identifying the molecular and clinical characteristics of medulloblastomas has ushered in a new era in basic and clinical research in paediatric neuro-oncology. Disease heterogeneity, once shrouded in ambiguity, is now clearly related to inherent molecular differences, which can be ascertained through the application of modern technology to tumour interrogation. This progress has already led to the generation of mouse models that more-accurately mirror the human disease. These tools and future models, alongside molecularly driven prospective clinical trials that are designed to answer questions regarding the outcomes of specific tumour subtypes, will predictably facilitate the discovery of novel therapies that are more effective and less toxic, even to the youngest children with medulloblastoma.
irradiation and high-dose chemotherapy with adapted craniospinal and conformal primary-site irradiation and high-dose chemotherapy with stem-cell rescue in the SJMB-96 trial. J. Clin. Oncol. 26, 1112–1118 (2008).


Chapter 77

Brain metastases†

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INTRODUCTION

Despite better prevention and treatment advances achieved during the last decades, cancer is still a major public health concern and remains one of the leading causes of death worldwide (Lopez et al., 2006). The central nervous system (CNS) is a frequent target for metastases from systemic cancer. The most common location of CNS metastases is the brain parenchyma, followed by the leptomeningeal space.

Parenchymal metastases differ from leptomeningeal disease in clinical presentation, treatment modalities, and prognosis. However, their combination is common: on one hand, superficial brain lesions may invade the subarachnoid space, and on the other hand, primary leptomeningeal carcinomatosis often invades the brain parenchyma via perivascular Virchow–Robin spaces.

This chapter deals only with metastases restricted to the brain parenchyma.

INCIDENCE AND PRIMARY TUMORS

The exact incidence of metastatic brain tumors is unknown. Most epidemiologic studies may underestimate their true incidence, in part because some brain metastases remain asymptomatic, in part because even symptomatic lesions are often ignored in severely ill patients with advanced primary disease (Gavrilovic and Posner, 2005). Autopsy and clinical studies suggest that brain metastases occur in 10–30% of adult patients with systemic malignancies (Posner and Chernik, 1978; Schouten et al., 2002), thus representing by far the most frequent neurologic complication of systemic cancer and the most common type of brain tumor in adults. They exceed the number of primary brain tumors at least fourfold.

The incidence of brain metastases is thought to be rising in the last few decades due to a combination of factors other than population aging. First, improvements in and wider use of neuroimaging have resulted in an increased and earlier detection of clinically silent metastases. For example, routine brain scans are performed during the staging evaluation in neurologically asymptomatic patients with newly diagnosed lung cancer (Shi et al., 2006) or metastatic melanoma (Gavrilovic and Posner, 2005). Second, more effective treatments for systemic disease have extended survival of cancer patients, leading to a larger population at risk for brain metastases. Third, some highly effective anticancer agents poorly cross the blood–brain barrier (BBB), and are thereby unable to eradicate dormant micrometastases in patients with controlled systemic disease.

Every malignant tumor is able to metastasize to the brain. However, only a limited number account for the vast majority of brain metastases. In adults, three tumors, lung and breast carcinomas and malignant melanoma, account for up to 75% of brain metastases (Nussbaum et al., 1996; DeAngelis and Posner, 2009). In children and very young adults, the primary tumors most likely to metastasize to the brain are sarcomas (osteogenic sarcoma, rhabdomyosarcoma, and Ewing's sarcoma) and germ cell tumors (Kebudi et al., 2005).

Lung cancers are the most common source of brain metastases, accounting for at least one half of the cases.

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†This chapter is dedicated to the memory of Professor Jerzy Hildebrand
Patients with small-cell lung cancer (SCLC), which accounts for only 15% of all lung cancers, are at special risk as up to 50% of them eventually develop brain metastases (Seute et al., 2004). Among non-small-cell lung cancers (NSCLC), adenocarcinomas metastasize to the brain more frequently than squamous cell carcinomas (Shi et al., 2006).

Breast cancer is the second most common source, responsible for about 15% of all brain metastases (Soffietti et al., 2006; DeAngelis and Posner, 2009). The risk is increased in estrogen receptor-negative and HER2/neu-positive tumors. Patients with HER2/neu-positive breast cancer are treated with trastuzumab (Herceptin®), and in that group the incidence of brain metastases is high. This may be due to increased patient survival and to the fact that trastuzumab, which does not cross the BBB, is unable to eradicate micrometastases (Stemmler et al., 2007).

Melanoma represents the third most common cause of brain metastases, accounting for 5–10% of the cases, despite its comparably low incidence (Majer and Samlowski, 2007). But its propensity to form brain metastases is very high, and 50% of patients dying of melanoma have brain lesions (Amer et al., 1978).

Genitourinary tract cancers, mainly renal carcinoma, and colorectal cancers come respectively as the fourth and fifth sources of brain metastases. In patients with prostate cancer, small-cell or neuroendocrine carcinomas represent less than 1% of the tumors, but are responsible for 26% of brain metastases caused by prostate malignancies (Flannery et al., 2010).

In up to 15% of the patients with histologically proven brain metastases, physical examination and laboratory investigations fail to identify the site of the primary tumor in the early course of the disease (Nussbaum et al., 1996). During follow-up most of these patients are eventually found to have lung cancer (Ruda et al., 2001).

### CLINICAL FINDINGS

Approximately two-thirds of brain metastases become symptomatic in the course of the malignant disease (Cairncross et al., 1980). Most of them are diagnosed in patients with already known systemic cancer (metachronous presentation) or found during diagnostic procedure of the malignant disease (synchronous presentation). The discovery of brain metastases before that of the underlying cancer (precocious presentation) is less common but this situation may prevail in departments of neurosurgery and neurology.

Tumor tissue plus the surrounding vasogenic edema and, in some cases, intratumor hemorrhage produce focal neurologic signs by compression rather than destruction of the CNS structures, and by ischemia (DeAngelis and Posner, 2009). Through mass effect and obstruction of cerebrospinal fluid (CSF) circulation, brain metastases may also cause intracranial hypertension, obstructive hydrocephalus, brain herniations, and false localizing signs such as sixth nerve palsy.

Most symptoms and signs caused by brain metastases evolve progressively over days to weeks. But some have a stroke-like onset caused by tumor hemorrhage, tumor emboli, or acute rise in intracranial pressure. However, in the authors’ experience, the mechanism of acute neurologic deficit remains often unexplained and could be related to acute worsening of edema.

Brain metastases may be located in all sites of the brain, and are multiple in about 50% of the cases (Nussbaum et al., 1996). Therefore, any new neurologic manifestation occurring in a patient with cancer should raise the possibility of a metastatic brain tumor. Certain features, however, are particularly common. They include headache, seizures, focal signs, cognitive and behavioral changes, and gait disorders.

*Headache* is the most common presenting symptom, occurring in approximately 50% of the patients (Forsyth and Posner, 1993). It is more common in patients with multiple and/or posterior fossa lesions. Headache caused by brain metastases is usually mild but increases in intensity and duration with time. Typically, it appears in the early morning, worsens with maneuvers that increase intracranial pressure such as straining, bending, or coughing, and may be accompanied by drowsiness, nausea, and vomiting. However, this “typical” presentation occurs in only one-quarter to one-third of the patients. In the majority of the cases headaches resemble primary disorders such as tension-type headaches, and occasionally migraine (Forsyth and Posner, 1993). Thus headache characteristics, other than recent worsening, fail to predict reliably the presence of brain metastases, unless focal deficits or papilledema coexist (Argyriou et al., 2006). Nowadays, papilledema is found in less than 10% of the patients due to earlier diagnosis of brain tumors (Young et al., 1974; DeAngelis and Posner, 2009).

*Seizures* occur as the first manifestation of brain metastases in 20% of the patients and appear during the course of the disease in a similar percentage of cases (Lynam et al., 2007). Patients with multiple lesions or metastatic melanoma have an increased seizure risk (Oberndorfer et al., 2002). Metastasis-related seizures are essentially focal with or without secondary generalization, and thus have a localizing value. The most characteristic presentation is sensorimotor focal fits with Jacksonian progression pattern. Postseizure palsy and dysphasia are common in patients with underlying...
tumor and may last longer than 24 hours (DeAngelis and Posner, 2009). The differential diagnosis of tumor-related focal seizures and nonepileptic fluctuation of focal deficits such as worsening of aphasia or focal weakness is often very difficult in patients with language or other cognitive disorders including memory impairment. Nonconvulsive status epilepticus, which may be caused by unknown brain metastases, is another challenging but less common diagnostic issue (Blitshteyn and Jaeckle, 2006).

Focal neurologic deficits such as weakness of one limb or hemiparesis with or without sensory changes, language disorders, and deficits of visual fields are common presenting signs occurring in up to 40% of the patients (Kaal et al., 2005).

Cognitive decline, including memory impairment or lack of concentration, and behavioral disturbances ranging from personality changes to depression are extremely frequent. They have been reported in up to two-thirds of patients with brain metastases (Young et al., 1974; Mehta et al., 2003; Chang et al., 2007), but are largely underdiagnosed because they may be subtle, are reported more often by family members than by the patient himself, and may occur in absence of other neurologic signs.

Gait disorders may be the initial manifestation of brain metastases even in the absence of lower limb weakness. They are typically caused by multiple, bilateral, small size metastases (Fig. 77.1). The patients usually complain of unsteadiness, and their gait is characterized by short steps and moderate widening of lower limbs.

**PATHOPHYSIOLOGY AND PATHOLOGY**

The metastatic process is a highly selective and nonrandom phenomenon, governed by a cascade of molecular and genetic changes (Chiang and Massague, 2008). The propensity to generate brain metastases differs not only between tumor types but also between cells of a single tumor. Not all cells of a given tumor are able to reach the CNS, and of those that do, not all will survive in the brain (Nathoo et al., 2005). Therefore primary tumors and their corresponding brain metastases can be biologically different, even when they appear pathologically similar (Morita et al., 1998).

This seed (the metastasis) and soil (the brain) phenomenon is complex and not yet fully understood. It consists of a series of linked sequential steps: (1) cell detachment from the primary tumor by downregulation of adhesion molecules (Bremnes et al., 2002), and invasion of the host tissue through upregulation of matrix-degrading enzymes such as metalloproteases (Egeblad and Werb, 2002); (2) entry into the bloodstream through more permeable tumor-induced endothelial cells (Chang et al., 2000); (3) escape from destruction in the circulation, mainly by the immune system (natural killer cells) (Nieswandt et al., 1999) but also by mechanical forces; (4) arrest and early extravasation from brain microvessels (Kienast et al., 2010), favored by specific adhesion to brain endothelial cells (Pasqualini and Arap, 2002); and (5) survival and proliferation in the brain tissue by production of appropriate growth factors and promotion of angiogenesis or vessel co-option (Marchetti et al., 2003; Kienast et al., 2010). Cell predisposition to
metastasize is determined by genetic alterations, and changes that predict brain metastases from lung (Kikuchi et al., 2006) and breast cancer (Albiges et al., 2005) have been identified. Every step of this cascade is relatively inefficient and must be accurately completed for the final development of a brain metastasis. This explains why only a very tiny percentage of primary tumor cells is able to form viable brain metastases (Liotta and Kohn, 2003).

Brain metastases are solid, usually rounded and well circumscribed space-occupying lesions (Wesseling et al., 2007). When present, the infiltration of the brain does not exceed 1 mm, except in metastases from SCLC and melanoma which may show a diffuse infiltration (Baumert et al., 2006). Distant infiltration may account for tumor recurrence after local therapy. Because tumor growth disrupts the BBB, brain metastases are often surrounded by vasogenic edema. Its extent, however, is not always proportional to the size of the malignant lesion.

Large and rapidly growing metastases may contain central necrosis. Metastases of adenocarcinomas may contain collections of mucoid material (Wesseling et al., 2007). Microbleeds are common, and can be due either to invasion of blood vessel walls or to associated neovascularization. Large bleeds occur in up to 50% of metastases of melanoma and choriocarcinoma, followed by renal, thyroid, and testicular tumors (Mandybur, 1977). Metastases of lung cancers bleed in about 5% of the cases, but are a leading cause of tumor hemorrhage because of their high frequency. A recent study has shown an unexpectedly high rate of intratumoral hemorrhage in patients with metastases of breast or prostate cancers (Navi et al., 2010), but this observation is unusual and may reflect local experience.

The histological appearance of brain metastases is generally similar to that of the primary tumor, even though they may differ biologically and genetically. In patients with unknown primary cancer and uninformative histological examination, appropriate immunostaining can suggest the nature and the location of the primary tumor (Becher et al., 2006).

Brain metastases tend to develop at the junction between gray and white matter (Delattre et al., 1988; Hwang et al., 1996). Melanoma metastases are more likely than other metastatic tumors to invade the gray matter. Although direct extension into the CNS occasionally occurs from local tumors, most brain metastases reach the brain through hematogenous spread, and are believed to be entrapped in small size terminal arteries (Kienast et al., 2010). This mechanism may explain the propensity of brain metastases to develop in watershed zones of the cerebral circulation. The distribution of brain metastases, however, is roughly proportional to brain volume and blood flow: 80% are located in the cerebral hemispheres, 15% in the cerebellum, and 5% in the brainstem. One study indicates that tumors arising in the pelvis, mainly prostate or uterine cancers, or colorectal cancer have a special affinity for the cerebellum (Delattre et al., 1988). The reason for this predilection is unknown.

Based on imaging and autopsy data, one-half of patients with brain metastases have a single lesion (Posner and Chernik, 1978; Nussbaum et al., 1996), and an additional 20% two or three metastatic lesions (Delattre et al., 1988). Metastases from renal and colorectal tumors are often single, whereas lung cancers and melanoma are more likely to generate multiple lesions (Delattre et al., 1988).

**DIAGNOSTIC PROCEDURES**

In patients with known cancer the purpose of diagnostic examinations is to identify and locate brain metastases. In individuals not known to have a malignant disease and in whom neuroimaging suggests brain metastases, the aim is to rule out other brain diseases, and to determine the nature and the location of the primary tumor.

**Patients with known cancer**

Magnetic resonance imaging (MRI) is the best technique for detecting brain metastases, although there are no pathognomonic MRI features. MRI is superior to computed tomography (CT), even with double-dose delayed contrast, in visualizing small metastases and posterior fossa lesions (Schellinger et al., 1999). MRI has indeed a higher resolution, superior tissue contrast, and no bone artifacts. Furthermore, its versatile and multiplanar capabilities are useful in differential diagnosis and in planning surgery or stereotactic radiosurgery.

**Standard MRI** includes **T1WI** (T1-weighted imaging) with and without contrast agent, **T2WI** (T2-weighted imaging), and **FLAIR** (fluid-attenuated inversion recovery) sequences.

Most brain metastases generate low or intermediate intensity signal on **T1WI**. An increased intensity may correspond to a recent hemorrhage or to melanin deposit. Peritumoral edema appears as an area of decreased intensity. Brain metastases that reach a certain volume are enhanced after injection of paramagnetic contrast agent. Most brain metastases are spherical and sharply delineated. They may show peripheral ring enhancement with a nonenhancing core corresponding to central necrosis (Fig. 77.1). The enhancement is more conspicuous with magnetization transfer suppression (Knauth et al., 1996) and triple contrast dose (Sze et al., 1998), which may reveal very small lesions but also lead to false-positive findings. Conversely, treatment of the primary tumor with antiangiogenic agents may decrease...
contrast enhancement of metastatic brain lesions (Karimi et al., 2009).

T2WI and FLAIR sequences usually demonstrate an area of increased intensity encompassing both the tumor and the surrounding edema. The extent of edema is better appreciated on T2WI and FLAIR than on T1WI.

**Diffusion-weighted MRI (DW-MRI)** is especially useful in the differential diagnosis of ring-enhancing cerebral lesions. It shows high-intensity signal in abscesses (restricted diffusion, low signal on apparent coefficient diffusion (ADC) map), compared to low-intensity signal (unrestricted diffusion, high signal on ADC map) in cystic or necrotic tumors (Fig. 77.2). **Solid brain metastases** can appear as hyperintense lesions (restricted diffusion) depending on their cellularity, in which case DW-MRI is unable to differentiate metastatic lesions from acute or subacute ischemic stroke (Geijer and Holtas, 2002).

**Perfusion MRI** often shows an elevated relative cerebral blood volume (rCBV) in both metastatic and primary tumors. However, beyond the contrast-enhancing margins of the lesion, rCBV is usually increased in infiltrating primary tumors and decreased by edema in minimally invasive metastases (Law et al., 2002).

The **MRI spectroscopy** profile of solid metastases is characterized by increased choline peak, and decreased or even absent N-acetylaspartate and creatine levels. In metastases with necrotic areas, elevated lactate and lipid peaks may be found (Fig. 77.3). These findings are not specific and are also seen in primary tumors. Likewise in rCBV study, peritumoral measurements may help to differentiate primary from secondary brain tumors, thanks to their different infiltration capacity. Spectroscopic imaging demonstrates elevated choline levels in the peritumoral region of gliomas but not of metastases (Law et al., 2002).

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**Fig. 77.2.** Cerebral lesions with ring-enhancing appearance on contrast-enhanced T1-weighted image (upper images of panels (A) and (B)). The lesion in panel (A) is a breast adenocarcinoma metastasis, and its core is characterized by high signal on apparent diffusion coefficient (ADC) map (unrestricted diffusion). The lesion on panel (B) is an abscess, and its core is hypointense on ADC map (restricted diffusion). (Reproduced courtesy of Prof. M. Lemort.)
CT scan with and without iodine contrast is used when MRI is not available or when its use is prohibited by the presence of magnetic material. On nonenhanced CT scan, brain metastases appear as hypo- or isodense mass lesions, except when there is intratumoral bleeding or calcification. Lesion density is increased after contrast injection.

Brain positron emission tomography (PET) using 18 F-fluorodeoxyglucose (18 F-FDG) or amino acid tracers can be useful to differentiate hypometabolic postradiation focal necrosis from hypermetabolic malignant lesion (Hustinx et al., 2005).

When MRI or CT scan show, in an appropriate clinical setting, multiple mass lesions located near the gray–white matter junction and surrounded by an often disproportionate edema, the diagnosis of brain metastases is usually accepted without pathological confirmation. However, diagnostic biopsy may be indicated in certain circumstances such as: (1) atypical neuroimaging, (2) controlled systemic malignancy without evidence of pulmonary tumor, (3) primary cancer with low propensity to form CNS metastases, and (4) high risk of vascular or infectious brain lesion.
In brain metastases without leptomeningeal involvement, CSF changes are characterized only by an increased protein level. But lumbar puncture for CSF analysis may be hazardous and is not recommended.

EEG is helpful to support the diagnosis of epileptic seizures especially in patients with confusion, language or memory disorders, and unreliable history. But its interpretation is often complicated by nonepileptiform abnormalities due to the underlying tumor.

Patients not known to have cancer

In patients without obvious cancer, the identification of the primary tumor is part of the diagnostic procedure. The major dilemma is how far to pursue systemic investigations, which may delay potentially curative treatment such as radiosurgery and jeopardize its efficacy. The diagnostic work-up before considering surgery should consist of at least a clinical examination including full-body skin scrutiny, chest and abdomen CT, and whole-body 18F-FDG PET if accessible. Chest CT is the most fruitful and simple examination (van de Pol et al., 1996), as about 60% of patients with brain metastases have primary or metastatic lung tumor. CT of abdomen and pelvis occasionally shows an unsuspected cancer. Whole-body 18F-FDG PET scan is a sensitive tool for detecting systemic cancer, but its specificity in differentiating malignant from benign or inflammatory lesions is relatively low (Lan et al., 2008). When these examinations are inconclusive brain tumor resection or stereotactic biopsy is recommended. Both procedures help to establish histological diagnosis and orient to the location of the primary tumor (Becher et al., 2006). In addition, neurosurgery may be the first therapeutic step.

Specific serum tumor markers such as α-fetoprotein and β-human chorionic gonadotrophin for germ cell tumors, and other markers including CA 15.3 for breast, CA 19.9 for pancreatic, and CA 125 for ovarian carcinoma may help to orient the diagnosis of the primary cancer.

Differential Diagnosis

Up to 10% of structural brain lesions seen in cancer patients may not be metastatic, and correspond to primary tumors, vascular, infectious, granulomatous, or demyelinating lesions (Patchell et al., 1990). They are more likely to occur in patients with specific risk factors for these complications, in individuals with controlled systemic cancer without evidence of primary or metastatic pulmonary lesions, and in tumors with low propensity to invade the CNS. In patients who have undergone prior brain irradiation, clinical and radiological features of necrosis may resemble tumor progression.

Primary brain gliomas and primary CNS lymphoma (PCNSL) with atypical radiological presentation may be extremely difficult to differentiate from brain metastases despite progress made in DW-MRI, perfusion MRI, and MR spectroscopy (see diagnostic procedures), and definite diagnosis may require biopsy.

Hemorrhage caused by a small and previously unidentified metastasis may closely resemble primary brain hematomata. In some patients the definite diagnosis is made only after a prolonged follow-up.

In most ischemic lesions the shape, the location, and the lack of early contrast enhancement helps to differentiate vascular from malignant disease. But in cases with delayed neuroimaging, contrast enhancement and edema may mislead the diagnosis. Occasionally, systemic tumors may generate emboli made of malignant cells and/or mucin (mucin-secreting cancers) plus fibrin, which obscure cerebral arteries of various sizes and cause symptomatic stroke. In these patients, malignant mass may develop subsequently on the site of the ischemic territory (Nielsen and Posner, 1983).

Brain abscesses are rare (≤1/100,000 per year), but their incidence is increased after neurosurgical procedures (bacteria) and in immunosuppressed patients (fungi such as aspergillus). In about 15% of the cases brain abscesses are cryptogenic, and in patients without identified infectious source, inflammatory laboratory changes are subtle or even absent, making the diagnosis difficult. Nowadays DW-MRI allows the distinction between infectious and malignant disease (Fig. 77.2). Also in tuberculomas, inflammatory tests are often unable to distinguish tuberculous from neoplastic lesions. Most CSF analysis shows only an increased protein level because concomitant tuberculous meningitis is present in less than 10% of the patients (Arseni, 1958).

Due to migration, neurocysticercosis is no longer limited to endemic areas (Latin America, Africa, and Eastern Europe). Multiple brain cysts occasionally mimic metastatic lesions. The diagnosis is based on the occurrence of chronic seizures and serologic criteria.

Two viral CNS diseases may have a pseudotumoral presentation: herpes simplex encephalitis (HSE) and progressive multifocal leukoencephalopathy (PML). Both diseases may be diagnosed with polymerase chain reaction. However, the diagnosis may be challenging in HSE patients with minimal infectious signs, and in patients with PML who can mount an inflammatory reaction allowing enhancement of MRI lesions.

Sarcoidosis is a systemic disease characterized by noncaseous granulomas which involve leptomeninges and cerebral parenchyma in up to 10% of the patients. Occasionally, parenchymal location is the only obvious manifestation of the disease and may be mistaken for...
a neoplastic lesion. The diagnosis may require biopsy, and is sometimes made on surgical material.

Recent multiple sclerosis plaques are contrast-enhanced and may be occasionally mistaken for brain metastases. However, in our experience, the most likely diagnostic error is PCNSL, because in both diseases the lesions are multiple and have, typically, a periventricular location.

Brain lesions due to late-delayed toxicity of radiation therapy usually appear over 1 year following treatment, and therefore are not frequent in patients with brain metastases. The most common complication is diffuse leukoencephalopathy, causing cognitive and gait disorders which may mimic tumor progression. The main risk factors for this pathology are age, vascular risk factors, and concomitant chemotherapy. Focal radionecrosis is uncommon in patients treated with whole-brain irradiation using 30 Gy delivered in 10 days. But it occurs in up to 10% of individuals treated with stereotactic irradiation. Radiation-induced meningioma or glioma develop after a delay of 10–15 years.

**TREATMENT**

Several therapeutic options are available to treat brain metastases. Their choice is guided by following factors:

1. the extent, control, and pathology of the primary tumor
2. the size, location, and number of brain metastases
3. prior anticancer treatments.

A meta-analysis of the RTOG studies defined four independent prognostic factors for survival of patients with brain metastases: (1) age, (2) extent of systemic disease, (3) one versus several brain metastases, and (4) performance status (PS). Median survival of patients with four favorable factors was 7 months, whereas patients with only one favorable factor survived only 3 months (Diener-West et al., 1989).

Treatment of brain metastases cannot be considered in isolation as in about half of the patients death is caused by extraneural lesions. In most patients with widespread and uncontrolled cancer, the survival is unlikely to be significantly prolonged even by the most effective therapy of the cerebral disease. In such patients the primary aim of treatment of brain metastases is to improve or stabilize the neurologic deficit and the quality of life, with minimal inconvenience and morbidity. Whole-brain radiation therapy (WBRT) and corticosteroids usually fulfill these requirements. For example, in melanoma patients on fotemustine (a nitrosourea derivative) (Mornex et al., 2003) or in SCLC patients on teniposide (a podophyllotoxin) (Postmus et al., 2000), WBRT significantly prolongs the time to progression of brain metastases.

By contrast, in patients in whom the primary tumor is either undiagnosed or well controlled, who have a PS of 70% or more, and between one and three brain metastases, both quality of life and survival are primarily related to the treatment of malignant brain lesions. In such patients aggressive treatment of CNS-disease is warranted and several options summarized in the therapeutic algorithm are available (Fig. 77.4).

**External whole-brain radiation therapy**

WBRT is a mainstay of treatment and is used at some stage in malignant brain disease in most patients. The standard dose is 30 Gy delivered in 10 daily fractions.
There is no clear evidence to support that modified dose or fractionation schedule results in significantly better control of brain disease, longer median survival, or better cognitive outcome (Borgelt et al., 1980; Kurtz et al., 1981). Also there is no evidence that particular dose or fractionation should be based either on tumor pathology or radiosensitivity of the primary tumor, but this may be due to the limited number of studies explicitly addressing the issue.

The main indications for WBRT are as follows:

- **As already mentioned**, patients with poor outcome: advanced age, uncontrolled systemic cancer, low PS (<70%) and multiple brain metastases.
- **Following surgical removal** of brain metastasis. The use of postoperation WBRT is based on one randomized and prospective study (Patchell et al., 1998). The trial enrolled 95 patients with macroscopically total tumor resection, among whom 49 received 50.4 Gy irradiation. NSCLC was the predominant primary tumor. Irradiated patients had fewer tumor recurrences at the operative site (5/49 versus 21/46, \( p < 0.001 \)) and fewer distant brain metastases (7/49 versus 17/46, \( p < 0.01 \)). Also the time to recurrence was significantly \( (p < 0.001) \) delayed, and the duration of functional independence was prolonged in the irradiation group. However, the overall survival was not significantly increased (48 versus 43 weeks). The lack of survival benefit and the potential CNS toxicity of WBRT give support for delaying irradiation until symptomatic progression of brain lesions, but this alternative is not followed in all centers.
- **Following external stereotactic irradiation** WBRT significantly decreases the rate of distant CNS recurrences but does not affect overall survival (Kondziolka et al., 1999; Aoyama et al., 2006). However, early WBRT worsens cognitive disorders, and likewise following neurosurgery, a strategy of close monitoring with neuroimaging, with WBRT being delayed until recurrence, is advocated as a reasonable option (Chang et al., 2009).
- **Second WBRT** with 15–20 Gy is occasionally used, but the overall results are poor (Kurup et al., 1980; Hazuka and Kinzie, 1988).
- **Prophylactic WBRT** aims to eradicate microscopic metastases. Prophylaxis has been tested mainly in SCLC. Single prospective and randomized trials have shown a significant reduction of symptomatic brain metastases without effect on patients’ survival. But a meta-analysis including 987 patients with limited systemic SCLC also showed a 5.4% increase in survival 3 years after irradiation (Auperin et al., 1999), and a more recent trial indicates that prophylactic WBRT could also benefit patients with more extensive SCLC (Slotman et al., 2007). Neither of the two analyses reported the rate of leukoencephalopathy and cognitive decline due to irradiation, which may hamper the modest survival benefit. Therefore the use of prophylactic WBRT remains controversial, and is not a routine procedure in many centers.

**Surgery**

Unlike primary malignant tumors, most brain metastases do not deeply infiltrate the surrounding brain and may be totally removed. Nowadays surgery is in competition with stereotactic external irradiation (see below) in number of patients. Prospective and randomized studies comparing the two treatments are in progress. Retrospective comparisons of surgery plus WBRT versus single-dose stereotactic irradiation plus WBRT failed to show a clear advantage of one treatment over the other. However, neurosurgery retains its own indications in accessible tumors larger than 3 cm in diameter, and in lesions producing large mass effect and/or shift in midline superior to one centimeter. Tumor removal allows immediate improvement of focal deficits and relief of intracranial hypertension, particularly in tumors compressing the fourth ventricle. In patients with unknown primary tumor or with multiple primary cancers, surgery helps to establish the histological diagnosis and may reveal an unexpected pathology (Patchell et al., 1990).

The benefit of surgery plus WBRT over WBRT alone in terms of survival, recurrence of brain metastases, and probably duration of functional independence is based on two prospective randomized trials (Patchell et al., 1990; Vecht et al., 1993) (class I evidence), and three nonrandomized studies (Sause et al., 1990; Ampil et al., 1996; Rades et al., 2008). The study by Patchell included 48 patients and the Dutch trial 63 patients with a single metastasis. Most patients had NSCLC. A third randomized trial (Mintz et al., 1996) did not confirm the benefit of surgery, and this “negative” result has been attributed to a high proportion of patients with extensive systemic disease.

Taken together, the results of the six trials indicate that macroscopically total resection of solitary brain metastasis originating from NSCLC, and probably from other cancers including breast carcinoma (Wronska et al., 1997), prolongs the overall survival and improves quality of life for patients.

**Stereotactic external radiation therapy**

Stereotactic external radiation, also called stereotactic radiosurgery (SRS) when the treatment is given in a single dose, delivers a high-precision photon radiation to a
small target volume, while largely sparing the normal brain. The overall risk of focal necrosis is less than 10%. The radiation differential delivered to the target and the surrounding brain is inversely correlated with the size and the number of brain metastases. Therefore SRS is best indicated to treat solitary brain metastasis of less than 3 cm in diameter (Aoyama et al., 2006). Its efficacy is not related to tumor sensitivity to conventional irradiation doses as vascular endothelium may be its primary target (Szeifert et al., 2002). SRS is therefore a valid therapy in radioresistant tumors such as melanoma, renal or colorectal cancer. SRS can be used to treat any metastatic location including brainstem lesions (Koyfman et al., 2010). SRS is also used to treat a larger number of lesions, but its efficacy decreases with the increase in number and size of brain metastases.

SRS can be delivered either with a gamma knife, consisting of multiple (201) collimated cobalt-60 sources, or with a linear accelerator (Linac) where the radiation source is moving in a number of arcs around the target (Suh, 2010). Compared to the gamma knife, Linac allows treating larger and nonspherical lesions (stereotactic conformal radiotherapy) and can be delivered in multiple fractions. SRS requires precise location of the tumor and head immobilization, which is conventionally achieved with frames affixed to the calvarium. But various frameless systems are now available, making the procedure more comfortable (Nath et al., 2010; Suh, 2010), and probably as safe and efficacious as conventional frame-based technologies (Muacevic et al., 2010).

One year after its completion, SRS achieves local tumor control in 80–90% of cases. The risk of local failure increases with time but an apparent cure is maintained in a large number of metastatic sites. Level 1 evidence shows that, in patients with between one and three brain metastases, SRS plus WBRT provides a better local tumor control, a better functional status, and a significantly longer survival than WBRT alone (Kondziolka et al., 1999; Andrews et al., 2004). Whether WBRT should follow SRS immediately or be kept for recurrence has been discussed above. Occasionally SRS is used in patients having undergone neurosurgery plus WBRT for solitary metastases (Roberge et al., 2009).

**Chemotherapy, hormone therapy, molecular targeted agents**

The role of chemotherapy in the treatment of brain metastases is poorly defined, due to a limited number of adequate studies, and is underestimated for several reasons. Many primary tumors and their brain metastases are resistant to currently available drugs or develop resistance following drug exposure to chemotherapy. Also, p-glycoprotein is highly expressed in brain capillary endothelium and mediates efflux of several anticancer drugs including vinca alkaloids, taxanes, and podophyllotoxins (Deeken and Loscher, 2007). However, the potential limitation of poor drug penetration due to BBB mainly applies to micrometastases. Most metastatic lesions shown by neuroimaging enhance with contrast and have a largely disrupted blood–tumor barrier.

Clinical evidence shows that in metastases reaching a macroscopic size, treatment efficacy is related more to tumor chemosensitivity than to drug ability to cross intact BBB.

In metastases originating from very chemosensitive cancers such as germ cell tumors (Logothetis et al., 1982) or choriocarcinoma (Rustin et al., 1989), chemotherapy is the first-line treatment and may be curative.

Brain metastases of tumors with lower chemosensitivity also respond to chemotherapy. Out of 100 patients with breast carcinoma brain metastases treated primarily with drugs that do not readily cross the BBB, 10 patients achieved complete response and survived 39.5 months, 40 had partial response and a median survival of 10.5 months, and 50 patients failed to respond and had a survival of only 1.5 months (Rosner et al., 1986). Twelve studies including patients with SCLC brain metastases were reviewed by Kristensen et al. (1992). At diagnosis, 73% responded to chemotherapy (43% of complete responses), and 43% responded at tumor relapse (18% of complete responses).

Much lower response rates, of approximately 15%, have been observed in NSCLC and melanoma, reflecting the relative chemoresistance of these cancers. In addition several randomized and prospective trials including primarily patients with NSCLC failed to demonstrate an increase in survival when chemotherapy with carboplatin (Guerrieri et al., 2004), nitrosoureas plus tegafur (Ushio et al., 1991), or temozolomide (Antonadou et al., 2002; Verger et al., 2005) was added to WBRT.

Taken together, these data indicate that the use of chemotherapy in the treatment of brain metastases is heavily related to primary tumor chemosensitivity. In very sensitive cancers such as germ cell tumors or choriocarcinoma, chemotherapy is the frontline therapy and may be curative. In breast or SCLC metastases chemotherapy plays an important role. In relatively chemoresistant cancers, currently available drugs are of limited value.

Therapeutic effects of hormone and molecular target therapies are based on small series or single-case reports. In breast carcinoma with positive estrogen receptors, brain metastases may respond to tamoxifen (Pors et al., 1991), megestrol (Stewart and Dahrouge, 1995), or letrozole (Madhup et al., 2006). In patients with HER2/neu-positive breast cancer, treatment with
trastuzumab should not be discontinued when brain metastases become clinically evident (Bartsch et al., 2007). Epidermal growth factor receptor (EGFR) inhibitors have been shown to be effective in a few patients with brain metastases from NSCLC showing the appropriate EGFR mutation (Cappuzzo et al., 2003).

Renal cell carcinoma brain metastases may respond to antiangiogenic drugs. A recent retrospective analysis which included patients with CNS metastases from advanced breast cancer, non-small-cell lung carcinoma, and renal and colorectal cancer has shown that treatment with bevacizumab is safe and does not significantly increase the risk of bleeding in brain metastases (Besse et al., 2010).

Supportive treatments

Several drugs, not specifically directed against cancer, are frequently used in the management of patients with brain metastases. They include corticosteroids, antiepileptics, anticoagulants, antidepressants, and analgesics. All of them have potentially serious side-effects which may outweigh their benefit if improperly used.

Corticosteroids

Corticosteroids (CS) are a symptomatic treatment, except in lymphomatous lesions. Clinical experience shows that CS relieve neurologic deficits within few days. This improvement is due to reduction of vasogenic peritumoral edema, which can be demonstrated by neuroimaging. CS also improve clinical manifestations of acute radiotoxicity. But no data support that they have an effect on progression-free periods or the overall survival of patients with brain metastases. CS have additional beneficial effects in that they improve appetite, and decrease nausea, vomiting, and pain.

Dexamethasone is preferred to other forms of CS because of its lack of mineralocorticoid effect and long half-life. The widespread use of corticosteroids in neuro-oncology contrasts with the paucity of studies indicating the dose and the duration of their administration. Based on a study by Vecht et al. (1994), the starting daily dose of 4–8 mg dexamethasone seems appropriate in most patients, and should be tapered after 1–2 weeks to reach the minimal efficacious dose. Higher doses including an initial loading dose of 100 mg of dexamethasone may be recommended in patients with severe symptoms and impending cerebral herniation.

Prolonged administration of CS is associated with a long list of side-effects (Drapatz et al., 2007). Non-neurologic complications include: osteoporosis, osteonecrosis, gastrointestinal bleeding, bowel perforation, hyperglycemia, opportunistic infections, and glaucoma. Neurologic side-effects, which may be mistaken for signs of tumor progression, are pelvic muscle atrophy causing gait difficulty, psychotic, behavioral and cognitive disorders, and spinal lipomatosis. These complications may be minimized by avoiding unnecessary high doses and prolonged CS administration. Even during radiation therapy the daily dose of CS may be reduced (Weissman et al., 1991).

Antiepileptic drugs

The best drug or drug combination for the treatment of seizures caused by brain metastases is not known. It seems appropriate to choose molecules which do not induce liver enzymes such as valproic acid or newer antiepileptic drugs (AEDs) in order to avoid interference with both CS and chemotherapy. It is also preferable to avoid drugs with serious neurotoxic effects which may aggravate neurologic status and be mistaken for tumor progression. Therefore we try to avoid the use of phenytoin, phenobarbital, and even carbamazepine or oxcarbazepine, and we prefer beginning treatment with levetiracetam and, if necessary, adding valproate. We also avoid prophylactic treatment. This attitude is based on the results of the controlled study by Forsyth et al. (2003) which included only 60 patients with brain metastases and used phenytoin in 25 of 26 treated patients, and the recommendations of the American Academy of Neurology (Glantz et al., 2000). However, these recommendations are based primarily on the use of phenytoin and phenobarbital, which are no longer first-choice drugs. Therefore, the issue remains controversial, particularly for the subgroup of patients with melanoma in whom the risk of seizure is high.

Anticoagulation

The risk of deep venous thrombosis (DVT) is increased in cancer patients (Lee and Levine, 2003). It is particularly high in individuals with brain metastases and reduced mobility in whom DVT is twice as likely to occur in the paralyzed leg. Craniotomy also favors DVT (Sawaya et al., 1992).

Prophylaxis of DVT combines active and passive mobilization, compression stockings, and low molecular weight heparins. In patients undergoing neurosurgery prophylactic anticoagulation is started 24 hours following operation.

In patients with brain metastases and established thromboembolic disease the benefit of anticoagulation largely surpasses the danger of intratumoral bleeding, which is rare (Schiff and DeAngelis, 1994). When coagulation is contraindicated, inferior cava filter is a reasonable alternative.
CONCLUSIONS

Brain metastases are found in about 20% of the patients with systemic cancer, and their incidence is increasing. They are both the most frequent neurologic complication of cancer and the most common type of brain tumor. Lung and breast cancers and melanoma are responsible for up to three-quarters of metastatic brain lesions, although every malignant tumor is able to metastasize to the brain. Patients with brain metastases often exhibit neurologic manifestations such as headache, seizures, focal deficits, and cognitive changes, which severely impair patients’ quality of life. The overall prognosis is poor and depends on age, the extent and activity of the systemic disease, the number of brain metastases and PS. In about half of the patients, especially those with widespread and uncontrolled systemic neoplasia, death is directly related to extraneural lesions, and treatment of the cerebral disease does not significantly improve survival. In such cases the aim of treatment is to improve or stabilize the neurologic deficit and maintain the quality of life. Corticosteroids and WBRT can be used for this purpose. In contrast, patients with a limited number of brain metastases, good PS, and well controlled or limited systemic disease may benefit from aggressive treatment of brain lesions as both the quality of life and survival are primarily related to treatment of CNS disease. Several efficacious therapeutic options based on a combination of surgery, radiotherapy, and chemotherapy are available for these patients.

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Biology in prevention and treatment of brain metastases


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Brain metastases are common in cancer patients, may significantly diminish neurocognitive function and quality of life and carry a poor prognosis. Brain metastases differ from metastases in other organs such as liver, lung, lymph nodes and bone, both from a pathobiological and from a clinical perspective. Despite the high incidence of brain metastases, only relatively few studies aiming at better understanding of their pathobiology have been performed in the past. However, recently druggable targets have been identified in brain metastases of several tumor types and novel treatment approaches are becoming a feasible option for selected patients. In addition, scientific advances are elucidating some fundamental aspects of brain metastasis formation and may lead to effective strategies of drug-mediated prevention of metastatic brain invasion or inhibition of intracerebral outgrowth.

KEYWORDS: biology • brain metastases • cancer • prophylaxis • therapy

Brain metastases are frequent and affect up to 40% of cancer patients. Primary tumor types differ in their risks for development of brain involvement with lung cancer (incidence of approximately 15–20%), breast cancer (5%), melanoma (7%) and kidney cancer (6–10%) showing the highest incidence of brain metastases [1]. The clinical presentation is diverse and depends on the tumor location and size, but oftentimes involves headache, signs of increased intracranial pressure, seizures, personality changes and neurocognitive deterioration. In general, brain metastases are associated with poor quality of life and have a poor survival prognosis [2–4]. Standard treatment options include mainly local therapies such as radiotherapy, neurosurgery and radiosurgery. Cytotoxic therapy is used in poorly standardized fashion and based on only little evidence from adequate clinical trials [5]. Recent advances in the understanding of pathobiology of brain metastases have led to some promising results and promising concepts for therapy and prevention of brain metastases based on scientific insights. However, the design of clinical trials on targeted agents in brain metastases is challenging with regard to the choice of endpoints, standardization of response criteria and translational aspects [6]. This article summarizes current knowledge on the molecular pathology with a focus on potential drug targets in brain metastases of solid cancers.

Pathobiology & potential targeted treatment strategies

For successful spread from the primary tumor to the brain, tumor cells rely on many distinct molecular adaptations. A detailed understanding of these offers the possibility for targeted intervention.

From the tumor of origin to the brain

Brain metastasis formation requires dissemination of tumor cells from an extra-CNS tumor manifestation through the blood stream. Lymphatic tumor cell dissemination to the CNS does not occur, as the brain is, for the most part, not drained by lymphatic vessels [7]. It is unclear, however, whether the brain-metastatic tumor cells originate from the primary tumor itself or from other organ metastases (e.g., lymph node metastases). It has been postulated that metastasis formation may not necessarily be unidirectional and that circulating tumor cells may also colonize their tumors of origin in a process termed ‘tumor self-seeding’ [8]. This phenomenon has also been shown for brain-seeking cell lines and was shown to accelerate tumor growth, angiogenesis and stromal recruitment through chemokine signaling.

Another unresolved issue is the time point of brain metastasis formation during the disease course of a cancer patient. In animal models, tumor cells have been shown to be
able to stay dormant for prolonged periods of time in perivascular niches in the brain before growing to form macro-metastases [9]. Indeed, a surprisingly high percentage of patients with subclinical small metastases may be found in certain populations of cancer patients. For example, in the CEREBEL trial, clinically asymptomatic brain metastases were found through MR-based screening in 20% of patients with HER2–positive metastatic breast cancer [10]. Further studies are needed to better understand the time course of brain involvement in cancer patients, as of course potential prevention strategies heavily rely on this information. For example, approaches aiming at interception of circulating tumor cells or at disruption of tumor cell extravasation through the blood–brain barrier (BBB) are not likely to be effective against dormant tumor cells that have colonized the brain early on. In such instances, prevention of macro-metastatic outgrowth, for example, by inhibition of angiogenesis or intraparenchymal migration/invasion may be more promising.

Epithelial-to-mesenchymal transition (EMT) is regarded as an important mechanism in the detachment and dissemination of metastatic tumor cells from the tumor bulk of origin. EMT is characterized by loss of E-cadherin expression, disruption of cell adhesion and induction of cell motility and invasion. During colonization of the target organ, cancer cells may undergo a reverse process such as mesenchymal-to-epithelial-reverting transition (MErT) with re-induction of E-cadherin expression [11]. Indeed, E-cadherin expression is found in the majority of brain metastases, at least of lung cancer, and showed a strong positive correlation with a high Ki67 tumor cell proliferation index in a previous study [12].

On their metastatic journey, tumor cells need to adapt to the microenvironment of the blood, which differs significantly from that of solid tissues such as the tumor of origin or the target organ. In the blood stream, tumor cells may be exposed not only to attacks by the immune system but also to considerable physical stress through hemodynamic shear forces, particularly during attachment to endothelial cells and the extravasation process. Several studies seem to indicate that tumor cells may aggregate with platelets and leukocytes, for example, via podoplanin, TGF-β/SMAD, NF-κB or selectin signaling, in the blood circulation to induce an EMT phenotype, protect them from immunologic assault and to facilitate extravasation [13–15]. Indeed, inhibition of NF-κB signaling in cancer cells or ablation of TGF-β1 expression solely in platelets has been shown to protect against lung metastases in animal models. Tumor cells may use similar mechanisms as leukocytes during the so-called adhesion cascade [16]. During this process, the cell needs to switch between various locomotion strategies, such as floating in the blood stream, rolling on the endothelial surface, tumor cell arrest and crawling, and finally tumor cell transmigration through the vascular wall. This process is heavily understudied in cancer, but is likely to depend on complex molecular mechanisms that may be potentially targetable with the aim of reducing the efficacy of metastatic (brain) colonization. Hypothetically, inhibitors of integrins or other adhesion molecules (e.g., alpha-II-b-beta-3 integrin, alpha-v-beta-3 integrin, alpha-4 integrins, beta-2 integrins and VCAM-1) that have been developed as antithrombotic or anti-inflammatory agents could protect from metastasis including to the brain by interfering with this mechanisms [15]. In line with this notion, in human cancer patients, the use of antiplatelet agents such as low-molecular-weight heparins (LMWHs) seems to improve prognosis and maybe reduce the risk for metastasis [17].

**Crossing the BBB**

After successful attachment to the brain endothelial cells, the tumor cells need to migrate through the protective BBB into the brain parenchyma. Kienast et al. used a mouse model and multi-photon laser-scanning microscopy through a chronic cranial window to image the interaction of tumor cells with the brain vasculature during establishment of brain metastases in vivo [9]. Using this approach, they were able to follow the brain metastatic process over minutes to months and up to a depth of 500 micrometers in the live brain of nude mice after intra-arterial injection of lung carcinoma or melanoma cells. A marked regression of cancer cells after initial hematogenous spread was observed, indicating that the late metastatic process is quite inefficient. Four essential steps of successful establishment of intraparenchymal macrometastases were identified in this experimental model: arrest of cancer cells occurs primarily at vascular branch points, presumably because of reduced shear force of the dynamic bold flow at these sites; early extravasation within few days; persistent contact of tumor cells with the abluminal side of microvessels; and perivascular growth by vessel cooption or early angiogenesis. The main molecules involved in the process of cancer cell migration through the BBB include selectins, integrins (e.g., integrin alpha-3-beta-1, chemokines [CXCR4 and its ligand CXCL12], VEGF, cyclooxygenase 2 [COX2], heparin-binding EGF [HBEGF], alpha-2,6-sialyl-transerase ST6GALNAC5 and placenta-derived growth factor [PIGF]) [18]. Some of these may be feasible therapy targets. In concordance with this notion, PIGF down-regulation was shown to suppress small-cell lung cancer metastasis to the brain in animal models, which is noteworthy as anti-PIGF agents are in clinical development [19].

**Intraparenchymal growth**

After brain invasion, tumor cells need to secure adequate supply with oxygen and nutrients, and there are different strategies to do this. Some tumor cells tend to grow along preexisting brain vessels (vascular cooption) and this invasion type has been primarily seen in melanomas, but can also be observed in various epithelial tumors [20–22]. This type of invasion is characterized by collective tumor cell migration along the basement membrane and seems to rely on integrin signaling [23]. In a preclinical brain metastasis model, the vascular basement membrane promoted adhesion and invasion of cancer cells and was sufficient for tumor growth prior to any evidence of angiogenesis. Blockade or loss of the beta1 integrin subunit in tumor cells prevented adhesion to the vascular basement membrane.
and attenuated metastasis establishment and growth in vivo [24]. Furthermore, anti-alpha-v-integrin antibody intetumumab treatment prevented metastasis formation on MRI and decreased the number of metastases on histology in rats inoculated with HER2-positive breast cancer cells [25].

Some tumor types induce early neo-angiogenesis via the VEGF/HIF1-alpha pathway, rather than growing via vascular cooption. This type of angiogenic behavior has been observed primarily in non-small-cell lung cancer (NSCLC), and can also cooption. This type of angiogenic behavior has been observed primarily in non-small-cell lung cancer (NSCLC), and can also cooption. This type of angiogenic behavior has been observed primarily in non-small-cell lung cancer (NSCLC), and can also be observed in other tumor types [9]. Here, it was seen that lung cancer cells formed separate cell clusters in close proximity to each other in the brain of mice after inoculation into the internal carotid artery. Simultaneous growth and subsequent fusion enabled the clusters to overcome a critical mean diameter (147–195 μm), which was followed by angiogenesis that culminated in a period of fast and unrestrained growth. Importantly, VEGF blockade inhibited brain metastasis formation and a prospective clinical trial testing this approach in NSCLC patients has been initiated by the European Organization for Research and Treatment of Cancer brain metastasis platform.

Growth and invasion of tumor cells in the CNS is dependent on the interaction with the extracellular matrix (ECM). The ECM of the brain lacks some constituents, such as fibronectin and collagen, usually found in solid organs but is rich in proteoglycans, tenascin, laminin, heparin sulfate, chondroitin/dermatan sulfate and hyaluronic acid [26]. Invasion and growth in this surrounding requires expression-degrading molecules such as heparanases and matrix-metalloproteinases (MMPs). Suramin, which is used for treatment of human sleeping sickness, is an inhibitor of heparanase and suramin analogs inhibited heparanase-induced invasion and angiogenesis in a melanoma brain metastasis model [27]. Furthermore, MMP inhibition also significantly decreased invasive cell behavior in an experimental brain metastasis model [28]. Of note, in some brain metastases, considerable interstitial collagenous fibrosis can be observed and at this point the pathobiological genesis of this is not known. However, a correlation of interstitial fibrosis with imaging parameters with potential clinically relevant implications was reported [29].

Brain metastases elicit a strong reactive astrogliosis, as can be readily observed in tissue samples using immunohistochemistry for GFAP. Intuitively, one would infer that the astrogliosis is a protective effect of the brain aimed at counteracting invasion of tumor cells. However, several papers seem to implicate that the brain-metastatic tumor cells may exploit the reactive astrocytes for their own advantage. Xing et al. reported that the brain-metastatic breast tumor cells highly express IL-1β, which activates the surrounding astrocytes [30]. This activation in turn promoted the growth of metastasized cancer stem-like cells via notch signaling and treatment with a BBB-permeable notch inhibitor (Compound E) suppressed brain metastasis in vivo. Another paper showed that reactive astrocytes and tumor cells may form physical contacts and gap junctional communication leading to calcium transfer and chemoprotection of the tumor cells [31]. Recently, a specific metastasis-associated subpopulation of reactive astrocytes with expression of PDGFR-beta (at tyrosine 751; p751-PDGFR-beta) has been identified and could be decreased by treatment with a specific inhibitor in animal models [32]. These results highlight the important role of the microenvironment in brain metastases and implicate that targeting of specific interactions of tumor cells and brain parenchymal cells may be of therapeutic value in the clinical setting.

The inflammatory reaction to brain metastases is characterized by prominent activation of microglia/macrophages and pronounced phagocytosis, but seems to be insufficient in activating adaptive immunity [33]. The role of microglial cells in brain metastases is unclear and controversial data exist. Activated microglia have been reported to exert tumoricidal effects via nitric oxide release [34]. However, microglia may also promote metastatic spread into the brain [35]. Treatment strategies aimed at activating specific immunity, for example, with immunomodulatory antibodies such as the anti-cytotoxic T-lymphocyte antigen 4 (CTLA4) antibody ipilimumab or PD-1/PD-L1 inhibitors, may potentiate immune attack on tumor cells. Indeed, ipilimumab has shown clinical activity in a subset of melanoma patients with brain metastases, particularly in those with small and asymptomatic lesions [36].

### Drug penetration into brain metastases

Recently, therapeutic successes with therapies targeting onco-genic pathways have been reported specifically in brain metastases patients [37]. Special consideration has to be given to the BBB, which forms a selective barrier between the blood and the brain parenchyma. The BBB is composed of endothelial cells connected by tight junctions (mainly formed by claudins and occludins), the basement membrane and astrocytic end-foot processes. Recently, the BBB has been recognized as a part of the neurovascular unit (NVU) with a wide and dynamic permeability range that is controlled by complex intra- and intercellular signaling events [38]. Endothelial transporters allow the passage of small hydrophilic molecules, while large hydrophilic molecules such as many chemotherapeutic and biologicals (e.g., antibodies) cannot penetrate the parenchyma. Furthermore, the BBB expresses drug efflux pumps such as P glycoprotein (Pgp) proteins, which actively remove some compounds from the CNS. The composition and function of the blood–tumor barrier (BTB) is expected to be different from the BBB, because it results from pathological neo-angiogenesis and may be influenced by signaling molecules influencing permeability (e.g., VEGF). The permeability of the BTB in brain metastases may be influenced by the tumor size and was reported to be more permeable in larger and diffusely growing lesions [39]. Furthermore, the permeability of the BTB seems to be heterogeneous throughout the tumor tissue. Permeability tends to be highest at the tumor center, while the integrity of microvasculature is preserved in the border and infiltration zone. The levels of Pgp expression in melanoma and lung carcinoma brain metastases have been shown to be reduced to 5 and 40% of the levels found in the normal brain [40]. Novel experimental approaches...
of drug delivery may exploit receptor-mediated transcytosis using antibody–chemotherapy conjugates that may inhibit drug efflux pumps or pharmacologically increase the BTB permeability [41]. Other experimental approaches to enhance drug delivery to brain tumors include convection-enhanced delivery, osmotic BBB disruption or targeted ultrasound BBB disruption [41].

**Emerging targeted therapies in specific indications**

Targeted agents with proven or potential clinically meaningful activity are emerging in several tumor types, including lung cancer, breast cancer, melanoma and others (Table 1).

Lung cancer

Approximately half of the cases of brain metastases originate from lung cancer and some target aberrations for specific therapeutic inhibition have been identified in patient subgroups. Unfortunately, so far, there is a lack of adequate clinical trials to provide evidence-based guidance for the role of targeted therapies in lung cancer patients with brain metastases.

**EGFR**

A few studies indicate a potential activity of EGFR tyrosine kinase inhibitors against NSCLC brain metastases and the therapeutic benefit may be highest in patients with activating EGFR mutations [42–45]. EGFR mutations may correlate with radiological patterns of brain metastasis, as miliary growth has been described to affect predominantly mutation-bearing patients [46]. A potential prophylactic effect on brain metastasis development has been reported for patients with advanced EGFR-mutated NSCLC [47].

**ALK**

ALK translocations, most commonly with EML4, are present in 2–5% of NSCLC patients and the ALK gene status is consistent between primary tumors and brain metastases [48]. The oral ATP-competitive selective ALK inhibitor crizotinib inhibits phosphorylation of activated ALK and is active in ALK-positive NSCLC [49]. Conclusive studies on the therapeutic role of crizotinib on ALK-aberrant brain metastases are missing. However, case reports documenting both, response to crizotinib and failure of crizotinib therapy in ALK-positive NSCLC brain metastases have recently been published [50–52]. The ability of crizotinib to penetrate the BBB/BTB and reach active concentrations in CNS metastases needs to be elaborated.

**Other potential therapy targets**

A number of novel therapy targets, such as ROS1 rearrangements, FGFR1 amplifications, RET rearrangements, BRAF mutations, HER2 insertions, PI3K mutations, are emerging in lung cancer patients and the frequency of these aberrations in brain metastases as compared to primary tumors and extra-CNS tumor manifestations need to be elaborated to identify the proportion of brain metastases patients potentially benefiting from specific drugs [53].

**Breast cancer**

Among breast cancer patients, those with HER2-positive or triple-negative tumors have the highest incidences of brain metastases [54,55].

**HER2**

In breast cancer, current studies on drug treatment of brain metastases are mainly focusing on HER2-targeting agents. Recently, the results of a prospective, single-arm, Phase II study were published, which investigated the influence of the combination of the oral HER2-antagonist lapatinib with the oral cytotoxic agent capecitabine on the radiological CNS response rate [56]. Of 44 assessable patients, an objective response was seen in 29 (65.9%) cases. These results may indicate a clinical role of this drug regimen and further, optimally randomized studies are needed to clarify its value in relation to standard treatment options such as whole brain radiotherapy [57]. Unpublished experience with some patients in several centers (including ours) suggests that the novel antibody–drug conjugate trastuzumab emtansine (T-DM1) may be active in brain metastases of HER2-positive breast cancer.

**Prevention of brain metastases**

The CEREBEL trial investigated the incidence of brain metastases if HER2-positive metastatic breast cancer patients treated...
either with trastuzumab/capecitabine or with lapatinib/capecitabine. Although the results were not adequately powered due to insufficient accrual, there was no statistically significant difference in brain metastasis incidence between the two arms [10]. Several preclinical data point toward a potential role of specific inhibitors for prevention of brain metastases of breast cancer. The pan-Raf inhibitor pazopanib resulted in a significant decline of brain macro- and micrometastases in mice after left cardiac injection of HER2-positive brain-seeking cell lines [58]. Pazopanib was shown to decrease a specific population of metastasis-associated reactive astrocytes expressing phosphorylated PDGFR-beta, which may also contribute to the inhibition of metastatic brain colonization [32]. Furthermore, inhibition of polo-like kinase 1 and treatment with the anti-alpha-v-antibody intetumumab prevented brain metastases [25,59].

Melanoma

**BRAF**

Approximately half of metastatic melanoma cases harbor BRAF V600 mutations (most commonly of the V600E type) and BRAF inhibitors such as vemurafenib and dabrafenib have shown favorable efficacy in mutation-bearing cases. BRAF inhibitors induce also high CNS response rates in patients with BRAF V600-mutated melanoma, but are generally prone to fairly quick development of tumor resistance as mono-therapies [60-63]. However, some patients with disease progression in the brain with concurrent treatment response of extracranial disease sites to vemurafenib have been reported [64]. In line with this observation, the brain distribution of vemurafenib has been reported to be severely restricted by active efflux by P-glycoprotein and breast cancer resistance protein (BCRP) [65]. Co-administration of the dual P-glycoprotein and BCRP inhibitor elacridar may increase brain accumulation of vemurafenib [66]. Current studies are investigating whether the addition of MEK inhibitors, which counteract resistance, may increase the duration of treatment response.

**Ipilimumab**

The immunomodulatory anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) monoclonal antibody ipilimumab has been shown to improve survival in patients with metastatic melanoma and has been approved for therapy. Ipilimumab has also shown some activity in patients with brain metastases, particularly with small and asymptomatic metastases [36]. Of note, ipilimumab does not probably have to cross the BBB to be active against brain metastases, as it elicits an anti-neoplastic immune response. Optimal algorithms for the sequence of BRAF inhibitors and ipilimumab need to be elaborated, also with particular reference to patients with brain metastases [67,68].

**Kidney cancer**

Although conclusive studies are missing, several data sets indicate a value of tyrosine kinase inhibitors such as sunitinib and sorafenib for treatment of kidney cancer brain metastases [69-70]. Recent data suggest that treatment with the tyrosine kinase inhibitors sorafenib or sunitinib may reduce the risk of brain metastases in metastatic renal cell carcinoma [71].

**Expert commentary**

The current standard treatment options for brain metastases are effective to some extent, but almost certainly better understanding of brain metastases and the conduct of more well-designed experimental, translational, diagnostic and therapeutic studies will lead to significant improvements in patient management. Interestingly, however, despite the fact that brain metastases are a common problem in some of the most prevalent cancer types such as lung cancer and breast cancer, relatively little research has been done in this field. In fact, the research landscape on primary brain tumors, which are approximately 10-times less frequent than brain metastases, is much more active, diverse and comprehensive. Unfortunately, brain metastases remain an exclusion criterion for far too many trials. The quality of brain metastases research could profit from involvement of established, strong neurooncological groups, as many of the methodologies used in investigations on primary brain tumors may be well applicable to CNS metastases. For example, clinical trials in brain tumor patients should include formal and well-planned testing of neurocognitive function and quality of life, and there are specific modules to test these dimensions in neurooncological patients. Also, the definition of endpoints in brain tumor patients is far from trivial and needs to rely on high expertise with regard to the selection of radiological criteria and recognition of potential confounding factors. At the same time, proactive involvement of oncologists from the various organ specialties as well as radiotherapy specialists is of paramount importance to ensure optimal incorporation of their perspective and also to guarantee adequate patient enrollment into clinical studies. For these reasons, the European Organization for Research and Treatment of Cancer has recently implemented the brain metastasis platform that involves experts on several tumor types and disciplines such as breast cancer, lung cancer, melanoma, imaging, pathobiology and radiation oncology. It is expected that cross-sectional platforms like this example stimulate innovative and insightful research in a collaborative environment in order to improve the standard of care and methodology of clinical research.

**Five-year view**

After decades of slow progress in the field of brain metastases research, there has been recent flaire of novel insights into brain metastases, which have led to some new options for clinical management of selected patients, the identification of potential target molecules for prophylaxis and treatment of brain metastases (Table 2) and the initiation of further studies. At the international level, multidisciplinary and multinational study groups have been formed with the specific aim to increase understanding of and improve treatment options for brain metastases. One of the most interesting concepts is the prevention of brain metastasis development in high-risk patients and we may learn within the coming five years whether the
promising data generated in experimental investigations and retrospective clinical studies actually translate to the clinic via successful prospective clinical trials. In addition, ongoing translational studies will hopefully clarify which targetable molecular alterations are present in established brain metastases. In parallel, systematic investigations of the ability of specific inhibitors to overcome the BBB/BTB need to be implemented, for example, by a close interaction of medical (neuro-)oncologists, neurosurgeons, neuroradiologists, pharmacologists and biologists in studies involving pretreatment with investigational drugs of patients scheduled for resection of brain metastases and subsequent analysis of the tumor tissue for drug concentration and biological effects (‘treat–resect–analyze’). Such initiatives are currently being started. As a consequence of these research activities, five years from now, the diagnosis of brain metastases should no longer be automatically met with therapeutic nihilism, but should stimulate initiation of evidence-based active treatments.

Table 2. Potential target molecules for prophylaxis and treatment of brain metastases (noncomprehensive list).

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Presumed function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Survival of tumor cell in the blood stream – aggregation with platelets and leucocytes</strong></td>
<td></td>
</tr>
<tr>
<td>NF-κB</td>
<td>EMT, inhibition protects against metastasis</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Ablation of TGF-β1 on platelets inhibits metastasis</td>
</tr>
<tr>
<td>Alpha IIb beta 3 integrin</td>
<td>Attachment to brain endothelial cells and basement membrane</td>
</tr>
<tr>
<td>Alpha v beta 3 integrin</td>
<td></td>
</tr>
<tr>
<td>Alpha 4 integrine</td>
<td></td>
</tr>
<tr>
<td>Beta 2 integrin</td>
<td></td>
</tr>
<tr>
<td>VCAM-1</td>
<td></td>
</tr>
<tr>
<td><strong>Crossing the BBB</strong></td>
<td></td>
</tr>
<tr>
<td>Selectins</td>
<td>Adhesion to endothelial cells</td>
</tr>
<tr>
<td>Integrin alpha-3-beta-1</td>
<td>Adhesion to endothelial cells</td>
</tr>
<tr>
<td>Chemokines</td>
<td>Adhesion to endothelial cells</td>
</tr>
<tr>
<td>Cyclooxygenase 2</td>
<td>Adhesion to endothelial cells and migration trough BBB</td>
</tr>
<tr>
<td>Heparin-binding EGF</td>
<td>Adhesion to endothelial cells and migration trough BBB</td>
</tr>
<tr>
<td>Alpha-2,6-sialytransferase ST6GALNAC5</td>
<td>Adhesion to endothelial cells and migration trough BBB</td>
</tr>
<tr>
<td>PDGF</td>
<td>Regulation of tight junctions</td>
</tr>
<tr>
<td><strong>Intraparenchymal growth</strong></td>
<td></td>
</tr>
<tr>
<td>Beta1 integrin subunit</td>
<td>Adhesion to the vascular basement membrane, growth via vascular co-option</td>
</tr>
<tr>
<td>Alpha-v-integrin</td>
<td>Inhibition prevents metastasis formation</td>
</tr>
<tr>
<td>VEGF</td>
<td>Regulation of vascularization</td>
</tr>
<tr>
<td>Heparinases</td>
<td>Degradation of the extracellular matrix</td>
</tr>
<tr>
<td>Matrix-metalloproteinases</td>
<td>Degradation of the extracellular matrix</td>
</tr>
<tr>
<td><strong>Microenvironment</strong></td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td>Activation of surrounding astrocytes</td>
</tr>
<tr>
<td>Notch signaling</td>
<td>Communication of tumor cell with astrocytes, inhibition suppresses brain metastasis growth</td>
</tr>
<tr>
<td>PDGFR-β</td>
<td>Expression on astrocytes promotes metastasis</td>
</tr>
<tr>
<td>Adaptive immune system</td>
<td>Seems to be insufficiently activated in BM</td>
</tr>
<tr>
<td><strong>BBT and BTB</strong></td>
<td></td>
</tr>
<tr>
<td>P-glycoprotein, ABCG2</td>
<td>Efflux pumps at BBB</td>
</tr>
</tbody>
</table>

BBB: Blood-brain barrier; BTB: Blood-tumor barrier.
Brain metastases affect up to 40% of cancer patients and are 8–10-times more common than primary brain tumors. Brain metastases are most common in lung cancer, breast cancer and melanoma. Localization: 80% in cerebral hemispheres, 15% in cerebellum, 5% in the brainstem. Typically found at gray–white matter junctions and terminal watershed areas. Brain metastases have high morbidity and mortality and are associated with poor quality of life. Standard treatment options include neurosurgery, radiotherapy (whole-brain radiotherapy, stereotactic radiosurgery), while systemic cytotoxic chemotherapy seems to have limited efficacy. The blood–brain barrier/blood–tumor barrier limits penetration of some systemic agents into the brain and may limit intracerebral efficacy. Molecular targeted agents have shown evidence for clinical activity against brain metastases in specific patient populations: BRAF inhibitors and ipilimumab in melanoma, EGFR inhibitors in non-small-cell lung cancer, lapatinib in HER2-positive breast cancer. Prevention of brain metastases by targeted agents may be feasible and this concept is being tested in clinical studies, for example, with chronic low-dose anti-angiogenesis.

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1. of interest
2. of considerable interest


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**Implementation of advanced imaging modalities may inform clinical management and prognosis in brain metastasis patients.**


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ClinicalTrials.gov Identifier: NCT01266967.
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Spinal Cord Compression
Summary
This paper updated the 2005 Cancer Care Ontario practice guidelines for the diagnosis and management of adult patients with extradural malignant spinal cord compression. Surgery should be considered for good prognosis patients who are medically and surgically operable. Radiation (RT) should be given to nonsurgical patients. For those with poor prognosis, a single fraction of 8 Gy should be given; for those with good prognosis, 30 Gy in 10 fractions could be considered.

Purpose: To update the 2005 Cancer Care Ontario practice guidelines for the diagnosis and treatment of adult patients with a suspected or confirmed diagnosis of extradural malignant spinal cord compression (MESCC).

Methods: A review and analysis of data published from January 2004 to May 2011. The systematic literature review included published randomized control trials (RCTs), systematic reviews, meta-analyses, and prospective/retrospective studies.

Results: An RCT of radiation therapy (RT) with or without decompressive surgery showed improvements in pain, ambulatory ability, urinary continence, duration of continence, functional status, and overall survival. Two RCTs of RT (30 Gy in eight fractions vs. 16 Gy in two fractions; 16 Gy in two fractions vs. 8 Gy in one fraction) in patients with a poor prognosis showed no difference in ambulation, duration of ambulation, bladder function, pain response, in-field failure, and overall survival. Retrospective multicenter studies reported that protracted RT schedules in nonsurgical patients with a good prognosis improved local control but had no effect on functional or survival outcomes.

Conclusions: If not medically contraindicated, steroids are recommended for any patient with neurologic deficits suspected or confirmed to have MESCC. Surgery should be considered for patients with a good prognosis who are medically and surgically operable. RT should be given to nonsurgical patients. For those with a poor prognosis, a single fraction of 8 Gy should be given; for those with a good prognosis, 30 Gy in 10 fractions could be considered. Patients should be followed up clinically and/or radiographically to determine whether a local relapse develops. Salvage therapies should be introduced before significant neurologic deficits occur. © 2012 Elsevier Inc.

Keywords: Cancer, Malignant spinal cord compression, Radiotherapy, Surgery

Note—An online CME test for this article can be taken at http://astro.org/MOC.
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Introduction

Malignant spinal cord compression (MSCC) is one of the most dreaded complications of metastatic cancer. MSCC can be divided into intradural (intramedullary and leptomeningeal) and extradural (MESCC). Its natural history, if untreated, is usually one of relentless and progressive pain, paralysis, sensory loss, and sphincter dysfunction. A population-based study of cancer patients reported that MSCC is fortunately not common; between 1990 and 1995, 2.5% (n = 3458) of all cancer patients who died of their disease had at least one admission for MSCC (1). The incidence of MSCC varied widely by primary cancer site, from 7.9% in patients with melanoma to 0.2% in patients with pancreatic cancer (1).

Our group has published two previous evidence-based clinical practice guidelines for the diagnosis and management of MESCC in 1998 (2) and in 2005 (3). The latter guideline was formally developed and approved through Cancer Care Ontario’s Program in Evidence-Based Care, which recommends that the guidelines be reviewed regularly and updated when potentially practice-changing data have been published. Since the most recent guideline, several randomized controlled trials and one evidence-based guideline have been published; however, to our knowledge no clinical practice guidelines have been issued.

The objective of this update was to systematically review the literature since the most recent guideline and to summarize the data. In addition, whereas the previous guideline was more of an evidence-based summary, this update also includes clinical practice guidelines. The questions identified in the 1998 guideline were again addressed, including the role for systemic steroids in the management of MESCC; the indications for surgery and radiotherapy (RT); and recurrent MESCC in an area previously irradiated. The two questions added in the 2005 guideline—the clinical symptoms of MESCC and investigating suspected MESCC—were not addressed because there have been few data answering these questions.

Methods

For the 2011 clinical practice guideline update, this systematic review formed the basis of an evidence summary for use in Ontario, Canada (http://www.cancercare.on.ca/access_PEBCC.htm). The literature search strategy was adopted from the initial review by Loblaw et al. in 2005 (3).

For the questions regarding the symptoms and diagnosis of MESCC, MEDLINE (2004 to May 2011) and Cochrane Database of Systematic Review, Cochrane Central Register of Controlled Trials (2004 to May 2011) databases were searched by using the search strategy (“spinal cord compression” or “cauda equina/ and nerve compression syndromes”) and (“spinal cord neoplasms/ or spinal neoplasms/”) and (“CT scan” and “magnetic resonance imaging”) or (“CT scan” and “myelograph”) or (“magnetic resonance imaging” and “myelograph”). For questions on the use of steroids, surgery, RT, dosage of RT, and recurrent MESCC, the literature search strategy used in the original publication was adopted and included articles from 2004 to 2011. MEDLINE (2004 to 2011) and Cochrane Library (2004 to May 2011) databases were searched by using the search strategy (“spinal cord compression” or “cauda equina/ and nerve compression syndromes”) and (“radiation therapy” or “surgical resection”). All the terms were then combined with the search terms for the following publication types and study designs: practice guidelines, systematic reviews or metaanalyses, randomized controlled trials (RCTs), and controlled clinical trials. Also, abstracts published in the proceedings of the annual meetings of the American Society of Clinical Oncology (through May 2011) and the American Society of Radiation Oncology (2004 to May 2011) were systematically searched for evidence relevant to this systematic review.

In addition, the Physician Data Query clinical trials database (http://www.cancer.gov/search/clinical_trials/) was searched for new or ongoing trials. The Canadian Medical Association Infobase (mdm.ca/cpgsnw/cpgs/index.asp) and the National Guidelines Clearinghouse (www.guideline.gov/index.asp) were searched for evidence-based practice guidelines.

Table 1 describes the details of the inclusion criteria and outcome variables for each question addressed in this systematic review. For each question, only full English publications and abstracts describing adult patients with extradural cord compression, but not intramedullary and leptomeningeal cord compression, were included. Case studies, case series, and duplicate articles were excluded (the most recent article in a series was included).

Results

Evidence

The literature search identified 101 articles; 28 met the inclusion criteria. Reasons for exclusion were as follows: not directly addressing guideline questions (7), not English (1), duplicates (9), case studies (9), no access to article (3).

Evidence summary

Is there a role for systemic steroids in the management of MESCC, and if there is, what is the optimal dose? Since publication of the initial guidelines, was one RCT (4) investigating the role for systemic steroids in the management of MESCC. The feasibility study by Graham et al. (4) randomized 20 patients with MESCC treated with 30 Gy in 10 fractions to dexamethasone 96 mg/day or 16 mg/day. The study authors concluded that a larger trial could not be conducted in Australia. They were unable to report any differences in toxicity, ambulation rates, or survival in this small study.

What are the indications for surgery in the management of MESCC? Since publication of the 2005 guidelines, one RCT (included as an abstract in the previous guideline) (5), one meta-analysis (6), and five retrospective studies (7–11) have been published.

The strongest evidence comes from a randomized multi-institutional control trial by Patchell et al. that randomized 101 patients with MESCC confirmed by magnetic resonance imaging (cauda equina lesions excluded) to receive decompressive surgical resection with RT 14 days later, or RT alone of 30 Gy in 10 fractions (5). It was published in abstract and included in the previous guideline, but the full article has since been published. All patients were directed to receive dexamethasone 100 mg bolus plus 96 mg daily (dose reduced for patients with contraindications to high-dose steroids). Patients were stratified by institution, tumor...
type, ambulatory status, and spinal stability; 38% of accrued patients had spinal instability.

The authors reported that patients undergoing surgery in addition to RT (30 Gy in 10 fractions) were more likely to retain or maintain their ambulatory status longer than were patients receiving RT alone (84% vs. 57%, \( p = 0.001 \)). In addition, patients assigned to the combined modality arm experienced better ambulatory time (122 days vs. 13 days, \( p = 0.003 \)), urinary continence (74% vs. 57%, \( p = 0.005 \)), duration of continence (median 157 days vs. 17 days, \( p = 0.016 \)), functional status (maintenance of Frankel and American Spinal Injury Association (ASIA) ASIA scores, \( p = 0.001 \)). There was a difference in survival favoring the combined modality arm (median 126 days vs. 100 days, \( p = 0.033 \)).

In the study by Patchell et al. (5), maintenance of ASIA and Frankel scores was independently predicted by spinal instability, cervical spinal lesion, surgery, and baseline Frankel score. Of note, baseline Frankel score and use of surgery plus RT were independently predictive of time to ambulation (not spinal stability).

Citing concerns about small sample size and patient selection (it took 10 years to accrue 101 patients in a multicenter study) for the above study, Rades et al. performed a retrospective, 11-variable matched control study of RT vs. surgery plus RT (2:1 matching) (11). In that study, 108 patients who received decompressive surgery plus RT were matched to 216 patients receiving RT alone. Overall, there was no difference in any of the outcomes examined: improvement in motor function (27% vs. 26%, \( p = 0.92 \)), post-treatment ambulatory rates (69% vs. 68%, \( p = 0.99 \)), recovery of ambulation among nonambulatory patients (30% vs. 26%, \( p = 0.86 \)), 1-year local control (90% vs. 91%, \( p = 0.48 \)), and 1-year overall survival (47% vs. 40%, \( p = 0.50 \)).

Klimo et al. performed a meta-analysis of conventional RT vs. surgery for the management of MESC (6). Their search strategy ended in 2003; they identified four RT studies involving 578 patients and 24 surgical studies involving 1020 patients. Surgically treated patients were more likely to recover ambulation (85% vs. 64%) and to have better pain control (90% vs. 70%) than were patients treated with primary RT. No prognostic and predictive factors were adjusted for in their analysis, including bony retropulsion/spinal instability (3), pretreatment neurologic status, presence of visceral metastases, and tumor type (12).

Two retrospective studies examined outcomes for radioresistant tumors; neither compared outcomes with a group of patients treated with initial surgery. Rades et al. (13) reported the outcomes in 87 patients with renal cell cancer treated with RT. Freundt et al. reported on 51 patients with oligometastatic radioresistant disease (renal cell cancer, colorectal cancer, and melanoma) who were treated with 30 Gy or higher doses (8). Other studies included radioreistant tumors (14, 15) but have not reported outcomes separately. Those patients with renal cell cancer

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### Table 1  Summary of study inclusion criteria and outcomes of interest

<table>
<thead>
<tr>
<th>Question</th>
<th>Inclusion criteria</th>
<th>Outcomes</th>
<th>Number and type of studies included</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Is there a role for systemic steroids in the management of MESC, and if so, what is the optimal dose?</td>
<td>RCTs comparing the use of steroids with other steroid regimens or no steroids</td>
<td>Rate of retaining or regaining ambulation</td>
<td>1 RCT (4)</td>
</tr>
<tr>
<td>2. What are the indications for surgery in the management of MESC?</td>
<td>RCTs comparing surgical procedures with other procedures or no surgery at all</td>
<td>Rate of retaining or regaining ambulation</td>
<td>1 RCT (5)</td>
</tr>
<tr>
<td>3. What are the indications for radiotherapy in the management of MESC?</td>
<td>RCTs comparing radiotherapy with other treatments (i.e., surgery)</td>
<td>Rate of retaining or regaining ambulation</td>
<td>No new articles</td>
</tr>
<tr>
<td>4. Is there an optimal dose prescription for radiotherapy?</td>
<td>RCTs comparing dosages of radiotherapy</td>
<td>Rate of retaining or regaining ambulation</td>
<td>2 RCTs (14, 15)</td>
</tr>
<tr>
<td>5. What are the treatment options for recurrent MESC in an area previously irradiated?</td>
<td>RCTs comparing treatments for patients with recurrent MESC in an area previously irradiated</td>
<td>Rate of retaining or regaining ambulation</td>
<td>1 RCT (4)</td>
</tr>
</tbody>
</table>

**Abbreviations:** RCT = randomized control trial; MESC = malignant epidural spinal cord compression.
seemed to have the same ambulatory outcomes as did all MESCC patients treated with RT (Table 2).

No new studies have been identified that add to the above question on bony compression or tumors of unknown primary origin.

**What are the indications for RT in the management of MESCC?**

Since the initial guidelines were published, no studies have expanded the indications for RT for the management of MESCC.

**Is there an optimal dose fractionation schedule in the management of MESCC?**

In this update, the following studies were identified that address the role of RT in the management of MESCC: two randomized control trials (14, 15), two systematic reviews (16, 17), two prospective studies (18, 19), and three retrospective multicenter studies (13, 20, 21).

For patients with a poor prognosis, Maranzano and colleagues have reported two randomized control trials addressing the question of dose fractionation schedule. These patients were defined as all patients having tumors with poor histologic features (melanoma or lung, sarcoma, gastrointestinal, head-and-neck, or kidney cancers) or those having tumors with good histologic features tumors with any functional impairment or poor performance status. The first study, reported in 2005, randomized 300 patients to a split course of RT (15 Gy in three fractions, 4-day break, then 15 Gy in five fractions), or hypofractionated RT (8 Gy in two fractions of radiotherapy)

The second study by Maranzano et al. reported in 2009, randomized 327 patients with a poor prognosis (as above) to 16 Gy in two fractions over 1 week vs. 8 Gy in one fraction (15). Dexamethasone 16 mg/day was given to both groups. The conditions of 303 patients were analyzable; the median follow-up time was not reported (but seems to be approximately 5 months, from the Kaplan-Meier plots). Again, no significant differences were reported between the treatment arms for ambulation, duration of ambulation, bladder control, pain response, and overall survival (Table 2). Of note, this study noted a nonsignificant trend toward greater in-field failures, favoring the two-fraction arm (2.5% vs 6.0%, p = 0.12).

Similar rates of post-treatment ambulation and overall survival were seen in earlier published retrospective (13, 20, 21) and prospective (22) studies regardless of dose fractionation schema (from 8 Gy in one fraction to 40 Gy in 20 fractions using conventional RT techniques). In a prospective two-arm, non-randomized study of short-course vs. long-course RT of 265 patients with MESCC, short-course and long-course RT (short course: 8 Gy in one fraction or 4 Gy in five fractions; long-course: 3 Gy in 10 fractions, 2.5 Gy in 15 fractions, or 2 Gy in 20 fractions) again resulted in similar functional outcomes (motor improvement 37% vs. 39%, p = 0.95) and 1-year overall survival (23% vs. 30%, p = 0.28). The RT schema was not predictive on multivariate analysis for either outcome. However, compared with short-course RT, long-course RT improved 12-month progression-free survival (72% vs. 55%, p = 0.034), and 12-month local control (81% vs. 61%, p = 0.005) (22). The systematic reviews did not provide a metaanalysis of those data (16, 17).

The second prospective study examined radiosurgery (12–20 Gy given in one fraction, median 16 Gy) (19). This study by Ryu et al. reported on 85 treated lesions in 62 patients. Of note, patients were required to have grade 4 or 5 power, so lesions associated with significant neurologic features and radiosensitive tumors (lymphoma, myeloma, small cell cancers, and germ cell cancers) were excluded. They reported reduced tumor area and increased thecal sac patency after treatment (p < 0.001 for both). More importantly, 33 of 35 (94%) neurologically intact patients at presentation remained intact; 17 of 27 (63%) of patients with objective neurologic deficits (21 with motor loss, 6 with sensory loss) improved (52% complete response, 11% partial response). Treatment was well tolerated, with no grade 2+ acute toxicities.

**What are the treatment options for recurrent MSCC in an area previously irradiated?**

Since the previous guideline, one nested prospective study (23) has been published on this issue. Using data from their two prospective RCTs, Maranzano et al. identified 24 patients (representing 4.2% of the 579 randomized patients treated with 8–30 Gy in one to eight fractions) who had an in-field recurrence. Twelve of these patients were retreated with RT (4–20 Gy in one to four fractions; cumulative dose <120 Gy2). Six of 7 (83%) ambulatory patients before reirradiation maintained ambulation. None of the nonambulatory patients recovered ambulation. No patient experienced radiation myelopathy.

**2011 clinical practice guidelines**

**Is there a role for systemic steroids in the management of MESCC, and if there is, what is the optimal dose?**

If not medically contraindicated, steroids are recommended for any patient with neurologic deficits suspected or confirmed to have MESCC, particularly if they are being treated with short-course RT (five or fewer fractions). Patients being treated with surgery will often need postoperative RT (5) and therefore should receive maintenance steroids. Those treated with protracted

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**Table 2** Outcomes in patients treated with single vs. multiple fractions of radiotherapy

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Hypofractionation study (14)</th>
<th>Single-fraction study (15)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>30/8 (n = 134)</td>
<td>16/2 (n = 142)</td>
</tr>
<tr>
<td>Ambulation</td>
<td>71%</td>
<td>68%</td>
</tr>
<tr>
<td>Duration of ambulation (med)</td>
<td>3.5 mo</td>
<td>3.5 mo</td>
</tr>
<tr>
<td>Bladder control</td>
<td>90%</td>
<td>89%</td>
</tr>
<tr>
<td>Pain response</td>
<td>59%</td>
<td>56%</td>
</tr>
<tr>
<td>Overall survival</td>
<td>4.0 mo</td>
<td>4.0 mo</td>
</tr>
<tr>
<td>Acute toxicity, grade 3</td>
<td>3.7%</td>
<td>2.1%</td>
</tr>
<tr>
<td>In-field failure</td>
<td>—</td>
<td>2.5%</td>
</tr>
</tbody>
</table>

* p = 0.0.
courses of RT (six or more fractions) who have no neurologic deficits or those prescribed high-dose steroids (5) can have the steroids weaned over at least 2 weeks once treatment is started. A bolus of 8 to 10 mg dexamethasone (or equivalent) can be given, followed by 16 mg/day (usually in twice-daily to four-time-daily doses for tolerance)(14, 15). Patients with dense paraparesis (Grade 3 or worse) should be considered for higher bolus (100 mg) and maintenance doses (up to 96 mg per day) (5, 24), but the risk of serious adverse events should be considered (24, 25). Patients with radiographic MESCC but no neurologic deficits do not require steroids (26).

What are the indications for surgery in the management of MESCC?

Surgery is associated with significant morbidity, which needs to be considered in the decision between surgery and RT for medically operable patients with a single area of compression and no spinal instability or bony compression. Surgery should be considered for patients with a good prognosis (Appendix E1) who are medically operable. Minimally invasive techniques are being more widely performed, and every effort should be made to minimize the surgical footprint without compromising the adequacy of the decompression or spinal stability. Complications are to be avoided, and postoperative resolution times need to be as short as possible (27). Technical factors that allow proper fixation/stabilization (if required) need to be considered for any technique. A surgical consultation should be provided within 24 hours of the radiographic diagnosis of MESCC to prevent the neurologic progression associated with delays (28, 29). Prophylactic stabilization should be considered in impending cases of MESCC.

What are the indications for RT in the management of MESCC?

All patients who are not treated with primary surgery should receive RT with or without steroids.

Is there an optimal dose fractionation schedule in the management of MESCC?

For patients with a poor prognosis, a single fraction of 8 Gy is recommended over more protracted courses of RT. Poor prognosis can be judged either by the Maranzano criteria or if the patient falls in Group I or II of the MESCC prognosis scale (Appendix E1). Patients should be followed up clinically and/or radiographically to determine whether a local relapse develops.

Patients with a good prognosis (ambulatory patients, those with good histologic features, or those in Group III of the MESCC prognosis scale) should be enrolled in clinical trials; further study is needed to demonstrate that higher doses of RT are beneficial in these patients. Inasmuch as a prospective nonrandomized study of short-course vs. long-course RT resulted in improved progression-free survival and local control (but no difference in functional outcomes or overall survival on primary or multivariate analyses) (22), outside a clinical trial, 30 Gy in 10 fractions could be considered, particularly when local control is of high value and/or close follow-up is burdensome.

An alternative way of biologic dose escalating is with radiosurgery/stereotactic body RT. This technique has the added advantage of cord sparing and reduction in treatment volume. This may translate into improved local control in patients with a good prognosis who have limited spinal metastases. There may also be a role for radiosurgical epidural decompression in a selected group of MESCC patients; however, this is yet to be defined, and radiosurgery/stereotactic body RT is unlikely to be able to be used as an emergency procedure, given the time required for planning and treatment verification (19). Radiosurgery is to be compared with conventional RT as frontal treatment for spinal metastases in a randomized trial. This study is being conducted by the Radiation Therapy Oncology Group and will include patients with a limited (one to three) number of spine metastases, with or without minimal epidural compression.

What are the treatment options for recurrent MSCC in an area previously irradiated?

Patients should be followed up clinically and/or radiographically to determine whether a local relapse develops. As with the first MESCC diagnosis, prognosis, the probability of neurologic recovery, and time to neurologic recovery are highly dependent on pretreatment neurologic status (3). Patients should be considered for surgical decompression with or without RT first, because salvage rates seem to be better despite higher complication rates (5). If a patient is not medically and surgically operable, RT with or without steroids should be given. The dosage and technique of RT should be chosen to keep the cumulative dose of RT less than 120 Gy (30, 31). Newer RT techniques such as stereotactic RT can be used to minimize cord dose (19, 31).

Ongoing Clinical Trials

The clinicaltrials.gov search revealed two ongoing studies and two terminated studies. The All Ireland Cooperative Oncology Research Group has been recruiting a 200-patient RCT of 20 Gy in five fractions vs. 10 Gy in one fraction (NCT00968643) since 2006. The same group is also recruiting to a Phase 2 study of reinirradiation (NCT00974168). The study of 40 patients opened in 2007 and was anticipated to close in 2011.

An international consortium of trialists is running a trial of 8 Gy in one fraction vs. 20 Gy in five fractions of RT (SCORAD III) for all-prognosis patients (ISRCTN97108008). The study was centrally activated in 2009; the sample size is 700 patients.

References


Anti-Tumour Treatment

Current and emerging concepts in non-invasive and minimally invasive management of spine metastasis

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SUMMARY

To provide a comprehensive review on the presentation, work-up and the management of spine metastasis with or without epidural spinal cord compression with focus on the roles of surgery and radiotherapy. Emphasis has been laid on the technological advances with recent development of stereotactic body radiotherapy (SBRT) or radiosurgery (SRS) and minimally invasive surgical approaches like kyphoplasty and vertebroplasty.

Introduction

Incidence of spine metastases (SM) is likely to increase as cancer patients live longer partly due to early detection and also because of improvements in medical care.1 In 2010, an estimated 1.53 million cases of newly diagnosed cancer were expected in the US alone.2 Bone metastases are a frequent complication of cancer and bones are the 3rd most common site for metastases following lung and liver.3 Approximately 350,000 people die each year with bone metastases, 2/3rds of which are due to advanced prostate and breast cancers.

Spine metastasis

Of the various bones, the spine is the most common site of metastases.4 More than 1/3rd of patients with cancer have vertebral metastases on autopsy.5 Within the spine the incidence varies, thoracic (68–70%), lumbo-sacral (16–22%) and cervical (8–15%).6 Median survival with SM usually ranges from 3 to 18 months.7–10 Patients with favorable histologies like thyroid, breast and prostate may live longer.11 The highest incidence of SM is found in the 40–65 age-groups as this is the period of highest cancer risk.12 Overt cord compression develops in 10–20% of patients with preexisting spinal disease and in 5–10% of all cancer patients.13 Vertebal and/or epidural (extradural) involvement is seen in 90–95% of SM.12,14 Intra-dural extra-medullary and intra-medullary seeding of systemic cancer is unusual.14 Lepto-meningeal disease occurs in about 10% of patients. Symptomatic spinal metastases may be the initial manifestation of malignancy in 12–20% of cases.15 Primary tumors most likely to metastasize to the vertebral column are breast (16–37%), prostate (9–15%), lung (12–15%), kidney (3–6%), and thyroid (4%).16 Variable involvement of the vertebrae can be seen with the metastasis to the vertebral body being most common (70%) followed by the lamina and pedicles (30%) and a mixed picture (10%). The posterior half of the vertebral body is usually involved first, with the anterior body, lamina, and pedicles usually affected later.

Pathways of spread

At least four paths of spread exist. First, the rich (growth factors) and vascular red marrow constitutes the arterial route.17 Second, spread via venous routes is often accomplished through the Batson plexus,18 the longitudinal network of valve-less veins, located in the epidural space between the spinal column bone and the dura mater, that connects vertebral veins with many other beds of venous drainage, including the caval, portal, azygous, intercostal, pulmonary, and renal systems. Third, direct invasion through the inter-vertebral foramina (e.g. lymphomas) can occur. And lastly, via lymphatics, though their role is not conclusive.

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Keywords:
Spine metastasis (SM)
Kyphoplasty
Vertebroplasty
Radiation therapy (RT)
Stereotactic radiosurgery (SRS)
Spinal cord compression (SCC)
Effects of SM and its clinical presentation

Two main effects include producing a mass effect and causing a vascular compromise. An epidural mass can cause cord distortion, resulting in demyelination or axonal destruction. Production of venous congestion and of vasogenic edema of the spinal cord can result in venous infarction and hemorrhage. Presentation can be highly variable and depends on the vertebral level and severity of involvement. The most common presenting symptom is axial bone and back pain in about 85–96% of cases.19 Pain almost always precedes the loss of neurological function.20,21 Sensory and motor dysfunction due to radiculopathy can be seen in up to 50%, and a smaller number present with have bowel, bladder or sexual dysfunction. Spinal cord compression fortunately develops only in 5–10% of cases. SM may be the initial presentation in about 20% of patients with systemic cancer.15

Diagnosis

A thorough physical and neurologic exam would help delineate the vertebral level of involvement and assess for any neurological impairment. Kyphosis is the most commonly observed clinical deformity secondary to compression fractures and decreased ambulation.20 X-ray findings become apparent only when vertebral trabecular bone destruction reaches 30–50%.22 Overall predictive value of plain X-rays is insufficient to warrant their routine use to investigate possible SM.23 Bone scan is a highly sensitive diagnostic tool and can detect SM as small as 2 mm in size. Compared to X-rays, it is able to detect bone metastases 3–18 months earlier.24 It has the drawback of low sensitivity for osteolytic lesions (e.g. Multiple myeloma, Renal Cell Ca, etc.) and also does not give an accurate anatomic spine level.

CT scan allows visualization of even small areas of vertebral destruction, assessment of extent of paravertebral soft tissue extension, extent and direction of impingement of spinal cord. It also best helps assess the osseous anatomy of surrounding spine prior to surgical stabilization. However the sensitivity is only 66% while its diagnostic accuracy is about 89%.25 MRI has the greatest sensitivity (98.5%) and specificity (98.9%) with an overall accuracy of 98.7%.25 It is superior in evaluating the neural elements (i.e. leptomeninges and spinal cord) and to detect multiple levels of vertebral involvement. It is the imaging modality of choice to detect SM.26,27 CT myelography can be performed for people who cannot get an MRI (due to metal implants/pacemakers) or when an MRI is not available to detect cord pathology.28,29 It is highly recommended to get MRI imaging of the entire axial spine.30 About 15% patients will have other lesions.31 MRI has also been shown to influence RT planning and in one study it led to plan changes in about 53% of patients with 21% being major changes.32 PET/CT scan has emerged as an imaging tool for initial staging of many malignancies.33,34 FDG-PET scans have a significantly higher sensitivity, specificity and negative predictive values as compared to a bone scan.35

Management

The goals of therapy are pain control and functional preservation. Pain can be easily and quickly assessed using a Brief Pain Inventory33 or a Visual Analog Scale. Performance status can be assessed using either the Eastern Cooperative Oncology Group (ECOG)34 or the Karnofsky Performance Scale.35 Primary tumor site is an important predictive factor for survival.11,13,14,36,37 Another very important prognostic indicator is the initial functional status.38 The ability to ambulate at the time of presentation is a favorable prognostic sign while loss of sphincter control is a poor prognostic feature and mostly irreversible. Most ambulatory patients remain ambulatory after treatment while few paraplegic patients are able to walk after treatment (outcome after treatment = neurological impairment before treatment). Thirty percent of patients who present with weakness progress to paraplegia within 1 week.39 The likelihood of neurologic recovery is poor when paraplegia has been present for $\geq$ 24 h. Neurologic impairment scales have been developed; American Spinal Injury Association (ASIA)40 and Frankel score.41

A multi-disciplinary approach is needed to best serve a patient. With improvements in chemotherapy, hormonal therapy, surgery and radiation therapy, survival has also improved.42

General

Management of overall health condition is essential as these patients likely have uncontrolled systemic disease burden and comorbidities. Hypercalcemia may require hydration, bisphosphonates and steroids. One needs to be cautious about overt signs of acute renal failure.

Pain

Pain can be localized and constant (due to periosteal stretching) or could be mechanical (position or movement related). If the nerve root is compressed, pain can be of sharp and shooting (radicular) quality.52 NSAID’s, gabapentin and narcotics can be used individually or in combination to manage this symptom. Evidence based standards for cancer pain management have been formulated.43

Medical

Depending on the type of primary (i.e. lymphomas, small cell lung cancer, germinomas, etc.) chemotherapy may have a role. They would typically be used in an asymptomatic patient. Hormonal therapy may be beneficial in endocrine dependent malignancies like breast and prostate.54,55 Bone strengthening agents like bisphosphonates (e.g. zoledronate) inhibit osteoclast-mediated bone resorption and induce osteoclast apoptosis.46 Denosumab is a new mono-clonal antibody designed to target RANK ligand (RANKL), a protein that acts as the primary signal to promote bone removal. Both zoledronic acid and denosumab have been shown to decrease the incidence of skeletal related events including pathologic fractures, spinal cord compression, severe pain requiring radiotherapy or surgery, and Hypercalcemia.47,48

Scoring systems

There are established scoring systems to help guide the decision making of surgery or RT. These are listed in Table 1. Harrington49 classified lesions based on structural integrity and neurological dysfunction. Tokuhashi et al.36 proposed a scoring system for pre-operative evaluation SM which was later revised.11 Other prognostic scoring systems by Tomita et al.,37 Van der Linden et al. and Bauer25 have also been established to guide treatment decisions. A modified Bauer scoring system excluding pathologic fracture as a prognostic factor has been proposed.50 Despite these, a recent analysis evaluating seven pre-operative scoring systems reported inaccuracies.50 Another decision making metric (NOMS) has been developed by Bilsky et al. which considers Neurologic factors (N), Oncologic factors (O), Mechanical Instability (M), and Systemic Disease including comorbidities (S) in making treatment decisions. It provides an algorithm based on these four categories which helps clinicians to make decisions about surgery vs. RT in the
treatment of spine metastasis. This remains to be validated prospectively.

A simple decision making tree to guide the choice of therapy has been depicted in Fig. 3. Expected life expectancy has a major determining role for surgery with preference for a non-surgical palliative approach if predicted to be less than 3–6 months. A word of caution, clinician assessment alone is inadequate in predicting survival in patients with osseous metastases.

Surgery

Historically the treatment of SM has consisted of RT ± surgery. It is estimated that less than 10% of patients with SM undergo surgery. Surgical risks must be weighed against life expectancy and quality of life to justify surgical intervention. Newer surgical techniques allow access to the vertebral body where 70% of spine metastases occur. The benefit of surgery is the ability to provide mechanical stabilization, pain relief and maintenance of neurologic function. Until recently there has been no accepted scoring system to assess spinal instability and the need for surgery. Recently the Spinal Instability Neoplastic Score (SINS) has been proposed by the Spine Oncology Study Group (SOSG) to identify the patients who require surgical stabilization. Spine instability is assessed by adding scores related to six factors including: location of the tumor within the spine, pain, lesion bone quality, radiographic alignment, vertebral body collapse, and posterolateral involvement of the spinal elements. SINS scores range from 0 to 18. A score of 0–6 indicates stability, 7–12 indicates indeterminate instability, and 13–18 is indicative of instability. Any patient with a score greater than 7 should have a surgical consultation. The SINS has been shown to have a sensitivity of 95.7% and a specificity of 79.5% in identifying potentially unstable or unstable lesions.

Another systematic approach for identifying spinal instability was proposed by Cybulski. It uses a three column model of the spine in which the anterior column consists of the anterior half of the vertebral body, the anterior longitudinal ligament and the annulus fibrosus. The middle column contains the posterior annulus, the posterior wall of the vertebral body and the posterior longitudinal ligament. The posterior column contains the neural arch, facets, ligamentum flavum, supraspinous and interspinous ligaments. Using these definitions spinal instability is considered to be one of the following: Anterior and middle column destruction (loss of 50% of vertebral body height), compression or collapse of 2 or more adjacent vertebral bodies, tumor involvement of the posterior and middle columns or iatrogenic, described as laminectomy performed without identification of existing anterior and/or middle column disease.

Goals of surgery in spinal metastases

(1) Decompression of neural structures (75% neurologic improvement), (2) pain relief (80–95%), (3) debulking or removal of tumor mass, (4) spinal stabilization to prevent deformity and allow mobilization.
Indications for surgery in spinal metastases

(1) Progressive neurologic deficit before, during or after RT, (2) intractable pain unresponsive to conservative treatment, (3) need for histologic diagnosis, (4) radio-resistant tumor histology (i.e. RCC, Melanoma), (5) spinal instability (i.e. vertebral collapse).

Surgical strategies

Decompressive laminectomy

Historically laminectomy was performed in patients with symptoms of compressive spinal cord metastases, but has been shown to have little to no added benefit over radiation therapy alone.61 Laminectomy is also likely to increase mechanical instability by destabilizing the posterior elements of the vertebrae while doing nothing to bolster the vertebral body. Since the vertebral body is affected in 70% of spine metastases and bears 80% of the axial load of the spine, surgical approaches which allow access to the anterior column for decompression and stabilization are more effective than laminectomy.62

Modern surgical approach

The development of improved perioperative care, surgical technique and spinal implants has allowed for better stabilization of the anterior spinal column.57 A recent prospective study by Patchell et al.63 compared surgical decompression and stabilization of spine metastases combined with postoperative radiation therapy versus radiation therapy alone. Surgical approach was determined by tumor location in the individual patient. Anterior or lateral approaches were used to access the vertebral body when indicated.

Kyphoplasty/vertebroplasty

Kyphoplasty and vertebroplasty are percutaneous techniques that provide stabilization and strengthening of compromised vertebrae. Vertebroplasty involves injection of polymethylmethacrylate (PMMA) cement directly into the compromised vertebral body under fluoroscopic guidance and requires an intact vertebral body cortex. Pain receptors are destroyed by an exothermic reaction of the cement and also due to the compression of small nerve endings. PMMA is stable to the effects of radiotherapy and could be done before or after RT depending on the clinical scenario (Weng et al.64). Kyphoplasty is a technique that involves percutaneous curettage of the affected vertebral body followed by inflation of a balloon in the vertebral body and subsequent injection of PMMA cement into the newly formed cavity. The balloon expansion allows for restoration of vertebral height in addition to stabilization.64 Working cannulas are then inserted bilaterally into the posterior aspect of the vertebral body under fluoroscopy. Balloons are then inflated bilaterally creating a cavity into which the PMMA cement is subsequently injected.65 This technique provides stability to the anterior column of the spine, reduces kyphotic deformity and relieves pain.66

Indications for kyphoplasty include painful vertebral body metastases or spinal instability without associated neurologic deficits. The benefit of kyphoplasty and radiation therapy over radiation therapy alone is the ability to stabilize the spine and prevent subsequent fractures. Additionally, kyphoplasty may be used in sequence with conventional radiation therapy or stereotactic radiosurgery techniques. Contraindications to kyphoplasty include allergy to PMMA, compression of neurologic structures, poor overall condition and short life expectancy.57 The SOSG has reviewed the pertinent literature related to vertebroplasty and kyphoplasty had recommended the procedures as safe and effective in improving pain and functional outcomes in patients with vertebral body metastases.67 A potential complication of kyphoplasty and vertebroplasty is cement leakage. It has been shown to occur in between 8%-73% of cases on radiograph and up to 93% of cases on CT, but this wide range may be attributable to variances in procedural technique and radiographic detection of leakage.57,68 Symptomatic side effects occur in only 10% of kyphoplasty or vertebroplasty cases and major complications occur in less than 5%.69-70

Pain relief

Complete or partial pain relief has been described in 50–100% of patients undergoing kyphoplasty or vertebroplasty for spine metastases, with most recent studies showing pain relief in 80–100% of patients.66 Table 2 shows the rates of pain relief described in the literature after vertebroplasty/kyphoplasty for spine metastases.

Quality of life scales

A review of the relevant literature reveals that ability to ambulate, Visual Analog Scale (VAS), morphine equivalents, Frankel score, Karnofsky, ODI, ASIA score and ECOG status have all been used to approximate quality of life in patients treated for spine metastases.63,74,76,81 The Edmonton Symptom Assessment System (ESAS)82 has also been used and has been shown to be accurate and reliable in assessing post treatment quality of life.81-83 Recently however the SOSG released a quality of life tool specific to patients with metastatic disease of the spine called the SOSG Outcomes Questionnaire (SOSGOQ). The content of the SOSGOQ has been validated based on the International Classification of Function and Disability (ICF) and has been shown to have superior content compared to previously reported quality of life tools.84

Timing of surgery and radiotherapy

RT has been used after surgery to achieve durable local tumor control. If RT is given immediately after surgery, the inflammatory response is inhibited and the number of inflammatory cells is reduced.85 Timing of post-op RT is at least 1 week after (preferably 2–3 weeks) to allow adequate soft tissue healing.86 RT could be delayed to 4–6 weeks if a bone graft is used. Preoperative RT has been described in the literature but has been shown to have adverse effects on surgical outcome, specifically with regards to wound healing. In a retrospective study 32% of patients who had RT before surgery had major wound complications while only 12% of those who had surgery prior to RT had such complications.87 The role

### Table 2

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>No. patients</th>
<th>Procedure</th>
<th>Pain relief (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton et al.</td>
<td>1996</td>
<td>37</td>
<td>VP</td>
<td>75%</td>
</tr>
<tr>
<td>Weill et al.</td>
<td>1996</td>
<td>37</td>
<td>VP</td>
<td>73%</td>
</tr>
<tr>
<td>Cortet et al.</td>
<td>1997</td>
<td>37</td>
<td>VP</td>
<td>97%</td>
</tr>
<tr>
<td>Fourney et al.</td>
<td>2003</td>
<td>56</td>
<td>VP/ KP</td>
<td>84%</td>
</tr>
<tr>
<td>Martin et al.</td>
<td>2003</td>
<td>32</td>
<td>VP</td>
<td>75%</td>
</tr>
<tr>
<td>Alvarez et al.</td>
<td>2003</td>
<td>21</td>
<td>VP</td>
<td>50%</td>
</tr>
<tr>
<td>Shimony et al.</td>
<td>2004</td>
<td>50</td>
<td>VP</td>
<td>82%</td>
</tr>
<tr>
<td>Bartolozzi et al.</td>
<td>2006</td>
<td>14</td>
<td>VP</td>
<td>100%</td>
</tr>
<tr>
<td>Cardoso et al.</td>
<td>2009</td>
<td>19</td>
<td>KP</td>
<td>100%</td>
</tr>
<tr>
<td>Sandri et al.</td>
<td>2010</td>
<td>11</td>
<td>KP</td>
<td>100%</td>
</tr>
<tr>
<td>Dalbayrak et al.</td>
<td>2010</td>
<td>31</td>
<td>KP</td>
<td>100%</td>
</tr>
</tbody>
</table>

KP: kyphoplasty; VP: vertebroplasty.

Cardoso et al. mixed samarium-153 with PMMA cement for intra-cavitary radiation dose delivery.
of postoperative RT is well established for both surgery and kypho/vertebroplasty. Addition of RT has been shown to not only to decrease the need for a second surgery but also to improve neurologic recovery.88 Recently spine radiosurgery has been used in the adjuvant setting delivering a single large dose for patients with SM89 and in metastatic epidural spinal cord compression (MESCC).90

Radiotherapy

Radiotherapy (RT) is the mainstay of SM management.55 The decision of who needs surgery followed by RT versus RT alone is guided by several factors. In general, the goals of therapy are to prevent neurologic decline, to alleviate pain, to restore lost neurological function and to stabilize the motion segments of the spine. Palliation and improvement of the quality of life is the central goal. In a randomized study of RT for painful metastases only 3% of treated patients progressed to a spinal cord compression.7

Conventional RT

It is the most common form of RT used. In most cases it entails using either an anterior and posterior beam or a posterior beam alone. Usually the treatment volume encompasses one or two vertebral bodies above and below. Most common conventional dose regimen is 30 Gy in 10 fractions.91 The ideal RT fractionation schedule is not well defined.92 Several dose comparison trials have been performed in bone metastasis literature in an attempt to minimize treatment duration.93–95 Patients with cord compression were uniformly excluded. All these studies have uniformly shown that a shorter course of even 1 fraction may be adequate in most patients with the end-point being pain control. A large meta-analysis96 of 16 trials showed equivalence between single and multiple fractions. Of note, the re-treatment rates are acceptably higher in the single fraction arms (Van der linden). This strategy is additionally beneficial in these patients as most have a poor overall survival.

A threshold dose of 8 Gy in 1 single fraction is an accepted standard and is the current randomized arm in RTOG-0631 trial testing conventional RT versus SRS. A Cancer Care Ontario guideline97 recommends single-fraction radiation as the standard for symptomatic, uncomplicated metastases (with the contraindications of previously irradiated areas, pathologic fractures, and spinal cord involvement or cauda equina) when the purpose is pain relief.

Stereotactic RT

The terms stereotactic radiosurgery (SRS) and Stereotactic body radiotherapy (SBRT) are used interchangeably in SM and are usually delivered in 1–3 fractions. This technique was originally developed in the late 1940’s by Prof. Lars Leksell to treat functional disorders of the brain and over the last decade has been applied to extra-cranial body sites. Essential requirements for Radiosurgery are a small and sharply defined target, high conformity of the RT dose, and accurate RT delivery systems. Dose-limiting structures (i.e. spinal cord) should be able to be defined and excluded from the target volume to limit the risk of radiation injury.

SRS uses highly conformal beams guided with 3-dimensional (3D) imaging to deliver a high dose of radiation to a small target and with rapid dose fall-off to avoid critical structures (Fig. 1). Several devices are available to deliver radiosurgery with variable advantages.98,99 Advances in imaging technology and CT-planning allow for the safe delivery of large doses of highly focused beams of radiation. Image guided localization provides an accuracy in the range of ±2–3 mm100,101 down to sub mm102 with precision of ±0.11 mm.100 Several phase I & II studies have documented the efficacy of SRS. Radio-biologically, delivering a large dose in 1–3 fractions would be expected to have a higher cell kill and thus potentially better tumor control.103 Compared to open surgery, SRS is a more cost-effective alternative.104,105

Indications of radiosurgery are not rigidly defined and will continue to evolve as more clinical evidence accumulates. In general, SRS is used for patients with limited (i.e. 1–3 metastases), no more
than 2 contiguous vertebral bodies, limited and/or controlled sys-
temic disease, good performance status and an anticipated survival of
greater than 3 months.\textsuperscript{106} Recently a recursive partitioning anal-
ysis (RPA) index has been proposed by Chao et al.\textsuperscript{107} which identi-
fies three classes predictive of overall survival. Class 1 was defined
as time from primary diagnosis (TDP) of >30 months and KPS of
>70; Class 2 was TDP of >30 months and KPS ≤ 70 or a TDP of
≤30 months and age <70 years; Class 3 was TDP of ≤30 months and
age ≥70 years. Median overall survival was 21.1 months for class 1,
8.7 months for class 2 and 2.4 months for class 3.

Unlike conventional RT which treats the adjacent vertebral
body in addition to the affected one, SRS targets only the affected
one. Ryu et al. reported no failures in the adjacent vertebral bodies
in 49 patients series treated for solitary metastases,\textsuperscript{108} Chang et al.
reported progression in adjacent vertebral in only one patient in a
63 patient series with 74 treated lesions.\textsuperscript{109} Gerszten et al. in their
large series of 500 cases reported no failures in the adjacent vertebral.\textsuperscript{110}

\textbf{Advantages of SRS}

(1) Avoids irradiating excess marrow, (2) does-not interfere
with on-going chemo, (3) single day out-patient treatment (es-
specially if the life-expectancy is a few months), (4) effective salvage
for previously irradiated area, (5) radio-resistant histologies – Mel-
oma, Sarcoma, Renal cell carcinoma, (6) possible rapid onset and
longer duration of pain control, (7) non-invasive.

\textbf{Response to RT}

Pain palliation after conventional RT is seen in about 57%-77%
cases.\textsuperscript{5–10} Approximately 85% of patients with pre-treatment pain
experience symptomatic relief after SRS.\textsuperscript{10} Of responding patients, 70%
have pain relief within 2 weeks and about 90% have pain relief
within 2 months. About half of the patients with neurologic dys-
function get recovery

Histology/primary pathology influences prognosis.\textsuperscript{13} In general
lymphoma, seminoma, myeloma, prostate and breast cancers are
considered favorable while renal cell carcinoma, lung cancer, melan-
oma, sarcoma and gastro-intestinal carcinomas are considered
unfavorable.\textsuperscript{8,10} In a prospective trial of 275 patients with MESCC,
Maranzano et al. found that patients with favorable histology (i.e.
breast cancer) recovered ambulatory status more often and had a
more durable local control as compared with unfavorable histolo-
gies (i.e. hepatocellular carcinoma).\textsuperscript{9} Patients with breast cancer
had an 80% response rate compared to 20% in patients with hepato-
cellular cancer. Further the durability of response was 10–
16 months for radiosensitive tumors as compared to 1–3 months
in radioresistant tumors.

A randomized trial also showed difference between the favor-
able and unfavorable histologies with improved duration of motor
improvement and median survival (6 vs. 3 months) for the favor-
able histologies.\textsuperscript{8} Gerszten et al. in the largest spine radiosurgery
study reported a difference in long term radiographic control based
on histology: breast (100%), lung (100%), renal cell (87%), and mel-
anoma (75%). Results of overall local control and radiographic re-
sponse after SRS reported are excellent 80–90%,\textsuperscript{10,111}

Combination of surgery and adjuvant SRS has also been shown
to be beneficial with a significant chance of stabilization and
improving neurologic outcomes with reported local control rate
of 94% in one study.\textsuperscript{89} SRS could also be performed after vertebral
cement augmentation with reported 92% local control rate in a
study of 26 patients.\textsuperscript{112}

The optimum SRS dose is not known but higher radiation doses
(≥24 Gy) have been shown to achieve better tumor control.\textsuperscript{90,111}

Most authors advocate a dose of 10–24 Gy given in 1–5 fractions
with extremely favorable symptom and local control rates. The
ongoing phase II/III RTOG study (0631) is testing the feasibility of
delivering a single fraction dose of 16 Gy (phase II component) and
also comparing the efficacy of 8 Gy conventional RT versus
16 Gy SRS in a randomized setting (phase III component), the pri-
mary end-point being pain control.

Summary of larger trials to date is listed in Table 3. Lower doses
and/or fractionation might be beneficial in re-irradiation patients.

\textbf{Complications of RT}

The exact radiation tolerance of the spinal cord is unknown. The
most feared complication is radiation myelopathy/myelitis which
may present after 9–15 months.\textsuperscript{120} A clinical study on canines indi-
cates that the onset of radiation myelopathy may be affected by
the volume or length of the spinal cord treated.\textsuperscript{121}

$$\text{Table 3}$$

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Patients/spine lesions</th>
<th>Previous RT</th>
<th>Dose (Gy)/fractions</th>
<th>Symptom/pain control</th>
<th>Local control</th>
<th>Follow-up (months)</th>
<th>Major complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryu (2004)\textsuperscript{108}</td>
<td>49/61</td>
<td>0%</td>
<td>10–16/1</td>
<td>85%</td>
<td>96%</td>
<td>Median – 6</td>
<td>None</td>
</tr>
<tr>
<td>Deges (2005)\textsuperscript{114}</td>
<td>38/58</td>
<td>53%</td>
<td>10–37.5/1–5</td>
<td>97%</td>
<td>95%</td>
<td>Mean – 11.7</td>
<td>None</td>
</tr>
<tr>
<td>Gerszten (2007)\textsuperscript{110}</td>
<td>336/500</td>
<td>60%</td>
<td>12.5–25/1</td>
<td>86%</td>
<td>88–90%</td>
<td>Median – 21</td>
<td>None</td>
</tr>
<tr>
<td>Chang (2007)\textsuperscript{109}</td>
<td>63/74</td>
<td>55.6%</td>
<td>30/5 &amp; 27/3</td>
<td>NR</td>
<td>84%</td>
<td>Median – 21.3</td>
<td>None</td>
</tr>
<tr>
<td>Gibbs (2007)\textsuperscript{115}</td>
<td>74/102</td>
<td>68%</td>
<td>16–23/1–5</td>
<td>84%</td>
<td>NR</td>
<td>Mean – 9</td>
<td>3 severe myelopathy</td>
</tr>
<tr>
<td>Ryu (2007)\textsuperscript{114}</td>
<td>177/230</td>
<td>0%</td>
<td>8–18/1</td>
<td>85%</td>
<td>NR</td>
<td>Median – 6.4</td>
<td>1 cord injury</td>
</tr>
<tr>
<td>Yamada (2008)\textsuperscript{111}</td>
<td>93/103</td>
<td>0%</td>
<td>18–24/1</td>
<td>NR</td>
<td>90%</td>
<td>Median – 15</td>
<td>None</td>
</tr>
<tr>
<td>Sheehan (2009)\textsuperscript{116}</td>
<td>40/110</td>
<td>0%</td>
<td>10–24/1–5</td>
<td>85%</td>
<td>82%</td>
<td>Mean – 12.7</td>
<td>Worsening kyphosis-16 cases</td>
</tr>
<tr>
<td>Sahgal (2009)\textsuperscript{120}</td>
<td>39/60</td>
<td>61.6%</td>
<td>7–40/1–5</td>
<td>NR</td>
<td>86%</td>
<td>Median – 8.5</td>
<td>None</td>
</tr>
<tr>
<td>Nelson (2009)\textsuperscript{121}</td>
<td>32/33</td>
<td>68.8%</td>
<td>14–30/1–4</td>
<td>91%</td>
<td>88%</td>
<td>Median – 6</td>
<td>None</td>
</tr>
<tr>
<td>Garg (2011)\textsuperscript{116}</td>
<td>59/63</td>
<td>100%</td>
<td>27–30/3–5</td>
<td>NR</td>
<td>76%</td>
<td>Mean – 17.6</td>
<td>2 Grade 3 neurotoxicity</td>
</tr>
</tbody>
</table>

F-SBRT, fractionated stereotactic body radiotherapy; Gy, gray; NR, not reported; RT, radiotherapy; SRS, stereotactic radiosurgery.
human spinal cord dose to give a 5% or less risk of radiation myelo-
pathy at 5 years is 50 Gy to <5 cm of the spinal cord.122 Reported
complications of SRS are generally mild and self-limited. In a large
retrospective study of 1075 cases treated with Cyberknife20, Gibbs
et al. reported six cases of radiation-induced myelopathy at mean
6 months post treatment. No specific predictive dosimetric param-
eters could be identified. Three of these six patients had received
previous radiation.123 Gerszten et al. observed no cases of spinal
cord injury in a large series of 500 cases with a median follow-
up of 21 months.110 Even in the post-operative setting SRS seems
to have very limited toxicity.89,90 Many patients with short life
expectancies do not experience the risks of late radiation sequelae.

Cost analyses

A recent matched-pair analysis by Haley et al.128 compared the
palliative efficacy and cost effectiveness of external beam radiation
therapy (EBRT) to stereotactic body radiation therapy (SBRT) as
primary treatment for bony metastatic disease to the spinal col-
umn in 44 patients (22 pairs). Patients who underwent SBRT had
the highest total gross charge and depending on technique, EBRT
treatments ranged from 29% to 71% of the SBRT charge. Patients
treated using EBRT had more acute toxicities, and more of these
patients underwent further intervention at the treated spinal level.
There were no late complications attributed to either treatment
modality.

Metastatic epidural spinal cord compression (MESCC)

Spinal cord compression is a dreaded complication that occurs
in about 5–10% of all patients with cancer and about 20% of pa-
patients with vertebral metastases.13 Although the first indicator
of cord compression is back pain in 83–98%,19,129–131 many cases
are asymptomatic.126 About 35–85% of patients have motor weak-
ness at the time of diagnosis19,129–131 and will progress to complete
paralysis in the absence of intervention.42 Sensory deficit is seen
in 61–78%,19,129 The most common autonomic finding is bladder dys-
function.132 MRI is the imaging modality of choice with excellent
sensitivity and specificity.25,26 In one study, MRI changed the
radiotherapy plan in 53% of patients (21% with major change).27
Approx. 10% of patients have multiple sites of involvement and
benefit from imaging of the entire spine.

Management of SCC requires a truly multi-disciplinary ap-
proach. Steroids (i.e. dexamethasone) should be instituted as soon
as possible. A study from Denmark comparing steroids versus no
steroids showed a clear benefit to the use of steroids with signifi-
cantly improved gait function and ambulation.133,134

Standard of care therapy is initial surgery followed by radiation
therapy.53 The randomized trial by Patchell et al. showed a longer
median survival in patients who underwent surgery followed by
RT (126 days) as compared to those treated with upfront RT alone
(100 days) (p = 0.033).53 Continence was maintained for a signifi-
cantly longer period in surgically treated patients (156 vs. 17 days,
p = 0.016). Significantly more surgical patients than RT only pa-
tients regained the ability to walk (62% vs. 19%, p = 0.01). The
authors used ability to ambulate as the primary endpoint in this
study, while also considering ASIA and Frankel scores as secondary.
Patients in the surgical arm were more likely to be ambulatory
after treatment than those who received only radiation therapy
(84% vs. 57%) and also remained ambulatory longer (122 days vs.
13 days). Surgery patients also had better ASIA and Frankel scores
and were not shown to have excess morbidity or mortality relative
to the radiation only group, demonstrating the utility of surgery in
properly selected patients, including those with neurologic deficits
and/or spinal instability and a life expectancy ≥3 months.57,63 The
patients who were initially treated with RT who crossed over to the
surgical treatment arm had inferior clinical outcomes. Timing of
surgery is thus important and if planned it should be performed
as early as possible preferably prior to RT. Similar results were ob-
erved in a recent large multi-center observational study.135 RT
dose in this study was 30 Gy in 10 fractions, but as discussed ear-
lier under SM, several dose fractionation regimens can be used.8,136

A phase III randomized study comparing 8 Gy in one fraction to
8 Gy in two fractions showed no difference in pain relief, motor
function or probability of survival.136 Short course schedules have
been shown to result in higher rates of in-field recurrence22 and
lower rates of progression-free survival and local control.137 Local
control defined as the absence of recurrent cord compression in
the conventionally irradiated field ranges between 61% and 89%.10

Role of radiosurgery in the adjuvant setting following decom-
pressive surgery has been documented.89,90 A study by Rock et al.89
reported on 18 patients receiving comparatively lower single-fra-
ction radiosurgery doses 6–16 Gy. The retrospective study by
Moulding et al.130 identified 21 patients (95% radio-resistant histol-
gies i.e. melanoma, renal cell ca& sarcomas) with high-grade ESCC
treated with surgery followed by higher single fraction SRS to 18–
24 Gy. Spinal cord dose was limited to a cord maximal dose of
14 Gy. Local control was 81% (21/24). Three of four local failures
occurred in patients receiving the lower radiosurgery dose sug-
B2 suggesting that higher dose predicted for better local control. The
authors identified a scoring scale for ESCC used in their practice
(Fig. 2). For patients with score of 1c to 3, they recommend operat-
ive decompression. In their opinion, without surgery, adequate
margin on the spinal cord cannot be achieved to deliver a high dose
of radiosurgery that would be required for tumor local control
especially in the radio-resistant histologies. This is a simple and
useful scale that can help aid decision making at time of
presentation.

![Fig. 2. Scoring scale based on degree of tumor invasion. Grade 0 – Tumor confined
to bone. Grade 1a – Tumor involves the epidural space without dural compression.
Grade 1b – Tumor compresses the dura but does not abut the spinal cord. Grade 1c
– Tumor abuts but does not compress the spinal cord. Grade 2 – Tumor compresses
the spinal cord but CSF is still visible. Grade 3 – Tumor compresses the spinal cord
and obliterates all visible CSF at that level.](image-url)
Radiosurgery has also been used in primary management of SCC as shown in the study by Jin et al. who delivered a single fraction of 16 Gy to 24 patients with 31 lesions. Complete radiologic response was observed in 81% with five out of seven patients who presented with neurological deficits experiencing improvement. In general however a high grade spinal cord compression remains a relative contra-indication and surgical decompression should be considered as the primary treatment. Further studies would be needed to validate the use of radiosurgery in the primary management of SCC.

Intraoperative RT (IORT) has also been studied in the setting of SCC status post posterior decompressive surgeries with good rates of improvement in neurologic function.

**Indications for RT alone**

(1) Patients with radiosensitive tumors, (2) expected survival of less than 3 months, (3) inability to tolerate an operation, (4) total neurological deficit for more than 24–48 h, and (5) multi-level or diffuse disease.

**Outcomes**

It is well established that a slower development of motor deficits before RT predicts for a better functional outcome and survival. Rapid deterioration within 48 h of the start of RT predicts a poor outcome. Patients who were ambulatory before treatment are more likely to maintain or improve their functional outcome.

**Conclusion**

The decision on management of SM should ideally be made in a multi-disciplinary setting with the goals of therapy best defined at the outset: improve the quality of life by preventing neurologic decline, achieving durable pain relief and local tumor control. It is reasonable to consider a single fraction conventional RT, especially in patients with life expectancy of less than 3 months. SRS has been shown to achieve excellent local control and pain relief with minimal toxicities with adequate sparing of the spinal cord. It is usually reserved for people with longer life expectancy and better prognosis. SRS can be used in the re-irradiation setting. Whether this modality should be the standard of care in the primary setting needs to be established via randomized trials. As imaging improves and SM’s get detected early, SRS may prove to be a very effective non-invasive first-line therapy. SRS is also being evaluated in the adjuvant setting post-surgical debulking which serves not only to stabilize the spine but may also help increase the safety of SRS by increasing the margin on the spinal cord. SRS in combination with kyphoplasty or vertebroplasty offers a less invasive modality for spinal cord stabilization. Surgery followed by RT remains the current standard of care for metastatic epidural management of spinal cord compression. Proper patient selection should ultimately guide therapy decisions.

**References**


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Paraneoplastic Syndromes
Update on clinical and mechanistic aspects of paraneoplastic syndromes

David Tarin

Abstract Many patients with cancer are afflicted with an array of severe ailments caused by remote effects of the neoplasm on distant organs, which it has not invaded or colonized. The growing local tumor dominates attention, but invisible chemical and cellular components released by this parasitic neoplastic growth can operate below the threshold of detection to derange feedback loops coordinating essential physiological functions. Ultimately, these changes cause serious signs and symptoms, collectively described as paraneoplastic syndromes (PNSs), which significantly diminish the patient’s quality of life. PNS can appear before, or after, detection of the cancer and are sometimes surprising or even bizarre. The patterns of clinical changes seen in these patients are caused by a range of mechanisms that reveal intricate networks of communications between different body systems, normally utilized for healthy function. For example, these disorders demonstrate that (1) hormones, peptides, and other long- and short-range signaling molecules produced by the tumor and (2) immune reactions to tumor-related antigens, can mediate diverse destabilizing effects. However, comparative analysis of numerous PNS reveals valuable information indicating that the primary pathogenetic events instigating these disturbances are much more fundamental. This article provides an overview of the diverse clinical manifestations of paraneoplastic disorders, with representative examples and presents evidence that inappropriate gene expression in the tumor, caused by loss of regulatory control, is a novel unifying explanation for such wide effects of the neoplasm on the host. It also discusses treatment options and issues relating to conducting randomized clinical trials on these disorders.

Keywords Inappropriate gene expression · Systemic effects of malignancy · Pathogenesis · Etiology · Cachexia · Randomised clinical trials

1 General and historical aspects

General malaise, weakness, pain, and discomfort are characteristic afflictions in patients with potentially fatal illnesses including cancer. In cancer patients, these problems are often attributable to organ failure caused by replacement of vital organs by tumor invasion or metastasis, or by side effects of therapy. However, summation of the incidence of PNS affecting various organ systems, reported by various investigators cited in this article, combined with the frequency of generalized malignant syndromes including cachexia and fever, leads to figures as high as 70 % of cancer patients being affected by some form of PNS. Secondary manifestations of disease on this scale require more intensive investigation if appropriate care is to be delivered to cancer patients.
This article presents a new vision of how PNS originate and of possible new approaches to therapy, which may help to alleviate some of the discomfort experienced by cancer patients. Many excellent review articles, book chapters, and monographs already provide thorough, extensively referenced, accounts of the variety of syndromes affecting many organ systems now known to exist and discussions of some of the possible mechanisms involved. However, this document focuses, instead, on the underlying principles involved and on what paraneoplastic syndromes as a group reveal about (a) the nature of neoplasia, (b) the organization of the human body, and (c) how this information helps to design new and more effective policies to treat cancer patients. It will use specific examples to anchor the discussion in confirmable facts and draw conclusions, which aim to have pragmatic value.

The healthy function of the human body depends upon intricate networks of humeral and neural communications in dynamic equilibrium regulated by feedback loops. As discussed below, paraneoplastic syndromes result from maladjustment of these balanced control mechanisms, caused by the tumor. The great variety of PNS that have now been described highlights the complexity of the multiple internal regulatory controls operating in the human body and the breadth of knowledge needed to effectively treat affected patients.

When a localized group of cells in the body becomes independent of this hierarchically organized system of controls, it becomes a neoplasm, which, in order to survive and grow, must continue to derive nutritional support from the host. Such support comes from stromal cells, blood vessels, and marrow stem cells attracted into the growing lesion by growth factors, cytokines, and other signaling molecules released by the cancer cells (see [1] for more information). However, the metabolic activities of tumors are not harmoniously integrated with the rest of the cellular communities from which they are derived and the neoplastic cells are free to initiate unregulated release of bioactive molecules that can disrupt communications necessary for maintaining efficient physiological functions and thereby exert widespread effects. However, this disorganized activity does not bear any consistent relationship to size, grade, or stage of the cancer, as demonstrated by the common clinical observation that the tumor becomes evident months or even 2 years after the PNS appears [2, 3]. Nor have PNS yet been related to any specific common genetic marker in malignancy (e.g., upregulation of estrogen receptor (ER+) epidermal growth factor (EGFR+) or Ras gene mutations).

These mechanisms can disturb multiple anatomical, physiological, and biochemical systems of the body and are therefore categorized as systemic effects of malignancy (SEM). Such effects may initially be below the threshold of perception by the patient, but, as they increase, they eventually cause symptoms related to the organs affected. Groups of symptoms and signs commonly occurring synchronously in patients with specific types of cancer are tied together by shared or interrelated mechanisms and are termed paraneoplastic syndromes (PNS). However, these mechanisms are secondary and tertiary manifestations of a more profound underlying disorder emerging from abnormal gene regulation within the tumor (see below).

The first recognition of a paraneoplastic syndrome is generally attributed to Trousseau (1801–1867) who described fleeting, multifocal, thrombophlebitis associated with pancreatic and gastric cancers [4]. He later diagnosed the same condition in himself, prior to his death from gastric cancer in 1867. Subsequently, a number of other French (e.g., Auché 1890) and German (e.g., Oppenheim 1888) physicians recognized tumor-associated systemic syndromes affecting the nervous and other organ systems in the 1880s and 1890s [5]. Since then a vast number of such syndromes have been recognized and a list of the better-known syndromes, grouped according to the main physiological system affected, is provided in Table 1. The types of cancer most commonly associated with each syndrome are also indicated in this table and in the text, although it should be understood that any syndrome can potentially be caused by any type of cancer. From this collection of disorders, it is possible to derive important principles about how tumors interact with their hosts to cause multisystem diseases classified as PNS. Formal scientific proof that a neoplasm is causing a specific syndrome in a particular patient is often difficult to obtain in clinical practice because of ethical considerations. However, in many of these paraneoplastic conditions, strong circumstantial evidence of an etiological relationship has accrued over time. In particular, regression of unusual symptoms after resection of a coexisting primary tumor and/or their return in association with a recurrence of the malignancy indicates that the tumor causes the condition and that further investigation may provide evidence of the mechanism.

In general, the wide variety of PNS identified to date can be grouped into two main categories, namely (1) those caused by secretion of hormones, cytokines, or other bioactive molecules synthesized by the cancer cells and (2) those caused by immune mechanisms directed against tumor-related products. However, there is a deeper explanation for activation of these two categories of cancer-related systemic malfunction, which is described below.

This article will discuss selected paraneoplastic syndromes under headings related to the organ systems affected and draw conclusions about the mechanisms involved as well as the implications for treatment. Its specific contribution is to provide a different perspective from other reviews [5–7] on the diverse array of syndromes experienced by cancer patients, by viewing them in the context of the whole organism, and thereby derive a unifying, clinically useful explanation for their causation. In addition, it considers factors affecting the conduct of clinical trials to find better treatments for these vexing side effects of malignant disease.
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(C) Springer
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2 Hematological disorders

2.1 Thrombophlebitis migrans

In this disorder, which can be caused by any type of cancer, but is most commonly associated with gastric and pancreatic adenocarcinomas, thrombi form in major veins in multiple parts of the body causing local pain and sometimes secondary embolic phenomena, which vary depending on the location of the affected vessels. The thrombophlebitis moves from vessel to vessel, causing a highly variable clinical picture, which can include chronic disseminated intravascular coagulopathy, platelet-rich microthrombi, microangiopathic hemolytic anemia and verrucous endocarditis with thromboembolic episodes. Investigation has shown that many mechanisms, including thrombocytosis, abnormalities in the production or activation of clotting factors, and release of tissue and endothelial factors by the tumor, can cause this phenomenon. Additionally, release of tumor cell products, such as epithelial mucins, into the circulation can bind selectins and activate thrombogenesis [8]. Clinical management depends upon identifying the exact mechanism causing activation of the coagulation cascade.

2.2 Polycythaemia

Increase in the red cell count in the blood is seen in some cases of renal carcinoma, secondary to the secretion of excess erythropoietin. It also occurs in myelofibrosis associated with mutation in the Jak 2 gene, which renders the erythropoietic progenitors more sensitive to erythropoietin [9] and in a number of other neoplasias including hepatoma. In turn, the increased viscosity of the blood can cause sluggish flow and thrombosis in scattered veins. Patients experience plethora, headaches, vertigo, hypertension, splenomegaly, and a raised hematocrit.

3 Cutaneous disorders

3.1 Erythema gyratum repens

This condition is rare but has a very high association (>80 %) with internal malignancy particularly small cell bronchogenic carcinoma. Patients display a serpiginous, often itchy, rash composed of parallel raised wavy erythematous lines resembling wood grain (Fig. 1) with mild hyperkeratosis, which moves across the surface of the skin at about 1 cm/day, so that the pattern changes every few hours [10, 11]. Histologically, there is a mild infiltrate of mononuclear inflammatory cells around dermal blood vessels (Fig. 2). It is unknown what causes the skin to respond in this way to the presence of the cancer deeper within the body, but, as it resolves after tumor resection [10], it is inferred that some tumor product, in the form of a vaso-active peptide or an immune complex containing a tumor-generated protein, is involved.

Other forms of cutaneous vasculitis, including polyarteritis nodosa and small vessel immune complex vasculitis with
associated fibrinoid necrosis, do occur in association with malignancy but not with sufficient frequency to be confident that the coexisting neoplasm causes them.

3.2 Acanthosis nigricans

Some patients present with darkly pigmented, velvety, corrugated thickening of the skin around the back of the neck, the axillae (Figs. 3 and 4), or the genital area [12, 13] or on the hands. Occasionally, this is seen in young female diabetics, but when the patient is a mature adult, this sign is a strong indicator of internal cancer, often gastric adenocarcinoma. The mechanism by which cancers cause this marked acanthosis, hyperkeratosis, and rugosity in specific regions of the bodies of these patients is unknown, but the features suggest that a humoral factor [insulin or insulin-like growth factor (IGF)], acting via an insulin-sensitive receptor, is involved [13]. It has also been reported that transforming growth factor-alpha (TGFα) is raised in some patients and that levels of this cytokine drop when the tumor is resected [14, 15].

3.3 Dermatomyositis

Proximal symmetrical weakness of the limbs coupled with skin rashes and heliotrope (violaceous discoloration of the skin) are suggestive of this disorder. Patients may find it difficult to raise their arms to comb their hair or to rise from a seated position and may find it painful to do so. If they also show Gottron’s patches, which are scaly erythematous eruptions or red papules overlying the knuckles, elbows, and knees, dermatomyositis (DM) should be strongly suspected. Biopsy of the skin and muscles shows perivascular chronic inflammatory cell infiltration, myocytolysis, and regenerating muscle fibers and electrophysiological studies show typical features of repetitive fibrillation discharges. The condition is well known to be associated with malignancy, often of the ovary, lung, GI tract or non-Hodgkin’s lymphoma [16]. In different case series [17, 18], about 15–20 % of patients with DM are harboring an occult or overt neoplasm and muscle antigens have been identified in tumors from some patients [19]. Hence, there is clear histopathological and immunological evidence of an autoimmune mechanism in most of these cases.

Several other dermatological conditions including cutaneous vasculitis, ichthyosis, bullous pemphigoid, etc. have been regarded as potential paraneoplastic syndromes on grounds of frequency of association, temporal coincidence, and other features, but firm mechanistic evidence of a causal relationship has not yet been documented.

4 Endocrine disorders

4.1 Cushing’s syndrome

The collection of signs and symptoms that can be seen in patients afflicted with Cushing’s syndrome is well known. Frequent among them are obesity, plethora, abdominal striae, diabetes mellitus, gastric ulceration, hypertension, cardiomegaly, lymphocytosis, and a “buffalo hump” due to accumulation of adipose tissue over the upper thoracic spine. When encountered as a paraneoplastic phenomenon, it is designated ectopic Cushing’s syndrome and is most commonly associated with small cell lung cancers and thymomas although cancers of the pancreas and lymphomas can also be responsible. The tumor, which indirectly causes this collection of abnormalities, can be large, small (e.g., < 2 mm), or undetectable, but its effects can be catastrophic. In such circumstances, the pituitary fossa is normal-sized or large, but the gland itself is severely shrunk

Fig. 3 Acanthosis nigricans. The skin in the axilla shows pigmented rugosity (arrow)

Fig. 4 Acanthosis nigricans. The skin on the back of the neck shows pigmentation and roughening (arrow)
(i.e., suppressed) and the adrenals are enormous (collective weight of ~150 g compared with a normal combined weight of 10–12 g. The excessive output of mineralocorticoids, glucocorticoids, and/or androgens by the hyper-stimulated adrenal cortex causes the extensive secondary effects described. These can in turn cause tertiary effects such as hypertension-induced cardiomegaly (~900 g heart compared with ~350 g normal weight) and cardiac failure, leading to the death of the patient. Ectopic synthesis of the adrenocorticotropic hormone (ACTH) by the tumor cells has been confirmed, by extraction of the protein from tumor tissue and from cultured tumor cells [20]. Occasionally, a tumor can secrete corticotrophin releasing factor (CRF) that, in turn, drives the patient’s pituitary to secrete excess ACTH [21]. The secreted ACTH product may be the whole hormone or a truncated version [20]. Inappropriate gene expression within the tumor cells is responsible for the ectopic production of either of these hormones.

4.2 Hypercalcemia

Raised serum calcium is one of the most common complications of cancer and, depending on its severity, causes a variety of symptoms including bradycardia, polydipsia, bone pain, nephrolithiasis, gastrointestinal effects, weakness, fatigue, and focal neurological signs including amaurosis fugax. According to different studies, hypercalcemia occurs in 10–30 % of cancer patients [22, 23]. Serum calcium may be elevated by several different mechanisms including direct destruction of bone by metastatic deposits or by coincidental parathyroid hyperplasia in a patient with another type of cancer. True examples of paraneoplastic hypercalcemia, however, occur in the absence of metastases or of parathyroid gland malfunction and are caused by a humoral agent secreted by the patient’s cancer. In some patients, the agent is either ectopic parathormone (PTH) or 1–25 (OH)₂ vitamin D₃, but humoral hypercalcemia of malignancy (HHCM) results most often (>80 %) from the release of parathyroid hormone related protein (PTHrP), a peptide which shares identity with PTH in the first 13 N-terminal amino acids. PTH is composed of 84 amino acids and PTHrP of 139–173 amino acids and the C-terminal portions of these peptides are completely different from each other. However, PTHrP folds into a configuration that can bind to the PTH receptor, although it can also bind to other receptors, via which it exerts different effects from PTH. PTHrP is produced in many different normal tissues [24] including the skin, anterior pituitary, and mammary gland. It can be produced as four different alternatively spliced messenger RNA transcripts, and at least two peptide isoforms are known to exist. It has effects upon tissues other than bone including smooth muscle and cardiac pacemaker cells and is essential for tooth eruption, mammary gland development, lactation, and maintaining the endochondral growth plate of long bones at a constant width. Deletion of the gene in animals causes embryonic lethality in their offspring, indicating that it has even wider effects in normal physiology. Consequently, PTHrP causes more widespread symptoms and signs than elevated PTH although there is some overlap. Convincing evidence of its production by tumor cells has been shown by immunohistochemistry [25] and by culture of cancer cells.

4.3 Hypoglycaemia

This is most commonly seen in patients with pleural mesothelioma, hepatoma, or islet cell tumors of the pancreas [26] and is important to recognize and manage appropriately because it can lead to hypoglycemic coma. The etiology is inappropriate secretion of insulin or insulin-like growth factor-2 by the tumor cells [27] demonstrable by extraction from the tumor, immunohistochemistry, and synthesis by tumor cells in culture.

4.4 Inappropriate antidiuretic hormone secretion

This condition is most often seen in patients with bronchogenic carcinomas and presents with water retention leading to hyponatremia in a euvolemic patient. Arginine vasopressin (ADH) is normally produced by the neurons in the hypothalamus when the plasma osmolality falls below 275 mOsm/kg or when the baroreceptors detect a hypovolemic state. It instigates the reabsorption of water in the distal convoluted renal tubules, without corresponding absorption of sodium. It has been demonstrated by cell culture, incorporation of isotope-labeled precursors and immunohistochemistry that a wide variety of cancers of different organs can produce ADH (reviewed in [28]) and that the syndrome resolves upon effective treatment of the patient’s cancer.

4.5 Mechanisms

The endocrine paraneoplastic syndromes as a group illustrate the important point that the deregulated genomic machinery and heterogeneity of cancer cell populations can trigger the secretion of highly bioactive molecules (including nonendocrine molecular messengers), which can cause dysfunction of several organ systems simultaneously, and these imbalances can, in turn, interact anomalously to cause further illness.

5 Neurological disorders

There is a whole constellation of neurological syndromes associated with cancer (see [29–31]), but many have not yet been firmly demonstrated to be caused by the malignancy, although signs of immunological reactivity to neural antigens are frequently detected. However, strong evidence confirms that some (see below) peripheral nervous system disorders are indeed initiated...
and sustained by mechanisms triggered by a coexisting cancer in the patient [31]. This suggests that many further neural manifestations, which regress when tumors are excised, but return when the lesions recur, are also secondary, remote effects of the tumor on its host. In circumstances where there has already been extensive neurological damage before treatment, recovery is of course very limited.

Table 2 lists the best documented examples of clinical neurological syndromes most commonly associated with overt or occult malignancy, grouped according to the affected region of the nervous system. They can be all associated with any type of cancer.

5.1 Syndromes affecting the Central Nervous System

5.1.1 Diffuse encephalitis

Patients can display a wide variety of disorders of cognitive, emotional, affective, and motor functions. Diffuse infiltration of brain parenchyma by lymphocytes and the presence of monoclonal immunoglobulin bands in Western blots of the blood and/or cerebrospinal fluid (CSF) confirm the diagnosis in the presence of an overt neoplasm and can indicate the presence of a cryptic cancer, if it is not already known. A high index of suspicion of malignancy should be maintained if antineuronal antibodies are found in a patient with this syndrome.

5.1.2 Bulbar encephalitis

When the encephalitis affects mainly the brain stem and/or hypothalamus, it causes disturbances in breathing, swallowing, motor coordination, speech, and/or temperature regulation (pyrexia). In some circumstances, these can be immediate life threatening emergencies.

5.1.3 Limbic syndrome

In 1968, Corsellis et al. [32] described a variety of encephalitis, which affects several parts of the limbic system, and this has been confirmed by several subsequent studies [33]. Patients display cognitive dysfunction, marked short-term memory loss, temporal lobe epilepsy sometimes combined with emotional (e.g., anger management), sensory (olfactory), and motor (opsoclonus) malfunctions. Histopathological examination of brain tissue reveals marked loss of neurons, gliosis, and sometimes a diffuse lymphocytic infiltrate focused on components of the limbic system. Modern imaging techniques, such as magnetic resonance imaging [3, 33] have confirmed the location of changes in the components of the limbic system and facilitated diagnosis of the condition. The etiology of the localization of the damage to the neurons in these components of the central nervous system (CNS) is not currently understood.

5.1.4 Cerebellar degeneration

This is manifested by disturbances in motor coordination, balance, gait, and signs of cerebellar shrinkage on imaging. It is almost always associated with extensive loss of Purkinje cells and the presence of antineuronal antibodies (mostly anti-Yo). In some cases, mononuclear cellular infiltrates are seen in the cerebellar cortex, deep cerebellar nuclei, and olivary nuclei [34–36]. Unless recognized and treated early, recovery may be very limited, and the resulting disability is incapacitating and permanent, due to extensive neuronal loss.

5.1.5 Mechanisms

It is now well established that many of these syndromes are associated with the presence of antibodies, in the patient’s blood, to intranuclear, cytoplasmic, or cell surface proteins of neurons. Accordingly, it has been widely speculated that this signifies that the neurological disturbances in these patients may be caused by immunological mechanisms, although in most of these syndromes, definitive evidence of such causality is still lacking. Many antibodies have now been described [37], and the ones most commonly associated with neurological syndromes include anti-Hu, anti-Yo, anti-Ri, anti voltage-gated calcium channel (VGCC), anti-acetyl choline receptor (AchR), anti neuronal nuclear antigen (ANNA 1,2 or 3), anti-amphiphysin and anti-Ma2. Convincing evidence of the frequent presence of the antibodies and the identity of the antigens against which they are directed has been documented by many investigators (see Darnell and Posner [5] for comprehensive review) and includes demonstration of monoclonal immunoglobulin in the blood and CSF as well as expression cloning of antigens from the tumor [38]. Although each antibody is more often associated with some syndromes than others, there does not appear to be any consistent relationship between an antibody and a specific
disease. Some of the antigens are normally only expressed in the CNS, an immunologically privileged site behind the blood–brain barrier, and it is reasoned that their inappropriate expression by tumors outside the CNS provokes the antibody production. Initially, it was presumed that such antibodies then cross-reacted with the corresponding antigen on normal neurons to induce the disorder. However, this simple explanation did not stand up to further scrutiny.

Binding of these antibodies to various central neural cell types has been confirmed by applying labeled purified antibodies obtained from patients to human tissue sections. However, included among the difficulties in assigning a pathogenic role to these antibodies are the facts that (1) a given antibody can be associated with different neurological syndromes, (2) a given syndrome can be associated with different antibodies in different patients, (3) the antibodies can be present although there are no signs or symptoms, (4) the antibodies can be absent in a patient with a syndrome, (5) antibodies are large protein molecules and one would not expect them to exit intact blood vessels or to cross the blood brain barrier to cause syndromes affecting the central nervous system, and (6) passive transfer of purified antibodies into mice has not resulted in inducing any syndrome, except when animals are inoculated with anti-VGCC from patients with Lambert Eaton myasthenia, in which circumstances they have been reported to cause weakness. Thus, although screening for antineuronal antibodies can be useful in diagnostic evaluation of patients with neurological syndromes of suspected paraneoplastic etiology, they probably have minimal role in the pathogenesis of most neurological PNS, (except for the neuromuscular disorders—see below). However, it has been observed that tumor resection or regression induced by chemo- or radiation therapy is the most effective means to induce remission or stabilization of the paraneoplastic syndrome [5, 39, 40] demonstrating that the continuation or progression of the syndrome is dependent upon some property of the neoplasm (e.g., an antigen or a secreted factor). Also, plasmapheresis, intravenous immunoglobulin, or immunosuppression with steroids or azathioprine have been reported to be effective in relieving symptoms in some patients with antibodies to neuronal cell surface proteins, but rarely in patients with other antibodies to neuronal intracellular proteins [39].

It has been proposed that this implies that cellular immunity to neural nuclear or cytoplasmic antigens, surface proteins or secreted products is the pathogenetic agent, but the data so far provided in support of this concept are weak and do not amount to more than class 4 clinical evidence (see [41] for discussion of formal classes of clinical evidence). Histopathologically, perivascular lymphocytic cuffing is seen in some patients [32] and in others degeneration and depletion of neuronal cell bodies can be detected. These observations could indicate that the neuronal cell loss is due to an autoimmune microvasculitis induced by the tumor, but no confirmatory evidence has yet been provided. The depletion of Purkinje cells in the cerebellum and of neurons in the dorsal root ganglia of patients with cerebellar degeneration and with demyelinating sensory neuropathy, respectively, is impressive, when seen, but this is not always detectable. Diffuse infiltration of brain parenchyma with lymphocytes, accompanied by microgliosis and neuronal degeneration without viral inclusions, is evident in patients with limbic or brainstem encephalitis. Oligoclonal expansion of helper or cytotoxic T lymphocytes with specific receptors to Hu or Yo antigens have been identified in some patients [3, 29, 42]. However, this is still only circumstantial evidence of immunological etiology and attempts to create paraneoplastic syndromes in animals with T cell transfer have not been successful so far [29].

In summary, the extensive data demonstrating immunological responses in patients with paraneoplastic syndromes affecting the central nervous system suggest that these reactions may play a role in the pathogenesis of the neurological disturbances, but do not confirm it. Even so, the presence of neurological symptoms and signs in a patient with an extracranial cancer, together with symptom relief after tumor resection, is sufficient to regard the condition as a paraneoplastic syndrome. It is also very important to remember that, while neurological syndromes associated with the presence of immunological abnormalities have dominated attention, secreted tumor products such as vaso-active peptides, hormones, cytokines, growth factors, and hormone fragments can also cause neurological malfunction in cancer patients. Such disturbances can also, via hypothalamic, brain stem, limbic, and cortical circuits, in turn, cause secondary complications such as excess hormone secretion, cachexia, and fever [43].

5.2 Peripheral neuropathy

5.2.1 Sensory neuropathy

Loss of proprioception and vibration sense, sometimes coupled with heightened pain perception, indicate a sensory neuropathy, which is most often associated with histological evidence of loss of neurons in the dorsal root ganglia, proliferation of satellite cells, and a monocellular infiltrate in the ganglia [44]. Secondary Wallerian degeneration with demyelination is seen in the gracile and cuneate tracts of the dorsal columns of the spinal cord (Fig. 5) following neuronal cell death. Anti-Hu or anti-CV2 antibodies may be detectable in the blood. Classically, this is associated with small cell lung carcinoma and is often accompanied by autonomic neuropathy.

5.2.2 Lambert–Eaton myasthenic syndrome (LEMS)

This is characterized by progressive weakness, beginning proximally in the arms and legs and sometimes affecting the pharyngeal and laryngeal muscles (bulbar palsy) as well as the ocular muscles. Reflexes are sluggish, but increase on repeat
testing. The underlying cause is the presence of antibodies against voltage-gated calcium channels (VGCC) in the presynaptic membrane of the motor endplate of neuromuscular junctions. The antibodies reduce calcium influx into the presynaptic axon endings and impede the release of acetylcholine thereby reducing muscle contractility and efferent autonomic nervous system activity. The antibody cross-reacts with calcium channels in the tumor cells. Passive transfer of the patient’s IgG or serum into mice temporarily replicates the condition in the animals [45]. About 60% of patients with LEMS will be found to have an associated cancer [usually small cell lung carcinoma (SCLC)] [3, 46] and symptoms regress for variable periods, upon removal of the tumor [46].

5.2.3 Autonomic neuropathy (Dysautonomia)

Patients with this category of neural disorders may manifest gastroparesis, distension, constipation or other gastrointestinal motility disorders, severe postural hypotension, or difficulties with sweating or micturition [47]. It often occurs in association with sensory neuropathies and presence of anti-Hu or anti-CVP antibodies. Autopsy studies have shown depletion of neurons in autonomic ganglia [47], but direct evidence of immune pathogenesis is still not available.

5.2.4 Mechanisms

In some paraneoplastic disorders affecting the peripheral nervous system, the antibodies to peripheral neuronal surface antigens have been decisively shown to be involved in the pathogenesis of the clinical signs and symptoms. Most convincing has been the passive transfer of the syndrome with purified antibodies and the demonstration of specific mechanisms of disease induction by binding to specific surface antigens or molecules involved in neuromuscular transmission (VGCC and AchR). The mode of generation of an antibody to an indigenous neuronal protein, which the body should presumably tolerate, is perplexing. It seems likely that the version of the protein produced by the tumor excites an antibody response because, due to genetic changes, it is sufficiently different in amino acid sequence, folding, or glycosylation to be considered foreign. However, it is evident that the antibodies generated do cross-react with the protein made by normal cells. Alternative mechanisms, which break tolerance to release normally suppressed minority T cell clones, as in thymomas (see below), may also be involved in some conditions.

6 Renal syndromes

In this category, the nephrotic syndrome takes precedence over other glomerulonephropathies in terms of frequency, but IgA nephropathy, focal and segmental glomerulosclerosis, mesangiocapillary glomerulonephritis, crescentic glomerulonephritis, amyloidosis and thrombotic microangiopathies, and minimal change disease all add to the catalog of renal diseases associated with cancers distant from the kidney [48].

6.1 Nephrotic syndrome

Proteinuria, hyperlipidemia, diffuse edema, and renal failure are sometimes seen in patients with malignant neoplasms, especially bronchogenic and colonic carcinomas [49]. Microscopy shows the deposition of immune complexes between the
glomerular endothelium and the adjacent basement membrane (Figs. 6, 7, and 8). Further investigation using immunofluorescence microscopy demonstrated that antibody eluted from glomerular immune complexes and antibody circulating in the patient’s blood, both reacted with antigens expressed on the tumor cell membranes, and this was confirmed by immuno-diffusion studies on Ouchterlony plates. These demonstrated identical lines of immunoprecipitation between the well containing tumor extract and those containing (a) antibodies from the serum and (b) the renal immune complexes [50, 51]. Resection of the primary carcinoma in such patients usually results in disappearance of proteinuria and other features of the nephrotic syndrome, confirming the etiological role of the immune complexes [52]. In some cases, return of nephrotic features has been reported in association with recurrence of the tumor [49].

7 Musculoskeletal disorders

7.1 Myasthenia gravis

This syndrome, in which the patient experiences muscle weakness and early fatiguability (i.e., rapidly decreasing function on repetitive contraction), is associated with thymomas and the presence of circulating autoantibodies to postsynaptic AchR in the membranes of skeletal muscle cells. It can also occur in the absence of neoplasia. The condition is usually generalized and can also affect the ocular muscles. Raw antibody titers do not relate well to the severity of the disease and are not useful for prognostic assessment, but measurement of their ability to block and promote degradation of the receptor correlate well [53]. The mechanism of antibody production in thymoma patients is not well understood, but involves inappropriate antigen processing by neoplastic thymic epithelial cells and myocytes and its presentation to residual clones of anti-AchR helper T cells, which are normally kept suppressed [54]. This example re-emphasizes the interactivity and interdependency of different cell populations in the human body in maintaining a stable, healthy internal milieu.

7.2 Arthritis polymyositis, myopathies, hypertrophic pulmonary osteodystrophy, and finger clubbing

These and several other rheumatic disorders have been linked with cancer in reviews [6, 16] and case series, but as the frequency of their coincidence is low and clear mechanistic relationships have not yet been established, it is not possible to exclude coincidental associations with confidence. Accordingly, these conditions need to be noted in this article but will not be discussed further.

8 Cachexia and fever

Patients with advanced cancer are often afflicted by anorexia and severe weight loss, manifested by diffuse loss of muscle and adipose tissue mass (cachexia) [43]. More than 50 % of cancer patients are affected by this syndrome. Until recently, the mechanisms involved were poorly understood, although (1) some evidence for a hormonal etiology was obtained from serum transfer experiments in animals and (2) competition by the tumor for vital growth resources was also considered likely. Rapid advances in understanding the regulation of appetite and body weight in health and disease, described in a superb article by Suzuki et al. [43], have now provided a wealth of new evidence, indicating that multiple secreted products inappropriately synthesized and released by the tumor cells contribute to this syndrome. These include various
inflammatory cytokines, leptin, and other peptides acting on the hypothalamic-gastrointestinal axis, to induce anorexia. Additionally, cytokines can cause malaise, weakness, fever [55] and increased catabolism in muscle and adipose tissue contributing to weight loss. The same article [43] also provides valuable recommendations for medical and psychological care of cancer patients affected by this complication.

9 Metastasis

It is unconventional to regard this type of cancerous behavior as a paraneoplastic syndrome, but the process shares etiological roots with other paraneoplastic syndromes (see “Unifying Concepts and Conclusions” below), which justify its inclusion. Thus, it results from inappropriate gene regulation within the cells of the primary tumor and causes deleterious effects upon distant organs. In this case, the systemic effects on the host body are exerted by release of malignant cells that can colonize remote organs and compromise their function. At first, the colonies are small and imperceptible, but, as they grow, they can eventually cause a variety of adverse clinical effects, including hemorrhage, ascites, and organ failure due to replacement of healthy tissue with malfunctioning tumor. Thus, this is yet another mechanism by which a localized tumor becomes a generalized disease, ultimately causing severe morbidity and death. The topic of tumor–host interactions in metastasis is too extensive to cover here and is considered in more detail in a separate article [56].

10 Therapy

Therapy for the wide range of paraneoplastic syndromes discussed above varies according to the specific organs and mechanisms involved. However, it is widely accepted that surgical removal of the primary tumor or reduction of tumor burden with chemotherapy and/or radiation is helpful [5, 10, 12, 28, 57], although often not curative, because of irreversible organ damage already sustained. In circumstances where the mechanism of the disorder is immunological, plasmapheresis and/or treatment with steroids, immunosuppressants (e.g., azathioprine, cyclophosphamide, cyclosporin), and intravenous immunoglobulins have ameliorated symptoms in some cases.

Where such treatments are not possible or effective, due to clinical circumstances, the secondary and tertiary physiological and metabolic consequences of the PNS need to be controlled in order to provide symptomatic relief and minimize continuing damage to target organs. Thus, for example, various drugs may be used to control motor incoordination due to encephalitis or opsoclonus, to treat epilepsy, and to diminish pain, hypertension, or thrombotic phenomena. In addition, bisphosphonates are used to reduce hypercalcemia, demeclocycline to counteract excess ADH or ketoconazole to block excess ectopic ACTH, as appropriate. Special diets, vitamin supplements, and appetite stimulants are necessary for patients with malignant cachexia [43] or hypoglycemic episodes.

Randomized clinical trials (RCT) have proved hard to organize because of the great variety of the syndromes described and difficulties in accruing sufficient suitable participants and controls. A recent search of the Cochrane database failed to identify any published RCT relevant to neurological paraneoplastic syndromes [58] and the six other non-RCT trials which recruited more than five patients were either not blinded or lacked important information such as outcome data and differences in treatment of the primary tumor or controls. The evidence obtained was reported as class 4 or below. A separate source [40] states that some studies of myasthenia gravis and Lambert–Eaton myasthenic syndrome have reached class 2 evidence status, but that published studies on other neurological syndromes did not even reach class 3 evidence.

Therefore, currently, the treatment of these syndromes depends upon the skill and judgment of the individual physician taking care of the patient, with little reliable guidance from clinical trials. As the main goal is alleviation of symptoms and prevention of further damage while detection and treatment of the underlying cancer is implemented, speed in making the correct diagnosis and implementing treatment and/or palliation is essential.

11 Unifying concepts and conclusions

This review has focused on specific examples of paraneoplastic disorders in which reasonable evidence of a causal role of the neoplasm in the mechanism of the systemic malfunction has been demonstrated. It is clear, however, from published reports that many other indirect effects of malignancy on organs remote from the tumor probably occur. It is, therefore, prudent to remain alert to the possibility of a paraneoplastic etiology when faced with clinical features that seem perplexing and inconsistent with known diseases, especially as the cancer is often occult when the syndrome first appears.

All of the ailments described in this article cause significant increased morbidity to cancer patients, already trying to cope with the side effects of surgery, radiation, and/or chemotherapy. It is important, therefore, for compassionate care of cancer patients who may be facing mortality, to take this group of syndromes seriously in the overall management of the malignant disease.

The wide range of ailments that are so far accepted as being paraneoplastic in etiology fall into two main categories; namely those that are caused by:

1. The unregulated secretion of hormones, growth factors, vaso-active peptides, cytokines, and/or other signaling
molecules by the tumor cells into the systemic circulation. In this group, the tumor cells responsible for synthesizing and secreting the protein products are sometimes of the same histogenetic origin as the normal counterparts responsible for producing the same molecule but frequently tumors of other cell types begin ectopic overproduction of the same substance.

2. Immune responses by the host to neo-antigens or other tumor products and the damaging effects of cross reactions of these with normal tissues and organs. In this category of syndromes, the tumor cells produce antigens mimicking products of other tissues and organs or neo-antigens, not normally present in healthy adult individual (e.g., fusion proteins etc.). The associated syndromes result from accidental collateral damage exerted by the host’s defenses upon bystander normal body tissues.

In addition, paraneoplastic disorders have been documented by veterinarians in domestic animals such as dogs and cats [59, 60], inbred strains of laboratory animals [61], and in wild animals, such as frogs [62]. They therefore represent a class of secondary diseases, which is more widely distributed in nature than generally recognized. This indicates that there is a common underlying causality for them, which is related to the mechanisms responsible for neoplasia itself.

This raises the question of what the pivotal mechanism underlying cancers and their secondary paraneoplastic effects might be and whether such information might be of direct clinical use. Analysis of the available data indicates that inappropriate gene expression (IGE) caused by genetic instability in cancer cells is a unifying explanation for the many manifestations of cancer and the effects the tumor has upon its host. The term IGE signifies the unscheduled appearance of a gene product at an unusual time of life or in an atypical cell, tissue, or organ. Such inappropriate expression is a characteristic feature of most cancers [56]. It can exert severe effects on patient health and comfort and results from the increasing dysregulation that occurs within the genome of affected cell population following the initial carcinogenic event and the faulty attempts to repair it.

This unifying perspective on the etiology of paraneoplastic illnesses and the underlying control and coordination mechanisms that they reveal is of value in maintaining a high index of suspicion of an occult malignancy, when faced with a patient with otherwise perplexing signs and symptoms, which can aid early diagnosis and treatment of both the syndrome and the neoplasm. It also raises awareness of possible wide-ranging subtle effects of the cancer upon multiple systems of the patient’s body, which may need therapeutic attention.

Although such understanding is not seen as leading speedily to a unifying cure of cancer and its secondary effects, it does promote a deeper understanding of the causes and consequences of the disease and enhance thinking about their treatment. Such insight leads to recognition that growing tumors interact parasitically with their hosts in many ways, including drawing essential resources from the surrounding tissues and exerting many deleterious effects on the whole organism, all of which require control to stop or diminish the progress of the illness. As all living organisms and humans in particular are mortal, it is impossible to cure all disease, and the more realistic aim is to extend useful life and ameliorate discomfort, an essential component of high quality cancer care. Recognition of the causes and consequences of paraneoplastic syndromes will help to advance diagnosis and treatment of these conditions and thus contribute to achieving these aims.

The practical clinical value of this unifying explanation is that it (1) raises awareness of the surprisingly high prevalence of these syndromes “hiding in plain sight” and (2) organizes the otherwise bewildering array of information about these conditions into a rational framework, which aids planning a systematic approach to treatment and clinical trials to design better therapy. Paradoxically, the paraneoplastic disorders also provide valuable insights into the dynamic interactions between different cell types, tissues, and organs, which create and maintain the stable equilibrium characteristic of healthy organisms.

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References


Paraneoplastic immune-mediated neurological effects of systemic cancers


Cancer patients may develop paraneoplastic neurological conditions associated with autoantibodies directed against neural or neuromuscular tissues. These syndromes are frequently manifested in advance of the cancer presentation by several months or years necessitating a detailed and expensive investigation to search for the presence of a malignancy. In such cases additional assistance may be obtained by the early employment of whole body 18F fluorodeoxyglucose positron emission tomography as a cancer screening imaging procedure for early cancer diagnosis and potential therapy. Effective therapy of the primary cancer consists the best current therapy for a given paraneoplastic syndrome. However, other forms of immune modulation, such as plasma exchange, intravenous gamma globulin, other immune therapies and symptomatic treatment for certain PNS may have additional benefit.

KEYWORDS: cancer • FDG PET • immunotherapy • paraneoplastic neurological syndrome

Paraneoplastic neurological syndromes (PNS) are remote effects of cancer involving the nervous system [12]. Some syndromes are considered ‘classical PNS’ if no other obvious etiology is found and comprise the limbic encephalitis, subacute sensory neuropathy, opsoclonus–myoclonus, cerebellar degeneration, myasthenic syndrome and peripheral nerve hyperexcitability [3]. In most cases, autoantibodies against nervous system tissue are detected indicating that autoimmunity may play a central role in the development of these syndromes [1,4]. However, in approximately 20–50% of patients, depending on the malignancy, paraneoplastic antibodies may be lacking [3].

PNS frequently present several months or years prior to cancer diagnosis necessitating its differential from other non-malignancy-related conditions. Thus, when a patient presents with a typical PNS but no cancer is identified with the routine cancer screening tests, the physician is faced with the dilemma to either observe the patient or perform additional diagnostic procedures to identify the responsible malignancy. Such procedures may include blood tests and imaging procedures that eventually may lead to identification of a suspicious lesion to biopsy and establish the diagnosis. Current imaging procedures include x-rays, ultrasonography, CT scans, MRI scans and nuclear medicine imaging examinations. Nuclear medicine imaging such as PET and single-photon emission computed tomography are useful for diagnosis and management of a variety of neurological diseases and malignancies [5–7]. In addition, single-photon emission computed tomography [8,9] and PET [10] scans may be used to assess tumor biologic behavior and estimation of overall prognosis.

In the present study, we reviewed the published literature of the last decade and included some older key references on diagnostic evaluation and therapy of patients with probable neurological paraneoplastic syndromes. The search strategy included English literature from PubMed (from 2004 to 29 November 2013) using the key words ‘paraneoplastic neurological syndrome’, ‘paraneoplastic syndrome and brain’, ‘paraneoplastic syndrome and spinal cord’, ‘paraneoplastic neuropathy’, ‘paraneoplastic syndrome’ alone and in combination with ‘therapy’ or ‘diagnosis’ or ‘PET’. The search produced 5361 documents, which after title and abstract review were reduced to 152 publications that were extensively reviewed. Some older key references were also included in this review.
PNS & immunity

The appearance of a PNS is frequently misleading and may imitate other clinical conditions [2]. The most common PNS and their associated autoantibodies are shown in Table 1. PNS are mediated by humoral or cell-mediated immunity. Autoantibodies related to PNS may aid in the diagnosis of the nature of cancer since paraneoplastic syndromes are usually manifested prior to cancer diagnosis [4]. Overall two types of immune mechanisms are implicated in paraneoplastic syndromes and associated with different responses to therapeutic interventions: the first or type I mechanism is associated with antibodies directed against intracellular antigens such as Hu, Ma2, CV2, amphiphysin and Ri and are predominantly dependent on T-cell-mediated responses. Type II is due to antibodies directed against cell membrane antigens, such as the voltage-gated calcium channels and anti-N-methyl-D-aspartate (NMDA) receptors. Although most cases are progressive leading to severe disability, occasionally they can be reversed or improve by effective treatment of the primary cancer, administration of intravenous immune gamma globulin (IVIg) and/or corticosteroids [2].

Contribution of 18-fluorine fluoro-2-deoxy-glucose PET in patients with PNS

18-Fluorine fluoro-2-deoxy-glucose (FDG) PET imaging represents an adjunct to cancer diagnosis and assessment of the extent of the structural or functional abnormality in the nervous system and its response to a given treatment [10]. The FDG PET/computed tomography (CT) takes advantage of the increased glucose uptake, a characteristic of tumor and inflammatory cells, in order to detect tumors and various inflammatory conditions such as paraneoplastic encephalitis [10].

FDG PET is a valuable imaging modality for cancer diagnosis in patients with PNS [11]. In patients with paraneoplastic antibodies, FDG PET may show the presence of a tumor undetectable by other imaging procedures; however, its value may be less in seronegative patients [3]. A retrospective review of 68 patients of whom 43 had a classical paraneoplastic syndrome, 18 patients had a positive FDG PET/CT suggestive of a possible cancer [12]. In a retrospective study of 27 patients with possible neurological paraneoplastic syndrome, FDG PET was abnormal in 6 patients suggesting possible cancer, which was confirmed histologically in 5 of them [13].

Overall, FDG PET scanning may detect small tumors in approximately one-third of patients with suspected PNS and negative workup for cancer [14]. In a retrospective study on 30 patients with suspected paraneoplastic syndrome and negative other diagnostic imaging tests including CT scans, FDG PET was positive in 6 of 7 biopsy proven patients with malignancies validating its usefulness in such cases [15].

FDG PET apart from assisting in detection of the primary cancer site [16-19], in some cases it may demonstrate multiple primary tumors requiring different therapeutic interventions [20,21]. Thus, FDG PET/CT even though is by itself an expensive test, it may represent a cost-effective procedure if it is used upon presentation of the PNS, because of its diagnostic value leading to early cancer detection and possibly improved outcome.

Diagnosis of common PNS (Table 2)

**Limbic encephalitis**

Paraneoplastic limbic encephalitis presents with seizures, behavioral abnormalities and memory dysfunction. It is often associated with small cell lung cancer (SCLC), testicular or ovarian tumors [22]. Cerebrospinal fluid examination may demonstrate mild lymphocytosis, elevated protein, normal glucose and presence of oligoclonal bands.

MRI of brain in paraneoplastic limbic encephalitis may be within normal limits [23], or shows high-intensity lesions of the medial temporal lobes on fluid-attenuated inversion recovery images [24]. FDG PET demonstrates hypermetabolism in one or both temporal lobes, a finding that can occur in viral encephalitis as well; however, in viral encephalitis focal areas of hypometabolism usually concur with the diffuse hypermetabolism [25]. In undetermined cases, assessment of paraneoplastic antibodies in the serum and PCR of the CSF for viruses are helpful to establish the diagnosis [26]. In addition to the temporal lobe abnormalities, FDG PET scan may also demonstrate striatal hypermetabolism, a finding that may also be noted in other autoimmune conditions, such systemic lupus erythematosus or antiphospholipid syndrome [27].

**Brainstem encephalitis**

Paraneoplastic brainstem encephalitis may be associated with limbic encephalitis or rarely may occur alone and linked to lung, testicular or gynecological cancers. The patient’s symptoms include vertigo, nystagmus, ataxia, diplopia, dysarthria and dysphagia. Anti-neuronal antibodies described in this syndrome include anti-Hu, anti-Ma2 and anti-Ri. Patients with anti-Ma2 antibodies may respond to specific treatment of the tumor or immunotherapy, patients with anti-Ri antibodies are unlikely to respond to any treatment, and patients with a-Hu tumor or immunotherapy, patients with anti-Ri antibodies are either unresponsive [19] or may stabilize after specific tumor therapy [28]. However, in rare cases effective treatment of the primary tumor may reverse anti-Hu encephalitis [29].

**NMDA-receptor encephalitis**

NMDA-receptor encephalitis may present with mental status changes, psychosis, catatonic reaction, spasticity and seizures, usually associated with ovarian tumors [2]. CSF evaluation may exhibit lymphocytosis and presence of oligoclonal bands. Serum NMDA-NR-1 receptor antibody titers are elevated. If a tumor is found, its resection may lead to regression of the syndrome [30]. If no tumor is detected, steroids or rituximab have been reported to be beneficial [31].

**Cerebellar degeneration**

Paraneoplastic cerebellar degeneration (PCD) is a cerebellar disease, involving both hemispheres and vermis. The patients clinically exhibit both appendicular and gait ataxia. It is frequently manifested prior to the diagnosis of malignancy and
less often after the cancer diagnosis \[32\]. The tumors that are more commonly associated with this syndrome include lung, ovarian and breast cancers and Hodgkin’s lymphoma. CSF may show elevated IgG index and presence of oligoclonal bands. Serum and CSF could demonstrate anti-neuronal antibodies such as anti-Yo, which are anti-Purkinje-cell autoantibodies typical of PCD. Apart from anti-Yo antibodies, some patients with PCD demonstrate anti-Tr antibodies especially

Table 1. Common neurological paraneoplastic syndromes.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Neurologic symptoms and findings</th>
<th>Cancer (s)</th>
<th>Type of immune reaction</th>
<th>Paraneoplastic antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limbic encephalitis [22,61]</td>
<td>Seizures, behavioral abnormalities, psychosis, memory dysfunction</td>
<td>SCLC, breast testicular cancer</td>
<td>I</td>
<td>Anti-Hu (ANNA-1) Anti-Ma2 (Ta)</td>
</tr>
<tr>
<td>Brainstem encephalitis</td>
<td>Vertigo, nystagmus, ataxia, diplopia, dysarthria, dysphagia</td>
<td>SCLC, breast, gynecological cancer, testicular cancer</td>
<td>I</td>
<td>Anti-Hu (ANNA-1) Anti-CV2 (CRMP5), Anti-amphiphysin, Anti-Ma2 (Ta)</td>
</tr>
<tr>
<td>NMDR encephalitis [62]</td>
<td>Seizures, fever, headache, involuntary movements, psychosis, catatonia</td>
<td>Ovarian teratoma</td>
<td>II</td>
<td>Anti-NMDA-R</td>
</tr>
<tr>
<td>Cerebellar degeneration [2]</td>
<td>Dysarthria, limb, truncal and gait ataxia, nystagmus</td>
<td>SCLC, ovarian, breast, Hodgkin’s</td>
<td>I (presence of specific CD8+ T cells to intracellular cdr2 antigen) [63]</td>
<td>Anti-Yo (CDR2) [64] Anti-Hu (ANNA-1) Anti-Tr [65]</td>
</tr>
<tr>
<td>Myelitis</td>
<td>Spastic weakness of legs, thoracic sensory level, bladder/bowel dysfunction</td>
<td>SCLC, lymphoma, breast, other cancers</td>
<td>I</td>
<td>Anti-CV2 (CRMP5), Anti-amphiphysin</td>
</tr>
<tr>
<td>Pure sensory neuropathy</td>
<td>Progressive numbness and pain of hands and feet; all sensory modalities affected</td>
<td>SCLC</td>
<td>I</td>
<td>Anti-Hu (ANNA-1) Anti-CV2 (CRMP5) Anti-amphiphysin [66]</td>
</tr>
<tr>
<td>Mixed sensory motor neuropathy</td>
<td>Various sensory decrease and motor weakness</td>
<td>NSCLC, various cancers</td>
<td>I</td>
<td>Anti-CV2 (CRMP5) Anti-amphiphysin</td>
</tr>
<tr>
<td>Myasthenic syndrome</td>
<td>Proximal muscle weakness, dry mouth and areflexia</td>
<td>SCLC</td>
<td>II</td>
<td>Anti-VGCC (not tumor specific) [67,68]</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>Eyelid ptosis, diplopia, weakness of various muscles</td>
<td>Thymoma</td>
<td>II</td>
<td>Anti/acetylcholine receptor (not tumor specific)</td>
</tr>
<tr>
<td>Peripheral nerve hyperexcitability (neuromyotonia) [46]</td>
<td>Spontaneous and continuous muscle overactivity, muscle cramps</td>
<td>Thymoma, lung cancer, lymphoma, plasmacytoma</td>
<td>II</td>
<td>Anti-VGCC (not tumor specific)</td>
</tr>
<tr>
<td>Dermatomyositis Polymyositis Necrotizing myositis</td>
<td>Proximal muscle weakness, muscle pain, serum elevated muscle enzymes</td>
<td>Non-Hodgkin’s lymphoma, lung cancer, breast cancer, other cancers</td>
<td>N/A</td>
<td>None</td>
</tr>
<tr>
<td>Stiff person</td>
<td>Stiffness and pain of axial and limb muscles</td>
<td>Breast, lung cancer</td>
<td>I</td>
<td>Anti-amphiphysin [52] Anti-GAD or anti-glycine receptor (not cancer associated) [47]</td>
</tr>
<tr>
<td>Opsoclonus/myoclonus</td>
<td>Multidirectional conjugate eye movements, ataxia, aphasia</td>
<td>Neuroblastoma, breast, ovarian, SCLC</td>
<td>II</td>
<td>Anti-Ri (ANNA-2), various other abs</td>
</tr>
</tbody>
</table>

Anti-VGCC: Autoantibodies against the voltage-gated calcium channels; CDR2: Cerebellar degeneration-related antigen 2; GAD: Glutamic acid decarboxylase; Hu: Binding protein to neuronal RNA/DNA; NMMDA: N-methyl-D-aspartate; Ri: RNA-binding protein; SCLC: Small cell lung cancer; VGKC: Voltage-gated potassium channels; Yo: DNA-binding protein.
with Hodgkin’s disease or less frequently with squamous cell carcinoma of the lung [33]. MRI of the brain may be normal or showing a nonspecific atrophy of the cerebellum. In PCD, hypometabolism is noted by FDG PET correlated with cerebellar atrophy observed with MRI [34]. In several published cases, the presence of the primary tumor was undetectable with conventional imaging but depicted by FDG PET [35].

Table 2. Diagnostic evaluation of most common paraneoplastic neurological syndromes.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>CNS MRI</th>
<th>PET</th>
<th>CSF studies</th>
<th>Other diagnostic tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limbic encephalitis [22,61]</td>
<td>Normal or temporal lobe non-contrast-enhancing T2 or fluid-attenuated inversion recovery hyperintensities</td>
<td>Temporal lobe or striatal FDG hypermetabolism</td>
<td>† cells (lymphs) † or normal protein Normal glucose</td>
<td>–</td>
</tr>
<tr>
<td>NMDR encephalitis [62]</td>
<td>Normal or temporal or brainstem T2 or fluid-attenuated inversion recovery hyperintensities</td>
<td>Frontal and temporal FDG hypermetabolism and occipital hypometabolism</td>
<td>† cells (lymphs) † or normal protein Normal glucose</td>
<td>–</td>
</tr>
<tr>
<td>Cerebellar degeneration [2]</td>
<td>Normal or cerebellar atrophy</td>
<td>Cerebellar FDG PET hypometabolism</td>
<td>† or normal cells (lymphs) † or normal protein Normal glucose ± oligoclonal bands</td>
<td>–</td>
</tr>
<tr>
<td>Pure sensory neuropathy</td>
<td>Normal</td>
<td>May show the primary cancer [10]</td>
<td>† or normal cells (lymphs) † or normal protein Normal glucose ± oligoclonal bands</td>
<td>↓ ↓ sensory action potentials in electrophysiologic tests [3]</td>
</tr>
<tr>
<td>Mixed sensory motor neuropathy</td>
<td>Normal</td>
<td>May show the primary cancer site</td>
<td>May be normal or nonspecific † or normal protein and cells</td>
<td>↓ Sensory and motor action potentials in electrophysiologic tests</td>
</tr>
<tr>
<td>Peripheral nerve hyperexcitability (neuromyotonia) [46]</td>
<td>Normal</td>
<td>May show the primary cancer site [69]</td>
<td>–</td>
<td>Spontaneous, continuous motor unit activation (neuromyotonia) in electromyography</td>
</tr>
<tr>
<td>Dermatomyositis Polymyositis Necrotizing myositis</td>
<td>May show increased signal intensity on T2 images of affected muscles [70]</td>
<td>May show the primary cancer site May show hypermetabolism in affected muscles</td>
<td>–</td>
<td>In muscle biopsy atrophy of muscle fibers and inflammatory infiltrates in dermatomyositis and polymyositis and necrosis of muscle fibers with limited inflammatory infiltrates in necrotizing myositis</td>
</tr>
<tr>
<td>Opsoclonus/myoclonus [56]</td>
<td>MRI negative or evidence of cerebellar dentate nucleus abnormalities [55]</td>
<td>FDG PET Uncertain abnormalities in brain; May show the primary cancer [71]</td>
<td>† or normal cells (lymphs) † or normal protein Normal glucose ↑ or normal IgG index ± oligoclonal bands</td>
<td>↑ Urinary VMA Pathological MIBG scintigraphy [72] (neuroblastoma)</td>
</tr>
</tbody>
</table>

**Polyneuropathy**
Paraneoplastic neuropathy in more frequently manifested as subacute sensory neuropathy and less frequently as sensorimotor neuropathy, Guillain–Barré syndrome and chronic inflammatory demyelinating polyneuropathy. The sensory neuropathy is manifested with pure sensory symptoms such as numbness, tingling and sensation loss of the hands and feet in a glove-stocking distribution without muscle weakness. The sensorimotor neuropathy causes both sensory symptoms and distal symmetric extremity weakness. The Guillain–Barré syndrome and chronic inflammatory demyelinating polyneuropathy are less frequent types and present with acute or chronic development of asymmetric extremity weakness and loss of tendon reflexes. All forms of neuropathies can be easily diagnosed with standard electrophysiological tests. CSF analysis may be unrevealing in sensory or sensorimotor neuropathies but it may show increased protein and no cells in Guillain–Barré syndrome and chronic inflammatory demyelinating polyneuropathy. Occasionally, autonomic neuropathies are also noted [36].

**Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy & skin abnormalities syndrome**
Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin abnormalities (POEMS) syndrome is a paraneoplastic syndrome. The syndrome is due to the underlying neoplasia of plasma cells [37]. Papilledema, generalized edema and increased levels of VEGF are common findings [38]. In a retrospective study on 14 patients with POEMS syndrome, the most frequent clinical manifestations included extremity numbness and weakness, lymphadenopathy, abdominal distention and skin hyperpigmentation [39]. The severity of the plasma cell disorder determines the prognosis. The bony lesions associated with POEMS syndrome are predominantly osteosclerotic and less often osteolytic as seen in multiple myeloma. For localized osteosclerotic lesions, radiotherapy may be sufficient. Systemic treatment should be reserved for diffuse disease and recurrences after radiotherapy and include steroids, alkylating agents and antiangiogenic agents [37]. Durable responses can be achieved using high-dose chemotherapy with peripheral blood stem cell transplantation [38].

**Neuromuscular junction syndromes**
**Lambert–Eaton myasthenic syndrome**
Lambert–Eaton myasthenic syndrome (LEMS) is a disorder of the neuromuscular junction, consisted of autoantibodies against the voltage-gated calcium channels blocking the release of acetylcholine by the presynaptic neurons. The patients exhibit proximal muscle weakness, dry mouth and areflexia. The syndrome is documented by the presence of specific LEMS autoantibodies in the sera of patients. Approximately 50% of patients with the syndrome are shown to have SCLC and in the remaining the syndrome is idiopathic [40]. Age at onset ≥50 years, smoking, weight loss, bulbar involvement, erectile difficulties and poor performance status are factors that increase the possibility of neoplastic origin of the syndrome [41]. The management consists of specific antitumor treatment; however, symptomatic treatment with 3,4-diaminopyridine (3,4-DAP) is effective to alleviate the symptoms [42]. 3,4-DAP increases the presynaptic release of acetylcholine by blocking the potassium channel efflux in nerve terminals resulting in increased duration of the action potential. This effect allows the forced opening of Ca²⁺ channels and acetylcholine release to stimulate the postsynaptic muscle fiber and improves muscle contraction. Various reports suggest that rituximab and immunosuppressive therapies may be occasionally beneficial with steroids or azathioprine [27].

**Myasthenia gravis**
Patients with myasthenia gravis may be classified in those with low-affinity acetylcholine receptor antibodies, those with antibodies to the postsynaptic low-density lipoprotein receptor-related protein 4, those with antibodies against muscle-specific kinase and those without antibodies [43]. Resection of a thymoma is the specific tumor management associated with symptomatic disease drugs as pyridostigmine and other immune therapies such as steroids, plasmapheresis [44] and IVlg or chronic immunosuppression with azathioprine.

**Muscle diseases**
Paraneoplastic muscle diseases include polymyositis, dermomyositis and necrotizing myopathy. All are manifested by proximal muscle weakness but the necrotizing myopathy has a more rapid course and elevation of serum muscle enzyme levels. Muscle biopsy demonstrates atrophy of muscular fibers with widespread inflammatory infiltrates in dermomyositis and polymyositis and necrosis of muscle fibers with limited or absent inflammatory infiltrates in necrotizing myopathy [17,45]. Therapies apart from tumor-specific treatment include steroids and IVlg [44].

**Peripheral nerve hyperexcitability syndrome**
This syndrome consists of spontaneous activity of the peripheral nerves resulting in muscular activity described clinically as neuromyotonia and associated with muscle cramps. It is an autoimmune disease, associated with antibodies against the voltage-gated potassium channel and in approximately 25% of cases associated with thymoma, lung cancer, lymphoma or plasmacytoma [46]. Treatment of the tumor, steroids, IVlg, plasma exchange and chemotherapeutic drug-induced immunosuppression usually improve the symptoms [3].

**Stiff-person syndrome**
Stiff-person syndrome is characterized by inhomogeneous association of muscle stiffness and painful muscular spasms that can be localized in various parts of the body. Over 50% of patients demonstrate presence in serum and CSF of antibodies against the glutamic acid decarboxylase [47,48] and less frequently against the glycine receptor (anti-GlyR) [49], which are not associated with malignancies. The enzyme glutamic acid decarboxylase is converting glutamic acid to GABA, an inhibitory neurotransmitter, thus its inhibition results in excessive
nerve terminus and is involved in endocytosis.

antibodies against amphiphysin, a protein located near the

cortex

strated significant decrease in GABA level in the sensorimotor

presence of the autoantibodies, MRI spectroscopy has demon-

neurotransmission and muscular stiffness. In addition to the

Brain and cerebrospinal fluid (CSF) analysis showed abnormal re-

activity. Amyloid angiopathy was also noted. In addition to the

Paraneoplastic stiff-person syndrome may be associated with

antibodies against amphiphysin, a protein located near the

nerve terminus and is involved in endocytosis [52]. Specific
tumor therapy [53] or symptomatic therapy with IVIg, plasma
exchange, botulinum toxin and muscle relaxants may success-

fully improve the clinical picture [48].

Opoclonus–myoclonus syndrome

Opoclonus–myoclonus syndrome (OMS) is a rare syndrome
characterized by sudden multidirectional conjugate eye

movements associated with brief muscle twitching. Ataxia and
aphasia may be also present, and sometimes may be misdiag-

osed as acute cerebellar ataxia [54]. The syndrome is noted pre-
dominantly in young children with neuroblastoma and in some
adults with either breast or SCLC. MRI may demonstrate non-
specific abnormalities in the pons or cerebellar regions [55]. In
children with neuroblastoma, the presence of the syndrome is
associated with better survival but higher incidence of long-
term neurologic sequelae.

A retrospective study in 21 adult patients with OMS from a
single institution showed the presence of a malignancy in only
3 patients (2 with breast and 1 with SCLC) [56]. Immunother-
apy resulted in either resolution or improvement in the major-
ity of patients. Review of 116 previously reported patients with
OMS revealed a cancer in 60 patients (SCLC in most followed
by breast carcinoma). The most common antibody was anti-
neuronal nuclear antibody type 2 (anti-Ri) [56].

Table 3. Effective non-tumor-related therapies for certain paraneoplastic neurological syndromes.

<table>
<thead>
<tr>
<th>Paraneoplastic syndrome</th>
<th>Drugs</th>
<th>Mode of action</th>
<th>Most important side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myasthenic syndrome [73,74]</td>
<td>First choice: 3,4-DAP, 20 mg p.o. q.i.d. [75,76] Second choice: Pyridostigmine, 30 mg p.o. t.i.d. Third choice: Immunosuppression with corticosteroids, azathioprine, IVIg, plasma exchange</td>
<td>3,4-DAP blocks potassium channel efflux in nerve terminals leading to opening of Ca^{2+} channels and acetylcholine release</td>
<td>3,4-DAP: paresthesias, epileptic seizures, arrhythmias</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>Pyridostigmine, 30 mg p.o. t.i.d. advance as needed to control symptoms</td>
<td>Inhibits acetylcholinesterase that hydrolyzes acetylcholine</td>
<td>Sweating, tearing, abdominal cramps, nausea/vomiting, pupill constriction</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>IVIg, plasma exchange [58]</td>
<td>Immune system modulation</td>
<td>Allergy, infection, thrombosis plasma exchange</td>
</tr>
<tr>
<td>Opsoclonus/myoclonus</td>
<td>[77] ACTH, 75 IU b.i.d. for 1 week, then 75 IU q.d. for 1 week, then 75 IU every other day for several months [78-80] Dexamethasone, 0.1 mg/kg/day Prednisone, 2 mg/kg/day [80] IVIg, 1 g/kg/day × 2 days, then 1 g/day every month [81,82] Rituximab 375 mg/m² IV weekly × 4 weeks [77] Cyclophosphamide 600 mg/m² IV infusion every month [57,77,82]</td>
<td>Immune system modulator drugs that reduce CCL21, a protein that binds to the CCR7 cell surface receptor</td>
<td>ACTH/steroids: Infection, hyperglycemia, hypertension, cushing syndrome IVIg: allergy Rituximab: allergy, infection, progressive multifocal leukoencephalopathy</td>
</tr>
<tr>
<td>Stiff-person syndrome</td>
<td>First choice: Diazepam, 20–40 mg p.o./day [83-85] Second choice: Baclofen, 5 mg p.o. t.i.d., advance as tolerated to relieve symptoms up to 80 mg/day [84,85] Third choice: Corticosteroids, azathioprine, IVIg [84-86]</td>
<td>Enhance the function of GABA (GABA agonists) Immune system modulator drugs</td>
<td>Diazepam: drowsiness, sedation, dependence Baclofen: drowsiness, ataxia in high doses</td>
</tr>
</tbody>
</table>

*The various drugs can be used either alone or in various combinations. Other dose schedules may be also used.

3,4-DAP: 3,4-Diaminopyridine; b.i.d: Twice a day; CCL21: Chemokine (C–C motif) ligand 21; q.d.: Once per day; q.i.d.: Four-times a day; t.i.d: Three-times a day.

3,4-DAP blocks potassium channel efflux in nerve terminals leading to opening of Ca^{2+} channels and acetylcholine release.
In children with untreated OMS, the serum level of CCL21, an inflammatory chemokine that mediates its effects by binding to the CCR7 cell surface receptor is high, but normalized after treatment with corticosteroids, ACTH or in combination with IVlg, rituximab or cyclophosphamide. These effects suggest that therapies that inhibit the inflammatory chemokines may be needed for successful treatment of this syndrome [57]. The syndrome may be responsive to corticosteroids, γ-globulin, treatment of the tumor and rituximab. OMS in adults is more resistant to any form of immunotherapy; however, it may occasionally respond to specific cancer treatment [28].

Management strategies (Table 3)
Although no randomized studies exist to evaluate various treatment modalities in patients with PNS, treatment strategies include specific tumor therapy, nonspecific immunotherapies such as plasma exchange and IVlg, symptomatic treatment and other immune therapies [58,59]. Presence of antibodies against surface (type II) rather than intracellular (type I) antigens may predict a favorable response to immunotherapy [12]. Specific cancer treatment may inhibit the evolution of type I paraneoplastic syndromes and may even improve the type II syndromes [4].

Type I paraneoplastic syndromes may also be managed with corticosteroids, IVlg and immunosuppression with inconsistent results. Patients with paraneoplastic conditions and antibodies against ion channel autoantibodies also respond better to immunotherapies [1]. Type II syndromes, in addition to tumor-specific treatment, may be managed with symptomatic treatment such as 3,4-DAP for LEMS and carbamazepine for PNH with acceptable results. Immunomodulatory treatments like IVlg and plasma exchange may be used as adjunct to the specific or symptomatic therapies. In resistant type II paraneoplastic syndromes, rituximab, a chimeric monoclonal antibody against CD20, may be beneficial [60].

Expert commentary
PNS are cancer-related manifestations not directly linked to tumor infiltration but to immunological processes with secondary involvement of the nervous systems and muscles. PNS may be associated either with antibodies directed against intracellular antigens that mediate T-cell immune responses and are resistant to therapeutic interventions, or with antibodies against cell membrane antigens, which depend on B-cell-mediated immunity and have better response to therapy. The most common PNS include neuropathy, cerebellar degeneration, encephalitis, OMS, POEMS syndrome, myasthenic syndrome, myasthenia, various muscle diseases, stiff-person syndrome and peripheral nerve hyperexcitability syndrome. Upon presentation of a patient with a probable PNS and no history of cancer, a careful evaluation should be performed to document the presence of a tumor. The investigation should include assessment of various known paraneoplastic antibodies in the sera of the patients and specific cancer-directed imaging modalities, which should include FDG PET. FDG PET may be performed if the conventional imaging tests are unrevealing or as an initial screening procedure because of its enhanced cancer detection capability. Therapeutic strategies include specific tumor therapy, nonspecific immunotherapies such as plasma exchange and IVlg, corticosteroids, and symptomatic therapies depending on the specific syndrome.

Five-year view
It is anticipated that over the next several years new paraneoplastic antibodies will be found reducing the percentage of ‘seronegative’ patients with paraneoplastic syndromes. This anticipation in addition to earlier cancer diagnosis using FDG PET at an early investigational stage could result in earlier and more effective treatment and improved prognosis.

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Paraneoplastic disorders of the central and peripheral nervous systems

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INTRODUCTION
Paraneoplastic neurologic syndromes (PNS) have been traditionally defined as an acute or subacute neurologic syndrome resulting from nervous system dysfunction that is remote from the site of a malignant neoplasm or its metastases. This definition excludes other differential diagnoses such as metastasis, carcinomatous meningitis, chemo- or radiotherapy toxicity, vitamin deficiency, or local spreading of the tumor. However, with respect to our current understanding of their pathogenesis, we may redefine these disorders as cancer-related dysimmune neurologic syndromes. PNS are rare conditions which may involve any part of the central or peripheral nervous systems including the neuromuscular junction.

A group of autoantibodies (Abs) directed against intracellular neuronal antigens and specifically associated with PNS has been early identified since the mid-eighties. The pathogenic role of these onconeural antibodies (ON-Abs) remains partially unknown and some of them are the witness of a predominantly T-cell-mediated autoimmune reaction.

The role of ON-Abs in the pathogenesis of such syndromes is reinforced by recent findings. Positive ON-Abs help to establish the diagnosis of PNS, provide evidence for the underlying pathogenesis, and thus have consequences for therapeutic strategies. The identification of ON-Abs in different clinical presentations other than those classically described leads to discover new cancer-associated syndromes such as encephalitis in N-methyl-D-aspartate receptor antibody (NMDAr-Abs)-positive patients.

In this chapter we first deal with the epidemiology and pathogenesis of PNS. In the second section we review the different classic paraneoplastic neurologic syndromes. Central and peripheral nervous system disorders have been treated separately. For each PNS, clinical features are detailed according to the associated ON-Abs. The third part of the chapter considers therapeutic approaches.

GENERAL CONSIDERATIONS
Epidemiology
PNS is a rare disease, though its prevalence may be underestimated. Early estimates were under 0.01% of the patients with cancer (Darnell and Posner, 2003); however, Abs associated with PNS were found in 0.9% of 60 000 patients with suspected PNS (Pittock, 2004). Prevalence is even higher if serologic screening is oriented by clinical context (up to 25% seropositive in a cohort of 649 cases consecutively analyzed) (Dalmau et al., 2008). These results emphasize the importance of the clinical criteria in the serologic screening of patients suspected to suffer from PNS.

Nevertheless some cancers are associated with a higher rate of PNS, such as thymoma and small-cell carcinoma, associated with myasthenia gravis in 15% of cases and with Lambert–Eaton myasthenic syndrome in 3% of cases respectively. Most of the tumors associated with PNS express neuroendocrine proteins, affect organs which belong to immune system, such as thymoma, or contain mature or immature neuronal tissue (Dalmau et al., 2008). Their incidence continues to grow as new syndromes are added. For instance, more than...
500 new cases of limbic encephalitis associated with NMDAr-Abs were reported in the first 4 years after its first description in 2007.

Diagnosis

The paraneoplastic neurologic syndromes are well known and clearly defined (Graus et al., 2004). Eight presentations have been individualized and are considered to be classic PNS: subacute cerebellar degeneration (SCD), limbic encephalitis (LE), paraneoplastic encephalomyelitis (PEM), opsoclonus myoclonus (OM), subacute sensory neuropathy (SSN), chronic gastrointestinal pseudo-obstruction, Lambert–Eaton myasthenic syndrome (LEMS), and dermatomyositis (Table 78.1). Cancer occurs within 3 years from the onset of a classic PNS. However, other rare and challenging clinical presentations can be considered as PNS. An expert panel has therefore attempted to define a set of criteria in order to identify PNS patients (Graus et al., 2004). These criteria are presented in Table 78.2. Furthermore a positive diagnosis can be made by looking for specific PNS-related antibodies in blood or cerebrospinal fluid (CSF) (Table 78.3).

Antibodies and pathogenesis

The underlying mechanisms of PNS probably differ according to the clinical syndrome and antibodies subtypes. Recent findings suggest a key role for neuronal cell surface antigens (NSA-Abs) such as NMDAr-Abs, Lgi1-Abs, CASPR2-Abs, GABABr-Abs and AMPAr-Abs in the pathophysiology of corresponding neurologic syndromes. All these Abs belong to the NSA-Abs group, which may impair neuronal function through their role on the target antigens (a membranar receptor in most cases). Conversely, T cell-mediated immune reaction plays a major role in PNS whose associated Abs target

| Table 78.1 |
| Paraneoplastic neurologic syndromes (from Graus et al., 2004) |

<table>
<thead>
<tr>
<th>Classic syndromes</th>
<th>Other clinical presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central nervous system</td>
<td></td>
</tr>
<tr>
<td>Limbic encephalitis</td>
<td>Brainstem encephalitis</td>
</tr>
<tr>
<td>Paraneoplastic encephalomyelitis</td>
<td>Optic neuritis</td>
</tr>
<tr>
<td>Opsoclonus myoclonus</td>
<td>Cancer-associated retinopathy</td>
</tr>
<tr>
<td>Subacute cerebellar ataxia</td>
<td>Melanoma-associated retinopathy</td>
</tr>
<tr>
<td>Muscle and nerve</td>
<td></td>
</tr>
<tr>
<td>Lambert–Eaton syndrome</td>
<td>Stiff person syndrome</td>
</tr>
<tr>
<td>Subacute sensory neuropathy</td>
<td>Necrotic myelitis</td>
</tr>
<tr>
<td>Pseudo-occlusion syndrome</td>
<td>Motoneuropathy</td>
</tr>
<tr>
<td>Dermatomyositis</td>
<td>Sensorimotor subacute neuropathy</td>
</tr>
<tr>
<td></td>
<td>Pandysautonomia</td>
</tr>
<tr>
<td></td>
<td>Neuromyotonia</td>
</tr>
<tr>
<td></td>
<td>Acute necrotic myositis</td>
</tr>
</tbody>
</table>

| Table 78.2 |
| Diagnostic criteria of paraneoplastic neurologic syndromes (from Graus et al., 2004) |

<table>
<thead>
<tr>
<th>Definite PNS</th>
<th>Possible PNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A classic syndrome and cancer that develops within 5 years of the diagnosis of the neurologic disorder</td>
<td>A classic syndrome, no onconeural antibodies, no cancer but at high risk for having an underlying cancer</td>
</tr>
<tr>
<td>A nonclassic syndrome that resolves or significantly improves after cancer treatment without concomitant immunotherapy, provided that the syndrome is not susceptible to spontaneous remission</td>
<td>A neurologic syndrome (classic or not) with partially characterized onconeural antibodies (targeting cell surface antigens or neurophils without further indications) and no cancer</td>
</tr>
<tr>
<td>A nonclassic syndrome with onconeural antibodies (well characterized or not) and cancer that develops within 5 years of the diagnosis of the neurologic disorder</td>
<td>A nonclassic syndrome, no onconeural antibodies, and cancer present within 2 years of diagnosis</td>
</tr>
<tr>
<td>A neurologic syndrome (classic or not) with well characterized onconeural antibodies (Hu, Yo, CV2, Ri, Ma2, amphiphysin, NMDAr, AMPAr, GABABr, Lgi1, CASPR2), and no cancer</td>
<td></td>
</tr>
</tbody>
</table>
intracellular antigens (Albert et al., 1998, 2000). These antibodies would be considered as paraneoplastic markers with no suggested direct pathophysiologic role. As the mechanism of the disease is probably related to the antibodies involved in it, we next review the main data available for each antibody.

### NEURONAL CELL SURFACE ANTIGEN ANTIBODIES

They are defined according to the epitopic target located on the surface layer of neurons or glial cells. Their direct pathogenic role is supported by immunologic studies.

#### VGCC-Abs

Clinical response to plasmapheresis (Lang et al., 1981) and to immunosuppressive medication (Newsom-Davis and Murray, 1984) provided strong evidence for an antibody-mediated immune mechanism. Subsequent experiments showed that the physiologic and morphologic features of LEMS could be transferred to mice by the injection of LEMS IgGs, and that this action was due to antibodies specifically targeting P/Q-type voltage-gated calcium channels (VGCCs) (Lang et al., 1981; Fukunaga et al., 1983). These antibodies may also be implicated in cerebellar ataxia occasionally associated with LEMS. Not surprisingly, cerebellar Purkinje and granule cells express P/Q-type VGCCs that can be blocked in vitro by LEMS IgGs (Lang et al., 1998). Passive transfer studies in mice later showed that the antibody-mediated downregulation of VGCCs expressed by cholinergic and adrenergic postganglionic neurons produced autonomic changes such as those seen in LEMS (Waterman et al., 1997).

#### VGKC-Abs, CASPR2-Abs and Lgi1-Abs

Recent findings proved that the real targets of VGKC-Abs are leucine-rich glioma-inactivated 1 protein (Lgi1) in patients with LE (Irani et al., 2010a; Lai et al., 2010) and contactin-associated protein 2 (CASPR2) in patients with neuromyotonia and Morvan’s syndrome (Vincent, 2009; Irani et al., 2010a). These two proteins coprecipitate with the potassium channel, thus explaining why VGKC was inaccurately identified by immune-precipitation as the targeted antigen in the previous studies. Lgi1-Abs may play a direct pathogenic role since this secreted protein is involved in excitatory synaptic transmission as demonstrated in a transgenic mouse model expressing a mutant Lgi1 similar to the one found in human autosomal dominant lateral temporal lobe epilepsy (Zhou et al., 2009).

#### NMDAr-Abs

NMDAr-Abs target the N-terminal extracellular domain of the NR1 subunit of the glutamate receptor NMDA and hamper the glutamatergic pathway by internalizing this receptor (Dalmau et al., 2007, 2008). NMDAr-Abs are present in patient’s sera and CSF as well, the latter showing a high antibody concentration and intrathecal synthesis too (Dalmau et al., 2008; Florance et al., 2009). In vitro, NMDAr-Abs were shown to tap down the NMDAr cluster density in a reversible manner when

### Table 78.3

<table>
<thead>
<tr>
<th>PNS + Ab</th>
<th>Tumor</th>
<th>Paraclinical examination</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hu-Abs (any PNS)</td>
<td>SCLC</td>
<td>FDG PET</td>
<td>Almost all patients have a cancer after 5 years of follow-up</td>
</tr>
<tr>
<td>Yo-Abs and SCA</td>
<td>Ovarian cancer</td>
<td>Explorative laparotomy if radiologic investigations remain negative</td>
<td></td>
</tr>
<tr>
<td>Ma2-Abs Diencephalitis</td>
<td>Testicular cancer</td>
<td>Bilateral orchidectomy must be discussed in case of negative cancer screening</td>
<td>Tr-Abs are fleeting</td>
</tr>
<tr>
<td>Tr-Abs</td>
<td>Hodgkin’s disease</td>
<td>CT of thorax, abdomen and pelvis + biopsy of lymph nodes + puncture of adenopathy</td>
<td></td>
</tr>
<tr>
<td>NMDAr-Abs and LE</td>
<td>Ovarian teratoma</td>
<td>Transvaginal ultrasound and/or pelvic MRI</td>
<td>Mature teratoma, whose metabolism is normal, not ruled out by FDG PET</td>
</tr>
</tbody>
</table>

PNS, paraneoplastic neurologic syndrome; Hu-Abs, anti-Hu antibodies; Yo-Abs, anti-Yo antibodies; Ma2-Abs, anti-Ma2 antibodies; Tr-Abs, anti-Tr antibodies; NMDAr-Abs, N-methyl-D-aspartate receptor antibodies; SCA, xxxxxx; LE, limbic encephalitis; SCLC, small-cell lung cancer; FDG PET, fluorodeoxyglucose positron emission tomography; MRI, magnetic resonance imaging; Ab, antibody.
removing the antibodies from the neuronal cell cultures (Dalmau et al., 2008). Using in vitro and in vivo studies, patient’s NMDAr-Abs have been shown to decrease the surface density and receptor localization of NMDAr clusters via antibody-mediated capping and internalization (Hughes et al., 2010). This effect does not depend on the presence of the complement and spares other synaptic proteins, AMPA currents, and synapse density. These changes on NMDA receptor surface density and localization correlate with the antibody titer and are reversible with its removal. Interestingly, synaptic current depends on receptor’s internalization and NMDA-mediated synaptic currents decrease with NMDAr-Abs exposure. In vivo CSF and purified IgGs from patients with NMDAr-Abs impaired the glutamatergic transmission and induced a susceptibility to AMPA infusion (Manto et al., 2011). Moreover, blockade of GABA\textsubscript{A} receptors reinforced the enhancing effect of NMDAr-Abs on glutamate concentrations (Manto et al., 2010), suggesting that NMDAr-Abs act mainly on the NMDA receptors of GABAergic neurons. The NMDAr-Abs thus seem to block the extracellular epitopes of the NR1 subunit of the NMDA receptor hence a hyperglutamatergic state in the brain with an imbalance between NMDA and AMPA pathways. In humans, a good clinical outcome proves to correlate with a significant decrease of NMDAr-Abs in the CSF (Dalmau et al., 2008).

GABA\textsubscript{B}-Abs

A first study using confocal microscopy showed that all patients’ antineuronal cell surface antibodies target the GABA\textsubscript{B} receptors, and that patients’ antibodies label almost all neuronal GABA\textsubscript{B} receptors (Lancaster et al., 2010). Pharmacologic (Enna and Bowery, 2004) or genetic (Prosser et al., 2001; Schuler et al., 2001) changes in these receptors in rodents were already known to induce epilepsy and symptoms similar to those observed in LE. Furthermore all reported patients have developed epileptic seizures. These clinical features may thus suggest a GABAergic pathway impairment through a receptor disruption in patients with GABA\textsubscript{B}-Abs but no experimental data are available as yet to reinforce this hypothesis.

AMPA\textsubscript{r}-Abs

As observed with NMDAr-Abs on NMDA receptors, AMPAr-Abs application on neuronal cell cultures reduces the receptor number and the localization of AMPAr clusters at postsynaptic and presynaptic sites. This effect is reversed by antibody removal from the neuronal cultures (Lai et al., 2009). However, there are no experimental data demonstrating a direct role for AMPAr-Abs in the neurologic symptoms.

**ONCONEURONAL ANTIBODIES**

Onconeuronal antibodies (ON-Abs) are defined as antibodies associated with cancer and targeting intracellular epitopes. The pathophysiologic role of ON-Abs is less clear than of NSA-Abs. These Abs are considered as being associated with a predominant T cell-mediated immune reaction. Most of them still have no identified pathogenic role and are simply a witness of the immune reactivity.

**Hu-Abs**

Hu-Abs is the most frequent paraneoplastic antibody. It was first identified in 1985 in a patient with subacute sensory neuropathy and SCLC (Graus et al., 1985). Hu-Abs recognizes a 35–40 kD family of proteins located in the nucleus and at a lower level in the cytoplasm of neurons of the central and peripheral nervous system. A pathogenic role of Hu-Abs has been initially hypothesized since an intrathecal synthesis was shown in patients with these antibodies (Furneaux et al., 1990a; Dalmau et al., 1991). Furthermore deposits of Hu-Abs were present in the nervous system at autopsy (Brashar et al., 1991). One study suggested that uptake of Hu-Abs was associated with neuronal degeneration in rats (Greenlee et al., 1993). However, mice, rat, and guinea pig models immunized with purified recombinant HuD fusion protein failed to develop any neurologic symptoms, although animals produced autologous antibody against HuD (Sillevis Smitt et al., 1995) and elevated titers of Hu-Abs were found in each of these models. In the same way, in patients with Hu-Abs, deposits vary across regions of the nervous system without correlation with neuronal death density or the areas involved in inflammation (Sillevis Smitt et al., 1995). In paraneoplastic encephalomyelitis associated with Hu-Abs, examination of frozen brain tissue from seven patients using immunohistochemistry and PCR suggests that an antigen-driven oligoclonal cytotoxic T cell response plays a role in the pathogenesis (Voltz et al., 1998). To reinforce this hypothesis, the recombinant HuD protein was used to stimulate in vitro peripheral blood mononuclear cells from patients with Hu-Abs and SCLC, patients with SCLC without Hu-Abs, and controls (Benyahia et al., 1999). Phenotype analysis of the peripheral blood lymphocytes revealed a significant increase of T cells activated against HuD protein in the group with Hu-Abs as compared to the other groups. HuD recombinant protein thus proved to be an antigenic target for autoreactive CD4\textsuperscript{+} T cells. The presence of a specific noncytotoxic CD8\textsuperscript{+} T cell in patients with Hu-Abs and paraneoplastic syndrome has also been demonstrated (Roberts et al., 2009). Injuries to the nervous system...
could consequently result from a cell-mediated immune reaction and Hu-Abs would only be a witness of this immune cell reaction. All these experiments exclude a direct pathogenic role for Hu-Abs.

**Yo-Abs**

Yo-Abs reacts with proteins which are mainly located in the Purkinje cells (Rodriguez et al., 1988; Hida et al., 1994). Yo-Abs also labels the patient’s tumor, suggesting a role for the underlying neoplasm in this immunologic response (Furneaux et al., 1990b). A recent publication showed that the uptake of Yo-Abs by Purkinje cells in cerebellar slice cultures resulted in their death, suggesting that Yo-Abs may be directly involved in the pathogenesis of paraneoplastic cerebellar degeneration (Greenlee et al., 2010). Nonetheless, no study to date has demonstrated a clear benefit of immunotherapy on the neurologic outcome.

**Amphiphysin-Abs**

Even though targeting an intracellular epitope, amphiphysin-Abs was shown to induce stiff person syndrome-like symptoms when intrathecally infused in rats (Geis et al., 2010). Moreover, a reduced presynaptic GABAergic inhibition was identified in this rodent model leading to the assumption that GABAergic synapses are vulnerable to defective endocytosis induced by anti-amphiphysin immunoglobulin G (Geis et al., 2010).

**SPECIFIC PARANEOPLASTIC NEUROLOGIC SYNDROMES**

**Paraneoplastic disorders of the central nervous system**

**Subacute cerebellar degeneration**

Clinical and biological characteristics of patients with paraneoplastic cerebellar ataxia

The first case of paraneoplastic cerebellar syndrome described was in 1919, that of a 60-year-old woman who suffered from pelvic cancer (Brouwer, 1919). The concept of paraneoplastic subacute cerebellar degeneration (SCD) was only demonstrated in 1983 with the description of anti-Yo antibodies (Greenlee and Brashear, 1983). SCD represents one of the most common PNS (Furneaux et al., 1990b; Graus et al., 2004; Giometto et al., 2010). The mean age of SCD patients is 63 (Shams’ili et al., 2003). The sex ratio is dependent on the antibody and the type of associated tumor (see below). SCD onset is typically subacute and becomes severe and debilitating within a few weeks or months. A faster onset within hours or days (Anderson et al., 1988b) or, at the opposite end, an extremely progressive form of ataxia have been described too (Peterson et al., 1992). Ataxia is usually symmetric. It may be associated with dysarthria, nystagmus, vertigo, and diplopia (Peterson et al., 1992). Prodromal signs such as a flu syndrome, nausea, and/or dizziness may precede cerebellar signs (Dalmau and Rosenfeld, 2008). The evolution is usually severe because only 34% of the patients are still able to walk once the stabilization of symptoms is achieved (Shams’ili et al., 2003). The type of associated onconeural antibodies indicates neurologic disability and predicts the outcome for patients with SCD. Tr-Abs and Ri-Abs patients live significantly longer (median: > 113 months and > 69 months, respectively) than Hu-Abs (median: 7 months) or Yo-Abs (median: 13 months) patients (Shams’ili et al., 2003). Cerebellar symptoms can be isolated or associated with signs of widespread nervous system dysfunction depending on the associated Ab. The CSF analysis is rarely normal (less than 20%), and typically shows pleocytosis, increased protein concentration, increased IgG level, and the presence of oligoclonal bands. Brain imaging by computed tomography (CT) or magnetic resonance imaging (MRI) is typically normal early in the disease. Cerebellar atrophy may be observed during the course of the disease, but is never seen at the onset of the cerebellar ataxia. Nonspecific abnormalities such as white matter or cerebellar cortex T2 abnormal hyperintense signals have occasionally been reported.

Clinical specificities according to the type of onconeural antibody

The most common antibodies associated with SCD described in the literature are Yo-Abs and Hu-Abs, which are detected in 70% of cases (Shams’ili et al., 2003). However, some Abs were not detected by all the laboratories suggesting some bias in the diagnosis. Some antibodies are specific to cerebellar syndromes (Yo-Abs, Tr-Abs, ZIC4-Abs) and some others are frequently observed, but not specific (Hu-Abs, CV2/CRMP5-Abs).

**SCD with Yo-Abs**

The cerebellar syndrome is most often pure and isolated, usually severe enough to cause major handicap, with 79% of the patients bedridden within a few weeks (Shams’ili et al., 2003). However, some Abs were not detected by all the laboratories suggesting some bias in the diagnosis. Some antibodies are specific to cerebellar syndromes (Yo-Abs, Tr-Abs, ZIC4-Abs) and some others are frequently observed, but not specific (Hu-Abs, CV2/CRMP5-Abs).

Survival is longer in patients with breast cancer (median: 100 months) than in patients with ovarian cancer (median: 22 months) (Rojas et al., 2000). The presence of Yo-Abs is significantly associated with the presence of a gynecologic cancer. The most frequent
tumors are ovarian carcinoma (38–47% of tumors) and breast cancers (27–35%) (Peterson et al., 1992; Rojas et al., 2000). Other gynecologic tumors may be responsible for SCD with Yo-Abs, such as cancers of the endometrium, fallopian tubes, or cervix (Peterson et al., 1992; Rojas et al., 2000). In case of negative results from mammography, chest, abdominal and pelvic CT, and ultrasound, it is advisable to perform an exploratory laparotomy to examine the ovaries and uterus (Peterson et al., 1992; Didelot and Honnorat, 2009).

SCD with Hu-Abs

A cerebellar syndrome is observed in 22% of the patients with Hu-Abs (Honnorat et al., 2009). Unlike patients with Yo-Abs, extracerebellar signs are frequently observed from the beginning of the neurologic symptoms, such as LE, cranial nerve involvement, brainstem disorders, dysautonomia, LEMS, or pure sensory neuronopathy (Honnorat et al., 2009). These symptoms are associated with extensive inflammation of the central nervous system, and neuronal destruction is not restricted to the cerebellum and Purkinje cells (Dalmau et al., 1992). Tumors associated with Hu-Abs are mainly lung cancer, and small-cell lung cancer (SCLC) is observed in 70% of cases (Honnorat et al., 2009).

SCD with Tr-Abs

The first case of SCD associated with Hodgkin’s disease and confirmed by autopsy was published in 1957. The association of Tr-Abs and SCD in patients suffering from Hodgkin’s disease was identified in 1997 (Graus et al., 1997). However, Tr-Abs identification is technically difficult. Suspicion of cerebellar syndrome with Tr-Abs (young man and/or known lymphoma) consequently requires special tests and must be clearly spelled out in the search for onconeuronal antibodies.

Clinically, cerebellar syndromes with Tr-Abs are characterized by subacute ataxia with, in some cases, diplopia, oscillopsia, vertigo, or influenza-like illness and headache (Bernal et al., 2003). SCD associated with Tr-Abs occurs mainly among young men with Hodgkin’s disease (Bernal et al., 2003; Shams’ili et al., 2003). Two studies revealed that in 83–86% of cases, the cerebellar syndrome preceded the diagnosis of Hodgkin’s disease (Bernal et al., 2003; Shams’ili et al., 2003).

SCD with CV2/CRMP5-Abs

CV2/CRMP5-Abs were initially identified in a patient with cerebellar ataxia, uveitis, peripheral neuropathy, and metastatic undifferentiated adenocarcinoma (Antoine et al., 1993). Cerebellar ataxia was observed in 46% of the patients with CV2/CRMP5-Abs (Honnorat et al., 2009). Patients with CV2/CRMP5-Abs are mainly males (70%) with a mean age of 62 years (Rogemond and Honnorat, 2000). The cerebellar syndrome is generally less severe than that observed with Yo-Abs. Other associated neurologic symptoms may be observed such as LE, chorea, or visual symptoms such as retinopathy or uveitis (de la Sayette et al., 1998; Rogemond and Honnorat, 2000). LEMS and peripheral neuropathy are also frequently associated with the cerebellar ataxia (Honnorat et al., 2009). The most frequently associated tumor is SCLC (60%), but malignant thymoma and uterine sarcoma have been described (Honnorat et al., 1996, 2009; Rogemond and Honnorat, 2000).

SCD with VGCC-Abs

Antibodies to calcium channels, particularly those directed against the P/Q type, have been primarily described in association with Lambert–Eaton myasthenic syndrome (LEMS) (Lennon et al., 1995; Motomura et al., 1997). However, studies showed that the incidence of cerebellar ataxia was particularly high in patients with LEMS (Clouston et al., 1992). In addition, patients with ataxia associated with LEMS presented more frequently with cancer than patients with isolated LEMS (Clouston et al., 1992). Antibodies to calcium channels of the P/Q type were also reported in patients with SCLC and SCD in the absence of LEMS (Mason et al., 1997). A study involving 39 patients with a cerebellar syndrome and lung cancer showed that 16 patients (41%) had antibodies to calcium channels of the P/Q type, while nine (23%) had exhibited Hu-Abs (Graus et al., 2002). Reported patients generally developed a pure cerebellar ataxia with a subacute onset.

SCD with Ri-Abs

A study of 50 patients with SCD showed that six of them had Ri-Abs (Shams’ili et al., 2003). Cerebellar syndrome may be isolated but is most often associated with other neurologic signs, such as opsomyoclonus and/or brainstem encephalitis. Ri-Abs are strongly associated with breast and lung cancers.

Other autoantibodies associated with paraneoplastic cerebellar ataxia

A few observations of SCD have been reported in association with anti-amphiphysin (Antoine et al., 1999a) or anti-Ma2 (Dalmau et al., 1999) antibodies. Some cases have also been reported with other autoantibodies, but in many cases, the specificity of these antibodies is unclear because only one or two cases have been identified.
Two patients with Ab directed against mGluR1, a metabotropic receptor of glutamate, have been reported (Sillevis Smitt et al., 2000). This antibody seems interesting because it could be directly responsible for cerebellar ataxia, as demonstrated by injection into rat cerebellum (Sillevis Smitt et al., 2000). However, since 2000, only one further case has been published (Marignier et al., 2010). Although the first two cases were associated with Hodgkin’s disease, the last one published had no cancer (Marignier et al., 2010). In the two first cases published, Hodgkin’s disease seemed not to be related to the cerebellar ataxia, suggesting that mGluR1-Abs antibodies could be associated with nonparaneoplastic SCD.

Anti-Zic antibodies are described in SCD with SCLC (Bataller et al., 2004; Sabater et al., 2008a). A recent study showed that these antibodies were observed in 15% of SCD patients with lung cancer (Graus et al., 2008). However, the diagnostic role of these antibodies is not clear (Graus et al., 2010) because they are also detected in patients who have lung cancer without associated neurologic syndromes.

One SCD patient with lung adenocarcinoma was described with antibodies against protein kinase C γ (Sabater et al., 2006), and other isolated cases have been published with uncharacterized antibodies. Although the significance of these antibodies is unknown, their presence indicates an unusual activation of the immune system that may play a role in the onset of cerebellar ataxia.

**Seronegative SCD.** It is interesting to note that a significant percentage (probably over 50%) of patients with SCD have no identified circulating neuronal antibodies (Anderson et al., 1988a; Mason et al., 1997). The etiology of the cerebellar ataxia in these patients without autoantibodies remains speculative; however, an autoimmune origin of the neurologic symptoms is probable because most of these patients have CSF inflammation, and pathologic lesions are not different from those seen in patients with onconeural antibodies (Mason et al., 1997). The clinical course of these patients is still difficult to discern owing to the lack of data.

**Paraneoplastic encephalomyelitis**

Paraneoplastic encephalomyelitis (PEM) is defined by disseminated neuronal loss and simultaneous inflammatory lesions in different parts of the nervous system. PEM may concern areas such as the hippocampus, the lower brainstem, the spinal cord, or dorsal root ganglia (Bataller et al., 2004). In patients with brainstem encephalitis, the pontine dysfunction precedes downward evolution in a half of the patients (Saiz et al., 2009). Sensory neuronopathy, LE, and cerebellar ataxia are the most common clinical syndromes observed in PEM. A failure of the autonomic nervous system is observed in 30% of the patients (orthostatic hypotension, urinary retention, pupillary abnormalities, impotence, and dry mouth) (Wabbels et al., 2004). Most of the patients present Hu-Abs, CV2/CRMP5-Abs or amphiphysin-Abs but a recent report shows a clinical pattern specific to Hu-Abs and CV2/CRMP5-Abs, respectively (Honnorat et al., 2009). A review of 200 patients suffering from PEM associated with Hu-Abs described comprehensively the clinical and paraclinical features associated with this disease (Graus et al., 2001). In this cohort, the mean age was 63 and 75% of cases were men. A subacute sensory neuropathy occurred in more than a half of the patients (54%), followed by cerebellar ataxia (10%), limbic encephalitis (9%), and multifocal involvement (11%). Interestingly in patients in whom the tumor diagnosis was the first event, PEM onset followed the progression or relapse of the underlying tumor. In 75% of the patients with PEM the underlying neoplasm is a SCLC. The treatment of the associated tumor, with or without immunotherapy, was an independent predictor of improvement of the neurologic symptoms. The factors independently associated with a higher mortality are: age over 60, a Rankin score over 3 at diagnosis, more than one area of the nervous system being affected, and the absence of treatment (Graus et al., 2001).

**Limbic encephalitis**

Limbic encephalitis (LE) is defined by an acute or a subacute anterograde amnesia associated with epileptic seizures and psychiatric disorders. Each symptom varies according to the associated Ab. Moreover, this concept is currently moving to a wider term of encephalitis due to the description of cases where observed symptoms suggested a widespread involvement of neurologic structures beyond the limbic structures. Hu-Abs, CV2/CRMP5-Abs and Ma2-Abs presented the vast majority of patients with LE until the recent description of Ab directed against synaptic proteins or receptors such as NMDAr, AMPAr or GABA_{A}R, Lgi1 and CASPR2 proteins. The clinical course and the specificity of LE according to the associated Ab are discussed below (Table 78.4).

**LE with NSA-Abs**

Since 2004, NSA-Abs have been described in some patients with LE. The clinical features and outcome of LE vary between patients with NSA-Abs and ON-Abs. In other words, many patients presenting with LE and NSA-Abs have no associated tumor and LE is often responsive to immunologic therapy.
### Table 78.4
Main features of limbic encephalitis (adapted from Didelot and Honnorat, 2011)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>NMDAr-Abs</th>
<th>VGKC/LGI1-Abs</th>
<th>Hu-Abs</th>
<th>Ma2-Abs</th>
<th>GABA&lt;sub&gt;B&lt;/sub&gt;-Abs</th>
<th>AMPAr-Abs</th>
<th>None</th>
<th>CV2-Abs</th>
<th>Amphiphysin-Abs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Published cases</td>
<td>248</td>
<td>150</td>
<td>29</td>
<td>217</td>
<td>88</td>
<td>65</td>
<td>17</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Antigenic target</td>
<td>SCA</td>
<td>EP</td>
<td>IC</td>
<td>IC</td>
<td>SCA</td>
<td>SCA</td>
<td>–</td>
<td>IC</td>
<td>IC</td>
</tr>
<tr>
<td>Age (range)</td>
<td>24 (18–80)</td>
<td>14</td>
<td>na</td>
<td>64 (9–84)</td>
<td>na</td>
<td>51 (22–82)</td>
<td>56 (24–75)</td>
<td>57 (38–87)</td>
<td>56 (28–67)</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>–</td>
<td>1/3</td>
<td>na</td>
<td>1</td>
<td>na</td>
<td>2.5</td>
<td>0.9</td>
<td>1.9</td>
<td>1</td>
</tr>
<tr>
<td>Percentage of cancer</td>
<td>58%</td>
<td>25%*</td>
<td>5%</td>
<td>12%</td>
<td>Almost all cases</td>
<td>92%</td>
<td>47%</td>
<td>70%</td>
<td>12%</td>
</tr>
<tr>
<td>Type of associated tumor</td>
<td>Teratoma</td>
<td>Teratoma and neuroblastoma</td>
<td>SCLC, Hodgkin, testicular germ cell tumor</td>
<td>SCLC and malignant thymoma</td>
<td>SCLC</td>
<td>Testicular cancer, SCLC</td>
<td>SCLC</td>
<td>Malignant thymoma, breast cancer, lung cancer</td>
<td>prostate cancer</td>
</tr>
<tr>
<td>Abnormal CSF</td>
<td>80%</td>
<td>43%</td>
<td>Frequent</td>
<td>78%</td>
<td>85%</td>
<td>90%</td>
<td>75%</td>
<td>66%</td>
<td>50%</td>
</tr>
<tr>
<td>Abnormal MRI at diagnosis</td>
<td>50%</td>
<td>78%</td>
<td>Frequent</td>
<td>74%</td>
<td>65%</td>
<td>60%</td>
<td>100%</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>Abnormal EEG</td>
<td>94%</td>
<td>71%</td>
<td>50%</td>
<td>100%</td>
<td>100%</td>
<td>75%</td>
<td>na</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

SCA, cell surface antigen; EP, excreted protein; IC, intracellular antigen; na: not available; SCLC, small-cell lung cancer; CSF, cerebrospinal fluid; EEG, electroencephalogram; MRI, magnetic resonance imaging; Abs, antibodies; r, receptor.

*Girls in all but two of the paraneoplastic cases of encephalitis with N-methyl-D-aspartate receptor antibodies in childhood.
LE with VGKC-Abs, Lgi 1-Abs and CASPR2-Abs

Antibodies supposedly to react against voltage-gated K⁺ channel (VGKC-Abs) were first described in 1995 in patients with neuromyotonia (Shillito et al., 1995; Hart et al., 1997; Vernino and Lennon, 2002), and few years later in some cases of Morvan’s disease (Lee et al., 1998; Barber et al., 2000; Liguori et al., 2001). In 2004, a larger published series of patients suggested a relationship between VGKC-Abs and LE (Vincent et al., 2004). However, recent immunologic findings have clearly demonstrated that voltage-gated K⁺ channel was not the direct target for the Ab. The accurate target of this NSA-Ab eventually proved to be leucine-rich glioma-inactivated 1 protein (Lgi1) in the case of LE (Irani et al., 2010a; Lai et al., 2010); contactin-associated protein 2 (CASPR2) is another epitopic target which may be more specific for neuromyotonia or Morvan’s disease (Irani et al., 2010a).

The mean age of patients with Lgi1-Abs and LE is 62 years (Irani et al., 2010a; Lai et al., 2010) and 67% of the patients are males. The clinical features are characterized by memory loss, epileptic seizures, and delusion. Sleep disorders such as insomnia may be observed and are probably due to a hypothalamic dysfunction. A hypotension is frequent (55%) and is due to antidiuretic hormone secretion (Pozo-Rosich et al., 2003). Brain MRI is normal in 45% of cases and the CSF analysis is normal in 59% of cases.

A tumor was identified in 5% of the reported patients with Lgi1-Abs and LE: two thyroid, one lung, one thymoma, one ovarian teratoma, and one renal cell carcinoma.

A significant clinical improvement was observed in 78% of cases with immunomodulator treatments such as corticosteroids, plasma exchanges, or intravenous immunoglobulins (Lai et al., 2010).

LE with Neuropil-Abs

This subtype of LE was described in 2005 with the identification of antibodies reacting against neuronal surface cell antigens with a different pattern than the one observed with VGKC-Abs (Ances et al., 2005; Vitaliani et al., 2005). Some Abs initially described as Neuropil-Abs were revealed to be NMDAr-Abs (Vitaliani et al., 2005), GABA₁r-Abs (Lancaster et al., 2010), or AMPAr-Abs (Lai et al., 2009). The subgroup of Neuropil-Abs should consequently cease to be used in future, with the more accurate identification of the antigens.

LE with NMDAr-Abs

Between 1997 and 2006, numerous cases of women presenting with ovarian teratoma and LE were reported (Nokura et al., 1997; Aydiner et al., 1998; Lee et al., 2003; Stein-Wexler et al., 2005; Yang et al., 2006; van Altena et al., 2008). All these cases occurred in young women and included psychiatric disorders such as delusions or behavioral disturbances. No antibody was described but NSA-Abs were not looked for. In 2005, four cases gave a clue to an association between this syndrome and antibodies reacting against the membrane of hippocampal neurons (Vitaliani et al., 2005). This was supported by another study (Shimazaki et al., 2007) in which isolated antibodies happened to colocalize with a brain-specific protein involved in the regulation of the dendritic development of hippocampal neurons (Vitaliani et al., 2005; Koide et al., 2007). Finally, by analogy of staining, a NR1 subunit of the N-methyl-D-aspartate glutamate receptor (NMDAr) was identified as a main target for these Abs (Dalmau et al., 2007). This subunit of the glutamate receptor is expressed at an elevated level in the associated ovarian teratoma as well (Dalmau et al., 2007).

The prevalence of LE appears to be higher with NMDAr-Abs than in the previously described groups of Abs. In fact the first 100 patients were reported within less than 1 year after the NMDAr-Abs discovery (Dalmau et al., 2008) and more than 400 patients with NMDAr-Abs were diagnosed within 3 years by the group of Josep Dalmau (Dalmau et al., 2011).

NMDAr-Abs were mainly observed in females (84%) who presented with stereotypical clinical features associating acute psychiatric disorders (delirium with visual or auditory delusions, mood disorders such as aggressiveness or irritability) followed by loss of consciousness and eventually epileptic seizures (Dalmau et al., 2011). A dysautonomia and/or an acute central respiratory failure occurred in one-third of patients and required resuscitation. Recent findings suggested that the disease should progress through two main stages (Irani et al., 2010b). The first stage comprises psychiatric and epileptic symptoms and is associated with CSF lymphocytosis; the second is represented by movement disorders, loss of consciousness, dysautonomia, and the presence of oligoclonal bands in the CSF.

Brain MRI was normal in 45% of cases and the CSF examination revealed clues for inflammation in more than 90% (elevated white cell count, mainly lymphocytes, mild hyperproteinorachia and/ or oligoclonal bands). Electroencephalogram was abnormal in more than 90% but remained unspecific. A paraneoplastic origin was observed in 60% of cases, the majority of which were associated with a teratoma (58% of all cases).
Even though LE with NMDAr-Abs was first described as a disease in young women, two other subgroups of patients could still be individualized. The first subgroup gathered LE with NMDAr-Abs in childhood and was described in a case series of 32 children aged 2–14 years (Florance et al., 2009). Clinical symptoms were similar to those observed in young women. However, brain MRI was more frequently normal (69% of cases) and EEG and CSF were abnormal in 100% and 94% of cases, respectively (Florance et al., 2009). LE and NMDAr-Abs in children were much less often paraneoplastic and a cancer was found in only nine children of the 33 reported cases (27%): eight Ataxia and one neuroblastoma (Florance et al., 2009; Lebas et al., 2010).

LE with NMDAr-Abs has also been identified in a few men. Again no significant clinical differences were observed as compared to the previous subgroups but only two of the nine reported men (22%) presented a cancer (a SCLC and an immature teratoma of testis) (Dalmau et al., 2008).

A good response to immunotherapy is observed in almost half of the patients. However, no study has yet demonstrated the superiority of one treatment over another. Intravenous immunoglobulin, plasmatic exchange, and corticosteroids are the most commonly used.

**LE with AMPAr-Abs**

To date, 12 patients (11 women) with AMPAr-Abs associated LE have been reported (Lai et al., 2009; Bataller et al., 2010; Graus et al., 2010). An accurate clinical description is difficult to obtain with these few cases. However, amnesic symptoms were constant and epileptic seizures occurred in only five cases (42%). CSF examination showed a mild lymphocytic pleocytosis (6–75 cells/mm³) in nine of the 12 cases (75%). Eight of the 11 patients (73%) in whom brain MRI was available showed T2-weighted and FLAIR (fluid attenuated inversion recovery) sequence abnormalities within the temporomesial structures. Eight patients had a cancer (four malignant thymoma, two breast cancers, one SCLC, and one non-SCLC), which had already been diagnosed at the time of LE in all but two cases (Lai et al., 2009; Graus et al., 2010). The first episode of LE responded positively in all patients, following immunotherapy and cancer treatment, suggesting that LE with AMPAr-Abs seemed to be associated with a good neurologic prognosis; however, the risk of relapse is high. For instance, many described patients had one to three relapses after the first episode of encephalitis (Lai et al., 2009). Recently, two cases of women over 50, presenting AMPAr-Abs and an acute late-onset psychosis, have been recently reported (Graus et al., 2010). This finding suggests that AMPAr-Abs could be present in patients with a larger clinical spectrum than isolated LE. Further clinical studies will be necessary in order to characterize clinical specificities of these patients.

**LE with GABAβ-F-Abs**

GABAβ-F-Abs has been identified in 2010 and targets the GABAβ1 and GABAβ2 receptor subunits (Lancaster et al., 2010). Patients with LE and GABAβ-F-Abs were characterized by early and prominent seizures as first symptoms in 15 of the 17 cases (88%) described to date (Lancaster et al., 2010). The brain MRI was abnormal in 11 patients (65%) and showed mesiotemporal hypersignal in T2-weighted sequences. The CSF examination was abnormal in 11 of the 13 patients in whom data were available (65%) and mainly showed an elevated white blood cell count and a mild hyperproteinorachia. Oligoclonal bands were present in five of the six patients (83%) who underwent this analysis. Interestingly, other antibodies can be associated with GABAβ-F-Abs in 60% of the patients (i.e., 9/17). GAD-Abs were found in five cases, TPO-Abs in four, VGCC-Abs in three, and Sox1 in one case (some patients had more than one associated antibody). Seven (41%) of the 17 patients with GABAβ-F-Abs had a cancer (six SCLC and one mediastinal adenopathy without histologic result). Eight of the 17 patients (i.e., 47%) experienced substantial improvement or fully recovered under various regimens of corticosteroids (65% of the patients received this therapy), intravenous immunoglobulins (35%), or plasma exchanges (two patients received this treatment, i.e., 12%) (Lancaster et al., 2010).

**LE with ON-Abs**

**LE with Hu-Abs.** Hu-Abs-associated LE was the most frequent type of LE before the description of NSA-Absa. LE is observed in 21.3% of PNS associated with Hu-Abs and its prevalence remains equal across several case series (Dalmau et al., 1992; Honnorat et al., 2009). The other main neurologic symptoms associated with Hu-Abs are sensory neuronopathies, observed in 86.1%, and cerebellar ataxia in 21.6% of the patients (Honnorat et al., 2009).

In Hu-Abs patients with LE, the temporal symptoms are rarely isolated and other neurologic symptoms such as cerebellar ataxia or a subacute sensory neuropathy are frequently present. An electroneuromyogram must consequently be performed in all cases in order to rule out an associated neuropathy. The association of LE with any other neurologic symptom must lead to the diagnosis of paraneoplastic encephalomyelitis (Graus et al., 2004).
LE with Ma2-Abs. Ma2-Abs was first described in 1999 in patients with testicular teratoma, limbic and brainstem encephalitis, and testicular cancer (Volitz et al., 1999). Ma2-Abs is rare and represents less than 5% of the patients with PNS reported in the European database (Giometto et al., 2010). An involvement of the limbic structures was observed in about 75% of the patients with Ma2-Abs (Rosenfeld et al., 2001); however, an isolated LE occurred in only 11% of the patients with Ma2-Abs (Dalmau et al., 2004). Testicular cancer, which is present in 53% of cases, must be ruled out in any man presenting Ma2-Abs. SCLC is the second most frequent associated tumor (21%) (Dalmau et al., 2004). Ma2-Abs are clearly associated with PNS even if 12% of the patients remained free of cancer after comprehensive carcinologic investigations; a neurosarcoidosis was reported in a woman with Ma2-Abs (Desestret et al., 2010). In male patients, the combination of the immunologic treatment with orchidectomy significantly improved clinical outcome in one-third of cases (Dalmau et al., 2004).

LE with CV2/CRMP5-Abs. The first case of LE with CV2/CRMP5-Abs has been described in a patient with malignant thymoma (Antoine et al., 1995). In a recent study of 37 patients with PNS and CV2/CRMP5-Abs, five (13.5%) patients had LE (Honnorat et al., 2009). More recently, another case of CV2/CRMP5-Abs was associated with myasthenia gravis and thymoma (Monstad et al., 2009). Unlike LE with Hu-Abs, CV2/CRMP5-Abs-related LE is more frequently isolated, even though a neuropathy was found in some cases (Honnorat et al., 2009). In two of the three cases where clinical features were available, it consisted of sudden psychiatric disorders with agitation and hallucinations. Memory loss was the most prominent symptom while epileptic seizures were less marked (Antoine et al., 1995). Brain MRI showed a limbic involvement in all patients but CSF was sometimes normal.

LE with Amphiphysin-Abs. To date, only six cases of LE with Amphiphysin-Abs have been reported in case series gathering 77 patients (Antoine et al., 1999a; Dorresteijn et al., 2002; Pittock et al., 2005). Three among them had other associated autoantibodies: VGKC-Abs, VGCC-Abs, CV2/CRMP5-Abs, Hu-Abs and GAD-Abs (Dorresteijn et al., 2002; Pittock et al., 2005). LE was the sole symptom in only one patient (Antoine et al., 1999a); in the other cases it was associated with various neurologic syndromes such as a sensorimotor neuropathy, a cerebellar ataxia, axial or limb stiffness, myoclonus, or choreoathetosis. Five of the six described patients with LE and Amphiphysin-Abs presented a SCLC and no tumor was found in the last patient (Antoine et al., 1999a; Dorresteijn et al., 2002; Pittock et al., 2005).

Paraneoplastic LE without antibody

The percentage of “seronegative” paraneoplastic LE is difficult to evaluate. Before the description of NSA-Abs, a retrospective study between 1987 and 1997, reporting 14 cases of LE with SCLC, described 50% of “seronegative” LE (Alamowitch et al., 1997). No relevant difference was observed between Hu-Abs positive and “seronegative” patients in terms of frequency of psychiatric disorders, percentage of abnormal CSF, or abnormal brain MRI. Even if the reported clinical features are too weak to form conclusions, most of these “seronegative” LE could be expected to belong to the later described antibody-associated PNS as seen with ON-Abs or NSA-Abs. Moreover “seronegative” LE without cancer has also been described (Bien et al., 2000) and the clinical features of some reported patients may correspond to VGKC-Abs patients presenting with LE.

In recent years, the frequency of “seronegative” LE decreased with the description of NSA-Abs. The immunologic findings in a case series of 39 patients with LE confirmed this assumption because only three of these patients (8%) eventually remained “seronegative” after immunohistochemistry screening (Bataller et al., 2007). Moreover, a recent study evaluated the prevalence of NSA-Abs in 45 patients suffering from LE, independently of the presence or not of ON-Abs (Graus et al., 2008). After NSA-Abs were identified in 29 patients, only five cases (17%) remained definitively “seronegative” despite comprehensive immunologic evaluations. The number of “seronegative” LE patients may continue to taper down with more antibodies to be discovered in the coming years. Nevertheless, the findings previously reported may still support the assumption that some LE genuinely are seronegative. This suggests that these LE may result from different mechanisms, which need to be characterized. Interestingly, within the eight “seronegative” cases which benefited from both ON-Abs and NSA-Abs screening, only one case was paraneoplastic (prostate adenocarcinoma) (Bataller et al., 2007; Graus et al., 2008). “Seronegative” LE thus seems to be rarely paraneoplastic. Conversely, the clinical outcome could be worse than in LE with NSA-Abs since only one of the eight reported cases (12%) improved following immunologic therapies (Bataller et al., 2007; Graus et al., 2008).

Opsoclonus myoclonus

Opsoclonus myoclonus (OM) associates large amplitude synchronous and chaotic eye movements (opsoclonus) with spontaneous muscle jerks (myoclonus) and ataxia. The eye movements are present during fixation, smooth
A paraneoplastic origin is only the dorsal root ganglia by cytotoxic T lymphocytes by primary damage of the sensory nerve cell body of Subacute sensory neuronopathy (SSN) is characterized an involvement of the cerebellar vermis (van Toorn et al., 2005) and a disinhibition of the cerebellum fastigial nucleus underlying the oculomotor symptoms (Huang et al., 2005; Ramat et al., 2005).

Paraneoplastic OM is rare (Ki Pang et al., 2010) and is usually observed in pediatric patients with neuroblastoma (Rothenberg et al., 2009) or adult females with Ri-Abs and breast cancer (Weissman et al., 1989; Luque et al., 1991). Other etiologies such as infection (Lyme disease, varicella-zoster infection, streptococcal infection and West Nile virus), celiac disease, or metabolic disorders must be considered and ruled out (Wong, 2007).

Most of the ON-Abs have been described as being associated with OM in single case reports and several other epitopic targets have also been proposed such as Zic2 (Bataller et al., 2003), neurofilaments (Noetzel et al., 1987), neuroleukin (Candler et al., 2006) or cell surface antigens (Blaes et al., 2005). However, the antibodies reacting against these different targets are not necessarily observed in paraneoplastic cases and none of them seems to be specific of this neurologic syndrome (Pranzatelli et al., 2002). Only a few adult patients presenting with seronegative paraneoplastic OM have been reported (Bataller et al., 2003). In these cases, a SCLC was almost always diagnosed.

Long-term outcome for children with paraneoplastic OM seems to be dominated by cognitive and behavioral problems rather than ataxia (Klein et al., 2007). In adults, relapses of opsoclonus may occur and residual gait ataxia tends to persist. Most patients who underwent treatment of the underlying tumors had complete or partial neurologic recovery (Bataller et al., 2001). Immunotherapy (such as corticosteroids or immunoglobulins) may also help recovery. A recent study provided clues to the efficiency of a combined treatment associating immunoglobulins, ACTH, and rituximab (Pranzatelli et al., 2010). The clinical improvement correlates with B cell reduction in the CSF (Pranzatelli et al., 2005, 2006, 2010).

Paraneoplastic disorders of the peripheral nervous system

SUBACUTE SENSORY NEURONOPATHY

Subacute sensory neuronopathy (SSN) is characterized by primary damage of the sensory nerve cell body of the dorsal root ganglia by cytotoxic T lymphocytes (Kuntzer et al., 2004). A paraneoplastic origin is only one of the causes of SSN (Molinuovo et al., 1998). The clinical presentation may vary between patients, but a recent study has put forward a fairly specific clinical and electrophysiologic pattern specific to SSN (Camdessanche et al., 2009). In this the clinical pattern would be an acute or subacute onset of painful paresthesias of all limbs with absent deep-tendon reflexes and impairment of all sensory modalities, in particular joint position sense. This clinical presentation differs from those observed in other neuropathies. Thus a large majority of patients present with sensory abnormalities which involve the distal part of the four limbs with a nonlength-dependent distribution, and paresthesia are very frequent. Ataxia is observed in more than two-thirds of the cases and may involve the upper as much as the lower limbs. A neuropathic pain is reported in about half of cases. Asymmetric distribution was present in 42% of patients. Increased proteins in CSF and an oligoclonal CSF pattern are significantly more frequent as compared to nonparaneoplastic SSN (Camdessanche et al., 2009). The electrophysiologic hallmark of SSN is a severe and diffuse alteration of sensory nerve action potentials. Motor conduction velocities are normal or mildly altered (Camdessanche et al., 2002). CSF can show elevated concentrations of protein, pleocytosis, or oligoclonal bands.

The tumor most often associated with paraneoplastic SSN is SCLC (Dalmau et al., 1992). More than 86% of the patients with Hu-Abs present with a SSN (Honnorat et al., 2009). This neuropathy is the most common symptom of the anti-Hu syndrome, but it is isolated in only 24% of the patients, the others having various combinations of central and PNS involvement (Graus et al., 2001). Other patients mostly present CV2/CRMP5-Abs (Antoine et al., 1999b) or amphiphysin-Abs. Yo-Abs and Ma2-Abs have been reported in only few cases (Tracy et al., 2006; Waragai et al., 2006). However, the peripheral neuropathies occurring in 57% of the patients with CV2/CRMP5-Abs are a little bit different than those observed in patients with Hu-Abs (Antoine et al., 2001). In patients with CV2/CRMP5-Abs the neuropathies are sensory or sensorimotor and predominate in the lower limbs. Furthermore, electroneuromyography shows an axonal or mixed axonal and demyelinating pattern (Antoine et al., 2001). Neuropathies associated with CV2/CRMP5-Abs are typically associated with cerebellar ataxia, limbic encephalitis, or ocular involvement.

NONCLASSIC PARANEOPLASTIC NEUROPATHIES

Motor nerves or motor neurons can also be affected in patients with PNS resulting in a motor or sensorimotor polyneuropathy (Antoine and Camdessanche, 2007).
A predominant or pure motor neuron syndrome is much less common (Verma et al., 1996). Lower motor neuron disease is reported in several cases of late-onset second PNS in patients with Hu-Abs and prolonged survival without tumor relapse (Ducray et al., 2010). A recent publication provides clues for an interaction between the so-called “survival motor neuron” gene and the HuD protein (Hubers et al., 2011). Hu-Abs may thus play a specific role in the pathophysiology of this disease.

Nerve vasculitis and demyelinating neuropathies are reported but are a very unusual presentation (Younger et al., 1994; Antoine, 1998). Neuropathies that occurred shortly after the discovery of cancer tended to be inflammatory, including those with Guillain–Barré syndrome, chronic inflammatory demyelinating polyneuropathy, and neuropathies with vasculitis (Antoine, 1999b). The improvement of the neuropathy after treatment of the tumor is a major criterion for the diagnosis of paraneoplastic disorders.

Ganglionic cholinergic receptor antibodies have been reported in patients with autonomic neuropathies and malignancies but these antibodies are not specific to cancer (Vernino and Lennon, 2000). Conversely, antiganglioside antibodies have been specifically associated with both cancer and neuropathy in few patients with melanoma (Weiss et al., 1998; Kloos et al., 2003).

### Lambert–Eaton Myasthenic Syndrome

Lambert–Eaton myasthenic syndrome (LEMS) is an autoimmune disorder of the neuromuscular junction characterized by muscle weakness and autonomic dysfunction (O’Neill et al., 1988; Newsom-Davis, 2004). First described in 1953 (Anderson et al., 1953), its electromyographic features were subsequently described, allowing differentiation of LEMS from myasthenia gravis (Lambert et al., 1956). Difficulty in walking because of proximal leg weakness is nearly always the first symptom (O’Neill et al., 1988). Proximal arm weakness is common, but ocular symptoms are less common than in myasthenia gravis. In rare cases, respiratory muscles can be affected (Nicolle et al., 1996). The cardinal features on examination are the augmentation of strength that occurs during the first few seconds of a maximum effort and the depressed deep-tendon reflexes that may show post-tetanic potentiation. Autonomic symptoms, even moderate (dry mouth, constipation, or erectile failure), are present in almost all cases (Khurana et al., 1988; O’Neill et al., 1988). In rare patients with paraneoplastic LEMS, a cerebellar ataxia can be observed (Fukuda et al., 2003; Romics et al., 2011). Conversely, some patients with paraneoplastic cerebellar degeneration can have VGCC-Abs but without clinical signs or symptoms of LEMS (Graus et al., 2002).

On electroneuromyographic examination, the compound muscle action potential has a small amplitude which increases from 100% to 1000% after maximum voluntary contraction. This facilitation phenomenon is also observed after repetitive nerve electrical stimulations at high frequencies (between 20 and 50 Hz). Nonetheless a decrease in the amplitude of the compound muscle action potentials occurs with repetitive nerve stimulations at low frequencies (between 1 and 5 Hz), as seen in myasthenia gravis. Almost all patients have a decremental response to low-frequency nerve stimulation in at least one hand muscle; in these patients a reproducible postexercise increase in compound muscle action potentials is considered to be specific to LEMS and is more easily demonstrated in distal muscles (Sanders, 2003).

Almost 60% of the patients with LEMS are paraneoplastic and SCLC is the main associated cancer, which will be detected mostly within the 2 years following the diagnosis of LEMS (O’Neill et al., 1988; Newsom-Davis, 2004). A prospective study in a cohort of patients presenting with SCLC showed the prevalence of LEMS to be 3% (Elrington et al., 1991). VGCC-Abs (voltage-gated calcium channel antibodies) are present in nearly all patients with LEMS, and these Abs do not differentiate between paraneoplastic and nonparaneoplastic forms. Sox1-Abs have been identified as a specific marker of paraneoplastic forms of LEMS (Graus and Saiz, 2005). In fact Sox1-Abs are present in 64% of the patients presenting LEMS and SCLC (Sabater et al., 2008b); on the other hand only one patient has been described to date with Sox1-Abs and an idiopathic LEMS (Titulaer and Verschuuren, 2008). Although more investigations are needed, Sox1-Abs seem to show a strong association with paraneoplastic LEMS (Tschernatsch et al., 2009).

Two randomized controlled trials have demonstrated a beneficial role for 3,4-diaminopyridine (McEvoy et al., 1989; Sanders et al., 2000). Symptomatic improvement can sometimes be obtained with a mild dose of pyridostigmine, but it is less effective than in myasthenia gravis. Guanidine can be used, either alone or with pyridostigmine, but its adverse effects include bone marrow suppression and renal failure. In LEMS patients with severe or life-threatening weakness, intravenous immunoglobulin therapy will often be followed by several weeks of improvement (Lang et al., 1981; Newsom-Davis and Murray, 1984; Bain et al., 1996).

### Autonomic Neuropathy

Pandysautonomia, autoimmune autonomic neuropathy, idiopathic autonomic neuropathy, or subacute autonomic neuropathy were also used for this clinical pattern, which rarely occurs solely. Autonomic neuropathy may overlap
with other paraneoplastic neuropathies. However, pure dysautonomia was reported associated with acetylcholine receptor-Abs (Vernino et al., 1998). Hu-Abs, CV2/CRMP5-Abs and ganglionic acetylcholine receptor-Abs are commonly present in autonomic neuropathy with a respective frequency of 25%, 31%, and 21% of cases (Koike et al., 2011). The diagnosis of autonomic neuropathy is of importance since PNS with autonomic neuropathy has been shown to be associated with a worse prognosis than the others (Giometto et al., 2010).

**Autonomic Neuropathy with Pseudo-obstruction**

Autonomic neuropathy with pseudo-obstruction may start as an isolated and severe constipation. The clinical symptoms then evolve to dysphagia and vomiting. Patients with autonomic neuropathy and pseudo-obstruction present with weight loss, persistent constipation, and abdominal distension due to neuronal damage of the enteric plexuses (De Giorgio et al., 2004; Lorusso et al., 2007). Some patients may present with dysphagia, nausea, and vomiting due to esophageal dysmotility or gastroparesis. Radiologic studies show bowel, colonic, or gastric dilatation; esophageal manometry may disclose spasms or achalasia. The most common Ab-associated tumor (mainly Hu-Abs) is SCLC.

**DERMATOMYOSITIS**

Dermatomyositis is defined by the association of a proximal motor weakness, Gottron’s papules (myxedematous lichen planus) over the knuckles, photosensitive erythematous patches spreading from the face to the upper limb, and a periorbital violaceous inflammation. The latter is almost pathognomonic (Cherin, 2004). In addition, a periangual erythema with a classic pressure-induced pain is highly suggestive of this diagnosis. The cutaneous signs may precede the myositis by several months. Patients may also present with asthenia, fever, and weight loss. A cardiac involvement with conduction disorders is not uncommon (15–20%). Respiratory disorders occur in 45% of cases and represent the second cause of death after cancer in dermatomyositis especially when resulting from a pharyngeal involvement.

In 1916, a published case of a dermatomyositis associated with a gastric cancer was the first to suggest the association between dermatomyositis and malignancy. According to the results of several subsequent studies, 25–30% of patients presenting with a dermatomyositis had an associated tumor (Sigurgeirsson et al., 1992). The resulting standardized incidence ratios of associated malignancies vary within four to six in those patients compared to the general population (Chow et al., 1995; Buchbinder et al., 2001). The excess of cancer incidence is maximal at the onset and within the first 3 years of dermatomyositis then it decreases with time. Conversely, more than a half of the cancers are diagnosed before the onset of the dermatomyositis (Buchbinder et al., 2001). The more frequently associated tumors are ovaries, lung, colorectal, and breast cancers (Buchbinder et al., 2001). The proportion of gastric cancer was particularly elevated (41%) among paraneoplastic dermatomyositis patients in a Japanese cohort (Azuma et al., 2011).

P155-Abs was shown to be specifically associated with myositis and cancer in adult patients (Targoff et al., 2006). Some recent studies proposed that these Abs may be involved in the transforming growth factor-β signaling pathway, which is inactivated in some malignancies (Vincent et al., 2009). A comprehensive cancer screening must be repeatedly performed in patients with p155-Abs. However, positron emission tomography (PET) scanning may be done only at the onset of dermatomyositis if these Abs are absent (Selva-O’Callaghan et al., 2010).

**CLINICAL MANAGEMENT AND PRINCIPLES OF TREATMENT**

**Identification of the tumor**

Detection of the associated cancer remains the main step in the treatment of patients suffering from PNS. In fact, PNS mostly precedes the diagnosis of the cancer, which is often of limited spread (Chartrand-Lefebvre et al., 1998) and could consequently be cured at a local stage. The type of carcinologic screening depends on the isolated Ab. There is an increasing number of Ab which are rarely associated with tumors. In cases such as GAD-Abs or NMDAr-Abs in children, a comprehensive carcinologic screening seems unnecessary. Conversely Hu-Abs and CV2/CRMP5-Abs are strongly associated with SCLC and screening for small mediastinal lymph nodes must be considered (Honnorat et al., 2009). Yo-Abs and SCD are highly suggestive of a gynecologic cancer and if mammography or pelvic examination is negative a surgical exploration of the pelvis must be discussed (Peterson et al., 1992; Rojas et al., 2000). Ma2-Abs in a male patient with risk of testicular cancer must lead to an orchidectomy if echography is not conclusive (Mathew et al., 2007). Women with NMDAr-Abs must have endovaginal ultrasound and pelvic MRI to rule out ovarian teratoma (Dalmau et al., 2008). Whole-body positron emission tomography with fluorodeoxyglucose (FDG-PET) is required in patients with paraneoplastic Ab when conventional imaging fails to identify a tumor or when lesions are difficult to reach for biopsy (Younes-Mhienni et al., 2004; Basu and Alavi, 2008).
Treatment

In case of ON-Abs, the best chance to stabilize or improve the syndrome remains induction of a complete response of the tumor (Keime-Guibert et al., 2000). The specific treatment for PNS is based on immunologic therapies but no randomized double-blind studies are available because of the low incidence of PNS. However, the European Federation of Neurological Societies (EFNS) Task Force published some recommendations for treatment (Vedeler et al., 2006). These suggest that the response to the treatment mainly depends on the subtype of Ab associated with the PNS. PNS with NSA-Abs may thus improve significantly under immunomodulatory treatment whereas the latter is inefficient for PNS associated with ON-Abs. A delayed response to the treatment may be observed in the particular case of LE with NMDAR-Abs in which repeated injections of immunoglobulins may be required before observing an improvement.

Another publication of the EFNS Task Force reviews the indications for intravenous immunoglobulins in neurology (Elovaara et al., 2008). This treatment shows efficacy for paraneoplastic LEMS and opsoclonus myoclonus especially in pediatric neuroblastoma patients. In contrast, intracellular Ab-associated PNS are constantly responsive to immunosuppressor treatment such as cyclophosphamide. Patients with Tr-Abs and Ma2-Abs and testicular cancer are more likely to improve than those with other intracellular Ab (Bernal et al., 2003; Dalmau et al., 2004).

According to these considerations, PNS management must be organized depending on the associated Ab. In some cases, LE may be associated with several subtypes of Ab (Bataller et al., 2007). Their prognosis and response to treatment seem to depend on the presence or absence of ON-Abs, which probably lead to a poorer outcome, when present.

Due to the low number of cases, the management of subacute cerebellar degeneration has not been codified. As a general rule a prompt treatment of the cancer is essential for neurologic stabilization in PNS. A study conducted on 50 patients demonstrated a neurologic improvement in only seven cases. All patients received antitumor treatment and were in complete remission (Shams’ili et al., 2003). The sensitivity to treatment is variable depending on the type of associated onconeuronal antibodies. In patients with Yo-Abs or Hu-Abs, improvement is rather rare, while a better outcome can be expected in Tr-Abs patients (Bernal et al., 2003). Regarding the specific management of PCD, immunomodulatory treatment or immunosuppressants may be proposed. Some clues based on case reports suggest a possible neurologic improvement with corticosteroids, cyclophosphamide (Thone et al., 2008), intravenous immunoglobulins (Phuphanich and Brock, 2007), or rituximab (Esposito et al., 2008). In cerebellar ataxia with anti-GAD, the effectiveness of immunoglobulins and steroids is often claimed but appears to be inconsistent. In some cases, periodic alternating nystagmus, reversible under GABAergic medication, has been reported (Tilikete et al., 2005). Finally, the management should be supplemented with the appropriate rehabilitation and psychological counseling.

ABBREVIATIONS

Abs antibodies
AMPA \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CASPR 2 contactin-associated protein 2
CNS central nervous system
CSF cerebrospinal fluid
CT computed tomography
FDG PET fluoro(deoxy)glucose positron emission tomography
GABA\(_A\) \(\gamma\)-aminobutyric acid A receptor
GABA\(_B\) \(\gamma\)-aminobutyric acid B receptor
GAD glutamic acid decarboxylase
IgGs immunoglobulin Gs
IVIg intravenous immunoglobulin
LE limbic encephalitis
LEMS Lambert–Eaton myasthenic syndrome
Lgi1-Abs leucine-rich glioma inactivated 1 protein
mGluR1 metabotropic glutamate receptor 1
NMDAr N-methyl-D-aspartate glutamate receptor
NSA neuronal cell surface antigen
OM opsoclonus myoclonus
ON onconeuronal
PCR polymerase chain reaction
PEM paraneoplastic encephalomyelitis
PNS paraneoplastic neurologic syndrome
SCD subacute cerebellar degeneration
SCLC small-cell lung cancer
SSN subacute sensory neuronopathy
VGCC voltage-gated calcium channel
VGKC voltage-gated potassium \((K^+\)\) channel.

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Neurofibromatoses
Neurofibromatosis type 1: a multidisciplinary approach to care

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Neurofibromatosis type 1 is a relatively common inherited disorder. Patients have a high predisposition to develop both benign and malignant tumours. Although many manifestations of neurofibromatosis type 1 affect the nervous system, other organs and tissues can also be affected. Because of the varying features and clinical heterogeneity inherent to this disorder, patients present to different medical and surgical specialists and, therefore, the association of clinical symptoms with neurofibromatosis type 1 might not be appreciated. Thus, for prompt diagnosis and to provide optimum care for patients with neurofibromatosis type 1, clinicians must be aware of the diverse clinical features of this disorder. We advocate a multidisciplinary approach to care, entailing a dedicated team of specialists throughout the lifetime of the patient. As our understanding of this disorder deepens through basic laboratory and clinical investigations, swift implementation of new effective treatments becomes feasible.

Introduction

Neurofibromatosis type 1 is a relatively common inherited disorder that affects about one in 2500 to one in 3000 people worldwide, irrespective of sex or ethnic origin.1,2 Individuals with neurofibromatosis type 1 are prone to develop benign and malignant tumours of the CNS and peripheral nervous system, in addition to malignant diseases affecting other parts of the body.3 Tumours that are commonly associated with the disorder include glioma of the optic pathway, glioblastoma, malignant peripheral nerve sheath tumour, gastrointestinal stromal tumour, breast cancer, leukaemia, phaeochromocytoma, duodenal carcinoid tumour, and rhabdomyosarcoma (table).4–13

Neurofibromatosis type 1 was first described by Friedrich von Recklinghausen in 1882. In 1987, formal diagnostic criteria were published by the National Institutes of Health (panel).14 In this Review, we describe benign and malignant features of neurofibromatosis type 1, focusing on diagnostic strategies, monitoring, and treatment of tumours located in the nervous system and elsewhere. Additionally, we highlight possible future therapeutic directions based on findings of preclinical drug discovery and evaluation studies in genetically engineered mouse models of neurofibromatosis type 1-associated malignant diseases. Finally, we discuss current and future clinical trials in patients with neurofibromatosis type 1.

Genetics and genetic testing

Neurofibromatosis type 1 is a dominantly inherited genetic disorder that results from a germline mutation in the NF1 tumour-suppressor gene. NF1 is located on chromosome 17q11.2 and encodes a 220 kDa cytoplasmic protein called neurofibromin. This protein functions, in part, as a negative regulator of the Ras proto-oncogene, which is a key signalling molecule in the control of cell growth.15 Affected individuals start life with one mutated (non-functional) copy and one functional copy of NF1 in every cell in their body. Although many of the clinical features of this syndrome are apparent from birth, complete loss of gene function is needed for formation of tumours, by acquisition of a somatic NF1 mutation in selected cells.16,17

About 50% of individuals with neurofibromatosis type 1 have no family history of the disease and the disease is due to de novo (spontaneous) mutations. With the advent of accurate genetic testing, early genotype-phenotype correlations are beginning to emerge, including the observation that people with genomic microdeletions affecting the entire NF1 gene have a more severe phenotype.18,19 For instance, this particular subgroup of

Panel: NIH consensus criteria14 for diagnosis of neurofibromatosis type 1

Two or more of the following clinical features are sufficient to establish a diagnosis of neurofibromatosis type 1:

- Six or more café-au-lait macules (≥0.5 cm at largest diameter in a prepubertal child or ≥1.5 cm in post-pubertal individuals)
- Axillary freckling or freckling in inguinal regions
- Two or more neurofibromas of any type or one or more plexiform neurofibromas
- Two or more Lisch nodules (iris hamartomas)
- A distinctive osseous lesion (sphenoid wing dysplasia, long-bone dysplasia)
- An optic pathway glioma
- A first-degree relative with neurofibromatosis type 1 diagnosed by the above criteria

NIH=National Institutes of Health.
individuals with NF1 microdeletions tend to develop neurofibromas at an earlier age, have a lower mean IQ, manifest abnormal facial features, and are at increased risk of developing malignant peripheral nerve-sheath tumours. At present, the diagnosis of neurofibromatosis type 1 is most commonly made using established clinical criteria, reserving NF1 genetic testing for unusual presentations or reproductive decision-making.

Non-malignant clinical features

Pigmentary abnormalities

Frequently, the earliest clinical manifestation of neurofibromatosis type 1 is café-au-lait macules, which usually develop within the first 2 years of life (figure 1). The presence of more than five café-au-lait macules (>0.5 cm in diameter before puberty or >1.5 cm after puberty) is one of the diagnostic criteria for neurofibromatosis type 1. These lesions have no malignant potential and tend to darken with sun exposure and fade with advancing age. For macules that reduce quality of life, individuals can be offered dermatological camouflage treatments.

Axillary and inguinal freckling is another common clinical feature of neurofibromatosis type 1 and is usually detected in affected individuals by age 5–8 years. These pigmentary abnormalities are typically the second diagnostic characteristic seen in children with the disorder, generally arising after development of café-au-lait macules. Freckles can also be found in areas where skinfolds are in apposition, including the neck and under the breasts in women.

Lisch nodules are benign melanocytic hamartomas of the iris, typically first noticed in children aged 5–10 years. Nearly all adults with neurofibromatosis type 1 have Lisch nodules. These pigmented lesions are best detected on slit-lamp examination by an experienced ophthalmologist. Lisch nodules do not impair vision or cause any medical problems.

Neurofibromas

Neurofibromas are benign Schwann-cell tumours composed not only of neoplastic Schwann cells but also of non-neoplastic fibroblasts, mast cells, macrophages, endothelial cells, pericytes, and perineural cells. There are four subtypes: cutaneous, subcutaneous, nodular or diffuse plexiform, and spinal.

Intracutaneous neurofibromas develop during late childhood or early adolescence and do not undergo malignant transformation. Owing to an abundance of mast cells associated with the tumours, these tumours might cause local pruritus. Intracutaneous neurofibromas can result in substantial discomfort or disfigurement when hundreds or thousands of these neurofibromas are present in a patient. In such instances, the tumours can be removed by a plastic surgeon. Spinal neurofibromas can occur at single or multiple nerve roots and are associated with both sensory and motor deficits.

Although neurofibromas are commonly found on the skin—presenting as subcutaneous, dermal, or exophytic masses—they can also be located deep within the body. Individuals with neurofibromatosis type 1 generally develop more neurofibromas as they get older, and some patients can have many deep neurofibromas without clinical symptoms. In these patients, tumour removal should be led by symptoms—such as pain and functional deficits—and findings of a risk-benefit assessment.

Plexiform neurofibromas

Plexiform neurofibromas typically manifest at birth but can continue to grow during adolescence and early adulthood (figure 2). In most individuals, these tumours enlarge most prominently during the first decade of life. Plexiform neurofibromas develop in about 30–50% of individuals
with neurofibromatosis type 1. They differ from cutaneous neurofibromas in that they arise from multiple nerve fascicles and can grow along the length of a nerve.30 These tumours can also extend into surrounding structures, causing substantial pain and bone destruction. Importantly, plexiform neurofibromas have a lifetime risk of malignant transformation. Although the best therapeutic option for symptomatic lesions is surgical removal, this approach is sometimes technically impossible.3

Chemotherapy is a potential therapeutic option for plexiform neurofibromas. In a recent phase 1 trial, pegylated interferon-alfa-2b (antiviral cytokine therapy) was assessed in 30 patients with plexiform neurofibromas who had radiographic progression before enrolment.31 Reported effects were pain reduction (in 11 of 16 patients), decreased tumour mass (13 of 14), and tumour shrinkage or stabilisation (three of four). Similarly, in a phase 2 trial of imatinib (tyrosine kinase inhibitor), 17% of patients with plexiform neurofibromas had a 20% or more reduction in tumour volume.32 In view of these initial encouraging results, several studies are now in progress to test the use of other biologically targeted therapies, including mammalian target of rapamycin (mTOR) inhibitors and mitogen-activated protein kinase kinase (MEK) inhibitors in these patients.

**Skeletal deformities**

People with neurofibromatosis type 1 can develop skeletal abnormalities, including osteopenia, scoliosis, sphenoid wing dysplasia, congenital tibial dysplasia, and pseudarthrosis (figure 3). Moreover, affected individuals tend to be shorter than expected for their age and frequently have low bone-mineral densities.33 In a large registry-based study of neurofibromatosis type 1, a roughly fivefold increase in fracture risk was reported for adults older than 40 years, and this risk was about threefold higher in children younger than 16 years.34 Lifestyle modifications such as increased exercise and calcium or vitamin D supplementation might be warranted in these patients. Low concentrations of vitamin D have been recorded in people with neurofibromatosis type 1.33,35–38 In a retrospective study of vitamin D supplementation, loss of bone-mineral density was reduced significantly in adult patients with neurofibromatosis type 1 whose vitamin D levels were maintained above 30 μg/L, compared with people who had not been supplemented.39 Although this finding suggests that all adults should be screened for vitamin D deficiency and appropriate replacements initiated, prospective studies are needed before practice recommendations are changed.

Scoliosis can affect 10–26% of individuals with neurofibromatosis type 1, making annual spinal examinations necessary during childhood and early adolescence.40 Mild curvature can be treated with bracing; however, more severe cases might need surgery to stabilise progressive spinal and chest-wall deformity and preserve lung function by minimising constrictive forces.1 The vertical expandable prosthesis titanium rib (VEPTR), which is used to diminish constrictive forces on the lung, has shown promise in clinical trials.41 It is noteworthy that some individuals with neurofibromatosis type 1 have dystrophic scoliosis with striking curvatures and generally less satisfactory surgical outcomes than those without striking curvatures.

Sphenoid wing dysplasia typically presents as a unilateral bony defect affecting the orbital plate and the frontal bone. Sometimes, thinning or absence of the sphenoid wing is attributable to the presence of an associated orbital plexiform neurofibroma, but it can occur as an isolated bony abnormality.42 These defects are usually seen in...
asymptomatic individuals after a careful physical examination in which one eye appears asymmetric or is proptotic or sunken. Congenital tibial dysplasia generally presents as anterolateral bowing of the lower leg, with cortical thinning evident on plain radiographs. The presence of bowing in an infant should warrant prompt radiographic assessment. Repeated fractures with failure to heal can lead to development of pseudarthrosis and, in some cases, limb amputation.41 Referral to a skilled paediatric orthopaedic surgeon is needed to initiate appropriate treatment and avoid this poor outcome.

Cardiovascular abnormalities

Individuals with neurofibromatosis type 1 can develop various cardiovascular abnormalities, ranging from congenital heart disease to vasculopathy and hypertension. Echocardiographic data suggest that up to 27% of patients with neurofibromatosis type 1 have a cardiovascular anomaly,42 and pulmonary artery stenosis accounts for as many as 50% of these abnormalities.43 The prevalence of abnormalities is likely to be an underestimate because a diagnosis is usually made only if symptoms develop. Therefore, all children born with neurofibromatosis type 1 should have a thorough cardiac examination and any murmurs should be investigated further by a skilled paediatric cardiologist.44

Neurofibromatosis type 1-related vasculopathy includes renal and cerebral artery stenosis, aortic coarctation, and arteriovenous malformations.45 The pathogenesis, clinical spectrum, and natural history of these anomalies remains poorly understood; however, impaired NF1 gene function in vascular endothelial cells results in increased proliferation and growth.46,47 Vasculopathy usually affects the arterial system, leading to cerebrovascular disease (eg, narrowed or ectatic vessels, vascular stenosis, aneurysm, or moyamoya disease) or renal artery stenosis.48,49 Individuals with neurofibromatosis type 1 who present with a new neurological deficit should be assessed for both cerebrovascular disease and brain tumour. Moreover, any patient with unexplained hypertension should undergo investigation for renal artery stenosis. Laboratory assessments (serum creatinine and electrolytes, plasma renin, and urinalysis), appropriate imaging studies, and arteriography are important. Although essential hypertension remains the most common reason for raised blood pressure in this population, other causes include coarctation of the aorta and phaeochromocytoma. Some of these abnormalities are likely to be congenital; however, whether vascular stenoses are actually present at birth is unclear. Further study is needed to resolve these uncertainties.

Neurocognitive deficits

Neurocognitive deficits are among the most common manifestations of neurofibromatosis type 1. Children should undergo neuropsychological screening assessments early in life, followed by more detailed testing when appropriate.50 Learning difficulties can include visuospatial and visuomotor deficits, language disorders, and fine and gross motor deficiencies. Furthermore, attention-deficit hyperactivity disorder, autism spectrum disorders, behavioural abnormalities, and psychosocial issues are prevalent in this population.51 Children with neurofibromatosis type 1 might benefit from a multidisciplinary approach, in which educational specialists, paediatric neuropsychologists, physical therapists, speech therapists, and occupational therapists work together to maximise abilities and optimise the chance for academic and social success. Pharmacological interventions with treatments such as lovastatin52 or drugs used to treat attention-deficit hyperactivity disorder (eg, methylphenidate) might be of benefit for some children.

Nervous-system tumours

Optic pathway and brainstem gliomas

About 15–20% of individuals with neurofibromatosis type 1 will develop low-grade glial neoplasms; roughly 80% are in the optic pathway, but some (15%) can be present in the brainstem,22 with rare involvement of the cerebellum, cortex, and subcortical regions.53 Optic pathway gliomas (figure 4A) typically present in children with neurofibromatosis type 1 who are younger than 7 years.54 These tumours are mainly WHO grade I glial neoplasms—termed pilocytic astrocytomas—and are indistinguishable histologically from gliomas that arise sporadically in individuals without neurofibromatosis type 1.55 Although many optic pathway gliomas are asymptomatic, up to half can cause clinical symptoms, most commonly resulting in reduced vision; some children present with precocious puberty.56

Owing to the frequency of optic pathway gliomas in young children with neurofibromatosis type 1, all patients who are younger than 13 years should undergo an
ophthalmological examination every year by a paediatric neuro-ophtalmologist. Screening MRI is not recommended unless children are unable to undertake the ophthalmological assessment and reliable measurements of visual acuity cannot be obtained. Of the optic pathway gliomas, current recommendations include an optic glioma, current recommendations include ophthalmological and MRI studies four times a year for the first year, followed by gradual lengthening of test intervals over the next 2–3 years. A two-line decrease in visual acuity, as measured by a standardised visual assessment procedure such as the Snellen chart, warrants referral to a paediatric neuro-oncologist for treatment.

Surgery plays little part in the treatment of optic pathway gliomas because it can result in permanent neurological damage. First-line treatment for most patients with symptomatic tumours is chemotherapy with carboplatin and vincristine. Other chemotherapy combinations have been used; however, no randomised trial data are available to support the use of one regimen over another. Cranial radiation therapy is not recommended for patients with neurofibromatosis type 1 because of the increased propensity of this population to develop second malignancies, vascular abnormalities, and neuropsychological difficulties.

Brainstem gliomas are the most frequently discovered brain tumor outside of the optic pathway in people with neurofibromatosis type 1. Similar to optic pathway tumours, brainstem gliomas (figure 4B) are usually pilocytic astrocytomas; however, they typically present later in the first decade of life. Affected children might come to medical attention with cranial neuropathies, lethargy, gait instability, or headaches. Chemotherapy is used to treat clinically progressive tumours, with drugs such as carboplatin or vincristine, as would be used to treat clinically progressive tumours, brainstem gliomas. The cumulative lifetime risk of developing malignant peripheral nerve sheath tumours in a patient with neurofibromatosis type 1 is about 8–13%. Malignant peripheral nerve sheath tumours can arise anywhere within the body. Risk for developing a malignant peripheral nerve sheath tumour is increased 20-fold in the area of an existing internal plexiform neurofibroma. Other risk factors for development of malignant peripheral nerve sheath tumours include previous radiation therapy and large germline mutations encompassing the entire NF1 gene (microdeletions).

Individuals with neurofibromatosis type 1 who report substantial or difficult-to-control pain, a rapid increase in the size of an existing plexiform neurofibroma, a change in tumour consistency (soft to hard), or a new neurological deficit warrant prompt assessment for malignant peripheral nerve sheath tumours. MRI is helpful to define the location and extent of the tumour, but it is not reliable for distinguishing between malignant disease and benign tumours. Over the past decade, 18F-fluorodeoxyglucose (18F-FDG)-PET has emerged as a highly sensitive and specific method for detection of malignant peripheral nerve sheath tumours (figure 5). Needle biopsy can be affected by sampling bias and might not allow the treating clinician to exclude a diagnosis of malignant peripheral nerve sheath tumour with confidence. Furthermore, patients with suspected malignant disease should be examined for evidence of metastatic disease (e.g., in the lung or bone). In view of the aggressive nature of malignant peripheral nerve sheath tumours, patients should be assessed and managed by a multidisciplinary team including neurologists, radiologists, surgeons, oncologists, and radiation oncologists to efficiently implement plans for biopsy (non-invasive or open) and treatment.

Surgery is the only curative treatment option for patients with malignant peripheral nerve sheath tumours. Even with surgical excision, 5-year overall survival rates are poor, and this cancer represents a substantial cause of mortality in individuals with neurofibromatosis type 1. Some findings suggest that survival is beginning to increase, particularly in women; however, this rise might be attributable to heightened recognition and identification of malignant peripheral nerve sheath tumours at an earlier stage. Although radiation therapy could delay time to recurrence, this treatment does not change time to death. Use of adjuvant chemotherapy is controversial. In some instances, chemotherapy can be used in the neoadjuvant setting to downstage tumours before resection; however, this practice has not been adopted widely. Clinical studies of promising chemotherapy drugs are underway in individuals with neurofibromatosis type 1-associated malignant peripheral nerve sheath tumours. In patients with metastatic disease, single-agent anthracycline is the most accepted form of palliative care.

Glioblastomas

Individuals with neurofibromatosis type 1 have at least a fivefold increased risk for developing other brain tumours, including WHO grade IV astrocytomas (glioblastomas), according to findings of several case reports and small retrospective studies. Glioblastomas usually present in young adults, in whom the overall prognosis is poor. Treatment is similar to that for people with sporadic glioblastomas and usually entails gross surgical resection followed by adjuvant radiation and chemotherapy (typically oral temozolomide).

Malignant peripheral nerve sheath tumours

Malignant peripheral nerve sheath tumours—sometimes referred to as neurofibrosarcomas or neurogenic sarcomas—are a subtype of sarcoma with a presumed Schwann cell origin. These tumours represent about 3–10% of all soft-tissue sarcomas, and a large proportion arise in individuals with neurofibromatosis type 1. Malignant peripheral nerve sheath tumours have a prevalence of 0.001% in the general population versus 0.1% in individuals with neurofibromatosis type 1. The cumulative lifetime risk of developing malignant peripheral nerve sheath tumours in a patient with neurofibromatosis type 1 is about 8–13%. Malignant peripheral nerve sheath tumours can arise anywhere within the body. Risk for developing a malignant peripheral nerve sheath tumour is increased 20-fold in the area of an existing internal plexiform neurofibroma. Other risk factors for development of malignant peripheral nerve sheath tumours include previous radiation therapy and large germline mutations encompassing the entire NF1 gene (microdeletions).
Non-nervous-system tumours

Gastrointestinal stromal tumours

Gastrointestinal stromal tumours are of mesenchymal origin and can develop anywhere along the gastrointestinal tract. Compared with the general population, individuals with neurofibromatosis type 1 are slightly younger at presentation (median age 50 years vs 60 years) and about 95% are asymptomatic. Moreover, patients with neurofibromatosis type 1 and gastrointestinal stromal tumours more frequently present with multiple tumours compared with the general population. The most common symptoms reported are abdominal pain, bleeding, intestinal perforation, and intestinal obstruction. Gastrointestinal stromal tumours can be an incidental finding during imaging studies, might be spotted during surgery to remove another tumour, or could be diagnosed from presenting symptoms.

In the general population, gastrointestinal stromal tumours are associated with increased expression of the KIT and PDGFRA transmembrane receptors. Activation of these receptor tyrosine kinases drives proliferation, providing the scientific basis for treatment with imatinib. By contrast, in patients with neurofibromatosis type 1, gastrointestinal stromal tumours typically do not overexpress KIT or PDGFRA, which limits the use of imatinib in this population. Currently, the only treatment option for neurofibromatosis type 1-associated gastrointestinal stromal tumours is surgery, if feasible. Clinical trials of other tyrosine kinase receptor inhibitors (eg, sunitinib) are ongoing in patients with metastatic disease.

Breast cancers

A fivefold increased risk for breast cancer has been reported in individuals with neurofibromatosis type 1, mainly affecting women younger than 50 years. Furthermore, mortality rates in women with neurofibromatosis type 1 and breast cancer are higher than those for women with breast cancer in the general population. Although reported studies include only a few patients, relative risks are similar to those for people with a family history of breast cancer. Women age 30–49 years with neurofibromatosis type 1 could undergo early mammography or MRI; however, large prospective studies are needed before formal recommendations can be made about screening in this population. Currently, treatment for neurofibromatosis type 1-associated breast cancer does not differ from that for women with breast cancer in the general population.

Leukaemia and lymphoma

Children with neurofibromatosis type 1 have at least a sevenfold higher risk for developing myeloid leukaemia compared with children in the general population, and the prevalence of chronic myelomonocytic leukaemia, juvenile chronic myelogenous leukaemia, acute lymphocytic leukaemia, and non-Hodgkin lymphoma is also raised. However, these tumours are rare in people with neurofibromatosis type 1. Moreover, no evidence is available to support a difference in prognosis between neurofibromatosis type 1-associated leukaemia and lymphoma relative to the general population. Treatments for neurofibromatosis type 1-associated leukaemia parallel those used for individuals without neurofibromatosis type 1.

Phaeochromocytoma

Phaeochromocytomas—catecholamine-secreting tumours of the adrenal medulla or other sites in the sympathetic nervous system—are seen at increased frequency in individuals with neurofibromatosis type 1. The age of onset (typically the fourth decade of life) is similar in neurofibromatosis type 1-associated and sporadic cases. Phaeochromocytomas should be suspected in an individual with neurofibromatosis type 1 who presents with unexplained hypertension, flushing,
headaches, sweating, or heart palpitations. Diagnosis is established typically with a combination of imaging studies (CT or MRI of the chest and abdomen or a metaiodobenzylguanidine scan or somatostatin scintigraphy if CT is negative) and biochemical assessments (eg, the amount of catecholamines in urine). Surgery is curative for resectable disease, whereas chemotherapy or radiopharmaceutical treatment with 131I-meta-iodobenzylguanidine is used for metastatic or unresectable cancers.

Duodenal carcinoids
Carcinoid tumours are neuroendocrine tumours that arise from endocrine cells within the gastrointestinal tract; they are reported in about 1% of individuals with neurofibromatosis type 1. The most common site for carcinoid tumours is the periampullary region; therefore, individuals usually present with jaundice and non-specific abdominal pain. In patients with neurofibromatosis type 1, these tumours generally present at a young age.

The diagnosis of carcinoid tumours is typically made with a combination of imaging studies—eg, CT of the chest, abdomen, and pelvis, somatostatin scintigraphy, and endoscopic ultrasound or endoscopy—and measurement of urinary and serum 5-hydroxyindolectic acid and chromagranin A, when clinically appropriate. Surgical resection should be done if possible; unresectable and metastatic disease is treated generally with somatostatin analogues or chemotherapy.

Rhabdomyosarcomas
Rhabdomyosarcomas are non-neurogenic sarcomas composed of small round blue cells that probably originate from the neural crest. Children with neurofibromatosis type 1 have about a 20-fold increased risk of developing these tumours. Rhabdomyosarcomas most commonly present as palpable masses. Management relies on surgical resection when feasible, whereas adjuvant chemotherapy and radiation therapy might be appropriate for some individuals.

Conclusions and future directions
Over the past 15 years, substantial advances have been made in our ability to discover, validate, and translate laboratory-based research findings to the clinical workplace. Many accurate preclinical models of neurofibromatosis type 1-associated malignant disease in genetically engineered mice have been developed and used as platforms to evaluate rational targeted treatments.

Although these models have some limitations, they have already proven useful in the design and implementation of human clinical trials.

After identification of NF1 as a negative Ras regulator findings of several preclinical studies in NF1-deficient mice have shown that inhibition of downstream targets of Ras attenuates NF1-deficient tumour cell growth in vitro and in vivo. For example, in studies of genetically engineered NF1 mice, rapamycin analogues (eg, everolimus) and MEK inhibitors were effective biologically targeted treatments for neurofibromatosis type 1-associated plexiform neurofibroma and chemokine receptor inhibitors were assessed for neurofibromatosis type 1-associated glioma and malignant peripheral nerve sheath tumours.

Models of optic pathway glioma and plexiform neurofibroma in genetically engineered NF1 mice showed that non-cancerous stromal cells (tumour microenvironment) have important roles in tumour development and growth. Loss of NF1 in Schwann cell and astrocytial cell precursors alone was not sufficient for tumorigenesis; loss of NF1 expression in Schwann cell or astrocytial cell precursors must occur in NF1+− mice (genetically comparable with individuals with neurofibromatosis type 1) for neurofibromas and optic gliomas, respectively, to form. Further investigation of participatory stromal cell types in models of optic pathway gliomas and plexiform neurofibromas in genetically engineered NF1 mice indicates an obligatory role for microglia and mast cells, respectively, in the genesis and maintenance of these tumours.

The finding that mast cells are important microenvironmental drivers of plexiform neurofibroma growth culminated in use of imatinib to inhibit c-kit function in preclinical studies in NF1 mice and the translation of these results to human clinical trials.

Although studies of these genetically engineered NF1 mice hold substantial promise, future studies need to report clearly positive findings to facilitate effective translation to human clinical trials. Thus, preclinical response criteria should incorporate the proportion of mice with significant radiographic responses, the durability of these outcomes, and the extent of tumour shrinkage. Pharmacokinetic and pharmacodynamic considerations will also need to be integrated into these criteria to ensure a high likelihood of success in patients.

With the establishment of the Neurofibromatosis Clinical Trials Consortium (NFCTC), therapeutic trials in large numbers of individuals with neurofibromatosis type 1-associated malignant disease can now be undertaken efficiently. Up to now, the NFCTC has initiated several clinical trials, including studies of sorafenib for neurofibromatosis type 1-associated plexiform neurofibromas (NCT00727233), bevacizumab and everolimus for malignant peripheral nerve sheath tumours (NCT01661283), everolimus for progressive neurofibromatosis type 1-associated glioma (NCT01158651).

Search strategy and selection criteria
We searched PubMed between January, 1970, and March, 2014, with the terms: "NF1", "skin", "bone", "cardiovascular", "neurocognitive", "plexiform", "malignancy", "optic pathway glioma", "GBM", "MPNST", "breast cancer", "leukemia", "GIST", "pheochromocytoma", "duodenal carcinoids", and "rhabdomyosarcoma". We also chose references from selected articles. We did not restrict our search by language.
MEK inhibitors for children with inoperable neurofibromatosis type 1-associated plexiform neurofibromas (NCT01362803), and MEK inhibitors for patients with tumours activated by RAS, RAF, or MEK, including those with mutations in the NFI gene (NCT01885195). Tumours that develop in patients with neurofibromatosis type 1 are heterogeneous from a molecular and cellular perspective and, therefore, represent complex cancers in which distinct cell types and growth-control pathways regulate tumour behaviour. With availability of accurate preclinical mouse models for most tumour types in neurofibromatosis type 1, a detailed understanding of neurofibromin-controlled signalling pathways, and availability of a clinical trials infrastructure for rapid drug evaluation, we can now envision a future in which effective treatments for people with neurofibromatosis type 1-associated tumours are imminent.

Contributors
ACH wrote the manuscript and prepared the figures. DHG edited the manuscript.

Declaration of interests
We declare no competing interests.

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Advances in the treatment of neurofibromatosis-associated tumours

Andrew L. Lin and David H. Gutmann

Abstract | Neurofibromatosis (NF) comprises two distinct genetic disorders—neurofibromatosis type 1 and 2 (NF1 and NF2)—in which affected individuals develop both benign and malignant tumours. NF1 results from germline mutations in the NF1 gene that encodes neurofibromin, while NF2 results from germline mutations in the NF2 gene that encodes merlin (or schwannomin). The major tumour types arising in individuals with NF1 include neurofibromas, malignant peripheral nerve sheath tumours, and gliomas, whereas NF2 is characterized by the formation of schwannomas, meningiomas, and ependymomas. With the identification of the NF1 and NF2 genes and the generation of robust preclinical mouse models of NF-associated neoplasms, novel treatments that specifically target the growth control pathways deregulated in these tumours have been discovered, some of which are now being tested in clinical trials in individuals with NF1 and NF2. In this Review, we will highlight the key clinical features of NF1 and NF2 and the advances in future clinical management based on an improved understanding of the function of the NF1 and NF2 genes and the development of small-animal models.


Introduction

Neurofibromatosis type 1 (NF1) and type 2 (NF2) represent distinct tumour predisposition syndromes that largely affect the nervous system. The major tumour types arising in individuals with NF1 are neurofibromas, malignant peripheral nerve sheath tumours, and gliomas, whereas individuals with NF2 are prone to the formation of schwannomas, meningiomas, and ependymomas. While these disorders share some similarities, they are distinct conditions with unique genetic aetiologies and pathogenetic mechanisms. The diagnosis of NF1 and NF2 is established using diagnostic criteria that was originally formulated by the National Institutes of Health Consensus Development panel in 1987, and was critically re-evaluated for NF2 in 2002.

NF1 is the more common of the two disorders, with a prevalence of approximately 1 in 2,500 to 3,000 worldwide. By contrast, NF2 has a much lower birth incidence, with estimates ranging from 1 in 25,000 to 1 in 40,000. Over half of individuals diagnosed with NF1 and NF2 carry de novo mutations and represent the first person in their family with the condition. As NF1 and NF2 are autosomal dominant syndromes with complete penetrance, once an individual has received a diagnosis, that given individual typically harbours a 50:50 chance of transmitting the disorder to their offspring. However, individuals with NF2 can also be mosaic for a mutation in the NF2 gene, resulting in less than a 50% risk of transmission to their offspring.

In a systematic review of death certificates, the mean age of death for individuals with NF1 was 54.4 years (versus 70.1 years in the general population) with death from malignancy disproportionately high among individuals that died before the age of 30. Similarly, individuals with NF2 have a reduced life expectancy from disease-related causes. In a recent observational study, the estimated life expectancy for individuals with NF2 was 69.0 years compared to a life expectancy of 71.5 years for individuals with NF1 and 80.0 years for the general population.

Prior to the 1990s, the treatment of NF-associated tumours was largely managed by surgeons and oncologists in an identical fashion to histologically-similar tumours arising in the general population. With the cloning of the NF1 and NF2 genes, the generation of preclinical mouse models for many of the NF-associated tumour types, and the establishment of a clinical trials consortium, there have been enormous advances in the therapeutic options available for affected individuals. In this Review, we discuss the clinical features, genetic aetiologies, and pathogenesis of neoplasia in NF1 and NF2. We also describe the current treatment options for patients with NF1 and NF2, and advances in clinical and preclinical science that will impact on the future treatment of these two hereditary tumour predisposition syndromes.

Clinical features of NF1

Individuals with NF1 have a predisposition to the development of Schwann cell neoplasms (neurofibromas, malignant peripheral nerve sheath tumours), gliomas (optic pathway gliomas, malignant gliomas), leukaemia, pheochromocytoma, and several other tumours (such as gastrointestinal stromal tumours, rhabdomyosarcoma, and breast cancer; Box 1 and Figure 1).

Neurofibromas, the most common type of tumour associated with NF1, are benign peripheral nerve sheath tumours (PNSTs) that arise from Schwann cell progenitors,
embedded in a microenvironment composed of perineurial cells, fibroblasts, mast cells and a rich collagenous extracellular matrix (Figure 1a). These benign PNSTs can occur on nerves anywhere in the peripheral nervous system. Cutaneous neurofibromas are the most common type of neurofibroma and can appear as nodular masses, peduncular lesions, or diffuse plaques. These tumours frequently begin to develop during early adolescence and continue to increase in number throughout adulthood. Importantly, cutaneous neurofibromas do not transform into malignant PNSTs (MPNSTs). Internal PNSTs are also very common and are present in as many as 60% of individuals with NF1, where they can present as intra- or plexiform neurofibromas. Intranuclear neurofibromas are fusiform expansions of a peripheral nerve, typically involving the spinal roots, which frequently develop a dumbbell appearance as they enlarge. By contrast, plexiform neurofibromas involve multiple fascicles of a nerve or a plexus and can extend down its branches (Figure 1b). Hence, plexiform neurofibromas may feel like a ‘bag of worms’ on palpation. These tumours are usually detected in young children and grow most rapidly during the first decade of life. These more diffuse PNSTs can exert a mass effect, compressing nearby structures (such as the trachea or blood vessels), and either stimulate bone growth or lead to bone erosion. PNSTs can also cause spinal cord compression, weakness, cranial neuropathy, disfigurement, and pain. Moreover, these tumours have a rich vascular network and can bleed profusely, especially during surgery.

Unlike cutaneous neurofibromas, plexiform neurofibromas can undergo malignant transformation into MPNSTs—an aggressive spindle-cell sarcoma. The lifetime risk for individuals with NF1 of developing a MPNST is between 8% and 13%, with a peak incidence in their mid-30s. MPNSTs are difficult to treat and can be widely metastatic to the lungs, soft tissue, and bone. Unfortunately, current therapies have shown little long-term benefit, and most individuals succumb to these cancers within 5 years following diagnosis.

Individuals with NF1 are also prone to the development of gliomas, most commonly pilocytic astrocytomas (WHO grade I) involving the optic pathway (optic pathway glioma [OPG]; Figure 1c). These tumours are predominantly observed in children younger than 7 years of age, with a mean age at presentation of 4.2 years. They can affect one or both optic nerves, the chiasm, or the postchiasmal optic tracks. Bilateral optic nerve involvement is common in children with NF1-OPG. Although the incidence of OPG in patients with NF1 is 15–20%, fewer than half of these tumours cause symptoms. When symptomatic, children typically present with decreased vision or precocious puberty secondary to tumour infiltration of the hypothalamus. In addition to OPGs, gliomas can develop in the brainstem. Brainstem gliomas occur with an estimated lifetime incidence of 4% or higher, and typically become symptomatic in children younger than 10 years of age. Many of these tumours occur in the medulla with occasional extension into the pons. They can result in brainstem dysfunction, including dysarthria, cranial neuropathies and incoordination, and can compromise the ventricular system, resulting in hydrocephalus and headache.

Most often, these tumours are pilocytic astrocytomas or, less commonly, grade II astrocytomas. In rare circumstances, cerebellar gliomas are observed, but when detected, these are typically pilocytic astrocytomas, which are usually asymptomatic at the time of diagnosis. Although the majority of brain tumours in this patient population are low-grade gliomas (astrocytomas) that occur during the first decade of life, adults with NF1 are at higher risk of developing high-grade tumours (WHO grade III and IV). Compared to the general population, adults with NF1 are 50 to 100 times more likely to develop a symptomatic non-optic pathway brain tumour. The majority of these tumours arise in the cerebral hemispheres (more frequently within cortical than subcortical areas), and only a minority are pilocytic astrocytomas. In this regard, 40% of non-optic pathway tumours reported in one cohort were grade II, 27% were grade III, and 13% were grade IV. The high-grade tumours (WHO grade III and IV) occurred in patients older than 20 years of age.

Other less-common cancers that occur in individuals with NF1 include leukaemia (juvenile chronic myelogenous leukaemia or myelodysplastic syndrome), rhabdomyosarcoma, gastrointestinal stromal tumours, and pheochromocytoma. Pheochromocytomas deserve particular mention owing to their association with unexplained hypertension in this at-risk population. These endocrine tumours generate excess catecholamines, leading to dramatic changes in blood pressure. It has also been shown that women younger than 50 years of age with NF1 have a fivefold increased risk of breast cancer. Secondary malignancies, such as MPNST or malignant glioma, are also reported in individuals with NF1, typically

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**Box 1 | Tumours arising in individuals with NF1 and their frequency**

<table>
<thead>
<tr>
<th>Tumours arising in individuals with NF1 and their frequency</th>
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<tbody>
<tr>
<td>Cutaneous neurofibroma: frequency 40–60%</td>
</tr>
<tr>
<td>Malignant peripheral nerve sheath tumour: frequency 8–13%</td>
</tr>
<tr>
<td>Malignant glioma: frequency 0.8%</td>
</tr>
<tr>
<td>Pheochromocytoma: frequency 0.1–1.3%</td>
</tr>
<tr>
<td>GIST: frequency 5–30%</td>
</tr>
</tbody>
</table>

Abbreviations: GIST, gastrointestinal stromal tumour; JMML, juvenile myelomonocytic leukaemia; NF1, neurofibromatosis type 1.
**Clinical features of NF2**

Tumours arising in individuals with NF2 are distinct from those seen in NF1 (Box 2, Figure 2). Vestibular schwannomas (VS) are a hallmark of NF2 and are present in >95% of individuals with this condition (Box 2; Figure 2a).43,44 Previously called acoustic neuromas, the name of these neoplasms was changed to reflect their Schwann cell lineage origins and the primary involvement of the vestibular, rather than the cochlear, branch of the eighth cranial nerve.44 Although the vast majority of unilateral VS occur sporadically, it is rare for an individual without NF2 to develop bilateral VS.7 Histologically, NF2-associated VS tend to be more lobular and less vascular relative to their sporadic counterparts, and become symptomatic earlier in life.34,54 In individuals with NF2, 44% of affected persons who become symptomatic as adults present with symptoms attributable to VS, including hearing loss, tinnitus, and vestibulopathy.43 Over time, progressive growth of these cranial nerve tumours can cause brainstem compression. When individuals with NF2 present as children, they are less likely to become symptomatic from an underlying VS; rather, they are more likely to become symptomatic as a result of mass effect from an intracranial or spinal cord lesion, a peripheral nerve tumour, or a cutaneous lesion.45

Over their lifetime, 24 to 51% of individuals with NF2 will develop a schwannoma of another cranial nerve in addition to VS, most commonly along the fifth, seventh, ninth, or twelfth cranial nerves.34,49 Schwannomas also develop near the spinal cord and along peripheral nerves, resulting in peripheral neuropathies, appearing as cutaneous tumours in 59–68% of individuals with NF2.43,48 Schwannomatosis49 is a syndrome that shares some clinical features with NF2, but is not caused by germline mutations in the NF2 gene. Instead, these tumors arise in individuals with mutations in the SMARCB1 tumour suppressor gene.51 Individuals with this condition can also develop multiple schwannomas, but in contrast to individuals with NF2, they rarely develop VS.52

Meningiomas occur in 45–58% of persons with NF2 (Box 2, Figure 2b).43,44 NF2-associated meningiomas tend to occur at an earlier age than sporadic meningiomas (during the third and fourth decade of life versus the middle of the sixth decade), and individuals with NF2 are prone to the development of multiple meningiomas.43,53 Meningiomas can affect the spinal cord and are difficult to distinguish from other spinal tumours arising in individuals with NF2. They may develop along the optic nerve, which can appear on neuroimaging studies as optic gliomas arising in the context of NF1.44

Finally, ependymomas are also observed in persons with NF2. These are intramedullary glial cell neoplasms most often involving the cervical cord (Box 2, Figure 2c).35,56 The frequency of these tumours in the NF2 population is unclear; however, estimates range from 33% to 53% of affected individuals.56 Fortunately, only a minority of these tumours cause symptoms and require treatment.

**Genetics of NF1 and NF2**

The genes mutated in NF1 and NF2 are tumour suppressor genes, which encode for proteins involved in divergent signalling pathways (Figure 3). While individuals with NF1 and NF2 start life with one functional copy of NF1 and NF2 genes in every cell of their bodies (with the exception of those with mosaic forms of NF1 or NF2), NF1-associated and NF2-associated tumours exhibit total loss of the protein products encoded by the respective genes.57–59

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**Box 2 | Tumour frequency arising in individuals with NF2**

<table>
<thead>
<tr>
<th>Tumour Type</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vestibular schwannoma</td>
<td>&gt;95%43,44</td>
</tr>
<tr>
<td>Other cranial nerve schwannoma</td>
<td>24–51%46</td>
</tr>
<tr>
<td>Cutaneous schwannoma</td>
<td>59–68%43,48</td>
</tr>
<tr>
<td>Intracranial meningioma</td>
<td>50%43</td>
</tr>
<tr>
<td>Peripheral nerve schwannoma</td>
<td>42%15</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>33–53%56</td>
</tr>
<tr>
<td>Mesothelioma, associated with asbestos exposure</td>
<td>124,125</td>
</tr>
<tr>
<td>Malignant schwannoma</td>
<td>rare126</td>
</tr>
</tbody>
</table>

Abbreviation: NF2, neurofibromatosis type 2.
The NF1 gene resides on chromosome 17 and codes for neurofibromin. Neurofibromin is a 220 kDa cytoplasmic protein containing a region of 300 amino acids with significant homology to domains found in GTPase-activating proteins (GAPs).\textsuperscript{60-62} GAPs comprise a family of proteins that function as negative regulators of the RAS proto-oncogene, an important regulator of cell proliferation, differentiation, apoptosis and migration.\textsuperscript{63} Ras cycles between one of two states—an active GTP-bound conformation and an inactive GDP-bound conformation. Interaction with neurofibromin accelerates the intrinsic GTPase activity of Ras, leading to Ras inactivation and reduced cell growth (Figure 3).\textsuperscript{62,64,65} In NF1-associated tumours, loss of neurofibromin results in high levels of activated Ras, leading to increased cell growth.\textsuperscript{53,63,67} NF1 loss leads to hyperactivation of the downstream effector proteins that transduce Ras signalling, including the mammalian target of rapamycin (mTOR) and mitogen-activated kinase kinase (MEK) signalling intermediates.\textsuperscript{68,69} In addition to its role in the negative regulation of Ras, neurofibromin is a positive regulator of adenylyl cyclase, the enzyme responsible for the generation of intracellular cyclic AMP (cAMP).\textsuperscript{70,71} In some cell types, including neurons, reduced neurofibromin levels lead to decreased intracellular cAMP levels and attenuated cell survival.\textsuperscript{72}

The NF2 tumour suppressor gene is located on human chromosome 22q and encodes another cytoplasmic protein called merlin (or schwannomin). Merlin is a 593-amino-acid protein with structural similarity to proteins of the Band 4.1 family.\textsuperscript{73,74} The shared band 4.1 domain spans 300 amino acids at the N-terminus of merlin, where it likely facilitates interactions with membrane-associated molecular partners, including SCHIP1 and fodrin (β2-spectrin).\textsuperscript{75} Merlin regulates the activation of several growth factor receptors (the EGFR and HER2 receptors) and suppresses the Rac1, mTOR, and Hippo/YAP intracellular mitogenic signalling pathways.\textsuperscript{76-79} Loss of merlin results in the increased activation of a number of these growth control signalling pathways (Figure 3), although there is currently no consensus as to which pathway is most important for tumorigenesis in any given tissue.

**Figure 2** Tumours arising in individuals with NF2. a | MRI shows bilateral vestibular schwannomas (asterisks) in a teenage girl with NF2. b | MRI shows a meningioma (asterisk) in a young girl with NF2. c | MRI shows multiple intraparenchymal spinal tumours, most likely ependymomas, in a young woman with NF2. Abbreviation: NF2, neurofibromatosis type 2.

**Treatment of NF-associated neoplasms**

Until recently, treatments for tumours arising in individuals with NF1 and NF2 were similar to those employed for histologically-similar tumours encountered in the general population. In the case of NF1, current therapies for cutaneous neurofibromas that cause disfigurement include surgery and, in some instances, CO2 laser treatment or electrodessication.\textsuperscript{80} Plexiform neurofibromas are largely debulked when clinically indicated, although their infiltrative nature presents significant challenges during surgery, and some patients will experience nerve damage or significant haemorrhage.\textsuperscript{80} As a result of advances in the understanding of NF1 at the molecular and cellular level, new investigational agents are now being evaluated in clinical trials (Table 1). One such promising therapeutic agent is imatinib, which was recently shown in a small phase II study to reduce plexiform neurofibroma growth by ≥20% in 17% of treated individuals.\textsuperscript{81} These individuals exhibiting therapeutic responses harboured plexiform neurofibromas in the neck and pelvic regions, and these results prompted a planned clinical trial focused specifically on imatinib treatment in this subgroup of NF1-affected individuals.

Malignant transformation of plexiform neurofibromas into MPNSTs is a significant problem in individuals with NF1 and is a leading cause of death. Individuals with NF1-associated plexiform neurofibromas must be monitored for a change in tumour growth and for signs and symptoms of transformation, including the development of pain, neurological deficit (weakness), or constitutional symptoms (weight loss, night sweats). While MRI can define the anatomic location and extent of a PNST, it does not provide accurate information regarding malignant transformation.\textsuperscript{82} FDG-PET is a helpful imaging modality for distinguishing benign PNSTs from malignant PNSTs (Figure 1d).\textsuperscript{83} In several studies, metabolically-active tumours with FDG standard uptake values greater than 4 were most often MPNSTs.\textsuperscript{84,85} Likewise, chest CT scans are useful for detection of metastatic disease, particularly when tumours spread to the lungs.\textsuperscript{86} Treatment of a MPNST involves surgical resection with subsequent
Neurofibromin and merlin growth control pathways. Neurofibromin accelerates the conversion of active Ras to inactive Ras. Ras in its active conformation increases cell growth by activating PI3K/AKT/mTOR signalling as well as increased Raf kinase and MEK signalling. In addition, neurofibromin functions as a positive regulator of AC to increase cAMP levels and inhibit cell growth. Merlin has been implicated as a negative regulator of Rac1, mTOR, and Hippo/YAP signalling, the activation of which leads to increased cell growth. Abbreviations: AC, adenyl cyclase; AKT, protein kinase B; cAMP, cyclic adenosine monophosphate; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; RTK, receptor tyrosine kinase; YAP, yes-associated protein.

**Figure 3** Neurofibromin and merlin growth control pathways. Neurofibromin accelerates the conversion of active Ras to inactive Ras. Ras in its active conformation increases cell growth by activating PI3K/AKT/mTOR signalling as well as increased Raf kinase and MEK signalling. In addition, neurofibromin functions as a positive regulator of AC to increase cAMP levels and inhibit cell growth. Merlin has been implicated as a negative regulator of Rac1, mTOR, and Hippo/YAP signalling, the activation of which leads to increased cell growth. Abbreviations: AC, adenyl cyclase; AKT, protein kinase B; cAMP, cyclic adenosine monophosphate; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; RTK, receptor tyrosine kinase; YAP, yes-associated protein.

**Table 1** Clinical trials for NF-associated tumours

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cellular or molecular target</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neurofibromatosis type 1</strong></td>
<td>pharmacologically</td>
<td></td>
</tr>
<tr>
<td>Thalidomide</td>
<td>Angiogenesis</td>
<td>Gupta et al. (2003)</td>
</tr>
<tr>
<td>13-cis-retinoic acid</td>
<td>(CRA) or interferon α-2a</td>
<td>Differences, angiogenesis</td>
</tr>
<tr>
<td>Pirfenidone</td>
<td>Tumour-associated fibroblasts</td>
<td>Babovic-Vukanovic et al. (2006)</td>
</tr>
<tr>
<td>Photodynamic therapy</td>
<td>Tumour tissue disruption</td>
<td>Kissil et al. (2010)</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>mTOR</td>
<td>Widemann et al. (2010)</td>
</tr>
<tr>
<td>Pegylated-interferon α-2b</td>
<td>Immune modulator and</td>
<td>Jakacki et al. (2011)</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>angiogenesis</td>
<td>Kim et al. (2013)</td>
</tr>
<tr>
<td>Imatinib</td>
<td>c-kit, PDGFRβ</td>
<td>Roberts et al. (2012)</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>EGFR</td>
<td>Albritton et al. (2006)</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>Dual inhibitor of Src/Abl</td>
<td>Schuette et al. (2009)</td>
</tr>
<tr>
<td>Topical rapamycin</td>
<td>mTOR</td>
<td>Koenig et al. (2012)</td>
</tr>
<tr>
<td><strong>Neurofibromatosis type 2</strong></td>
<td>pharmacologically</td>
<td></td>
</tr>
<tr>
<td>Lapatinib</td>
<td>EGFR/erbB2</td>
<td>Karajannis et al. (2012)</td>
</tr>
</tbody>
</table>

Abbreviation: mTOR, mammalian target of rapamycin.

The treatment of NF2-associated neoplasms predominantly entails conservative monitoring and, when appropriate, surgical intervention. In the case of VS, the surgeon aims to achieve a complete resection; unfortunately, this is often not possible owing to adherence of the tumour to the facial nerve or, rarely, to the brainstem, and instead, deliberate incomplete resections may be performed to preserve nerve function. The decision to operate requires the coordinated efforts of multiple medical services (otolaryngology, neurosurgery, medical oncology) to best determine when surgery is most likely to preserve hearing and result in minimal potential secondary consequences. In some centres, VS may also be treated with stereotactic radiosurgery. Hearing preservation has been reported in 40% of patients with NF2 3 years after radiosurgery to treat VS. However, similar to conventional surgery, radiosurgery can also result in facial and trigeminal cranial nerve dysfunction. Bevacizumab, an anti-VEGF monoclonal antibody, has emerged as a first-line treatment for VS in individuals with NF2. In the largest case series to date, 57% of individuals had a hearing response (improvement in word recognition), and 55% exhibited a radiographic response (20% decrease in tumour volume) following bevacizumab treatment. At 3 years, 61% had stable or improved hearing and 54% had stable or decreased tumour size. A phase II clinical trial (NCT01767792) is currently underway to further evaluate the use of bevacizumab for this indication. In another study, adults and children with NF2 and progressive VS were treated with lapatinib, a dual inhibitor of EGFR and HER2, with some success. In this study of radiotherapy and chemotherapy. The best outcomes are achieved following radical excision of the tumour with wide surgical margins. When chemotherapy is employed, doxorubicin and ifosfamide have been shown to be effective; however, there is currently no standard care for these deadly cancers. Unfortunately, long-term survival is rare when MPNST occurs in patients with NF1 because of lung and bone metastases as well as local tumour recurrence.

Another important consideration when managing patients with NF1 is the occurrence of OPG. Since the majority of NF1-associated OPGs are asymptomatic, surveillance in children younger than 12 years of age entails annual examinations by an experienced ophthalmologist. Age-appropriate visual screening tools should be employed, since vision loss typically occurs in young preverbal children. Screening neuroimaging is not recommended, as early identification of an OPG does not improve clinical outcome. When a patient with NF1-associated OPG exhibits clinical progression, as evidenced by declining vision or precocious puberty, treatment with chemotherapy is usually initiated. The typical first-line treatment is combination therapy with carboplatin and vincristine, although other alkylating agents (such as vinblastine) are sometimes used. Surgery is usually reserved for individuals with unilateral optic gliomas causing proptosis and a blind eye. Radiation therapy is not employed in individuals with NF1 because of the increased risk for radiation-induced secondary malignancies, the majority of which are high-grade gliomas. A retrospective study found a threefold increase in the relative risk of developing a second nervous system malignancy among 18 patients with optic gliomas treated with radiotherapy compared to 40 patients who did not receive radiation.
Surgery is the primary treatment modality for symptomatic and progressively enlarging NF2-associated ependymomas, non-vestibular schwannomas, and meningiomas. Mortality following surgery for meningiomas has been estimated to be two-to-three times higher in individuals with NF2 compared to the general population, since NF2-associated meningiomas tend to be larger at the time of surgery. Effective medical treatments for meningiomas are not yet available, although treatment with stereotactic radiosurgery and biologically-based therapies are currently being explored for these tumours in early phase studies.

### Advances in NF therapeutics

One of the most exciting advances in the field of NF clinical therapeutics was the development and intelligent use of mouse models of NF-associated malignancy. Over the past decade, numerous genetically-engineered mouse (GEM) strains have been developed and used in preclinical therapeutic studies of NF (Table 2). The availability of robust preclinical mouse models not only provides an experimental platform to discover new therapeutic targets for drug design, but also enables the rapid evaluation of new classes of compounds prior to testing in human clinical trials.

GEM strains for NF1-associated cutaneous neurofibroma, plexiform neurofibroma, optic glioma, MPNST, and leukaemia have been developed. Similarly, mouse models of NF2-associated schwannoma and meningioma have also been generated. While each of these models has its limitations, they have provided instructive information to guide the design and execution of human clinical trials. For example, NF1 GEM strains of optic glioma and plexiform neurofibroma have revealed the critical role of non-neoplastic stromal cells (tumour microenvironment) in cancer maintenance. In these studies, mice lacking NF1 expression in Schwann cell or astroglial cell precursors alone do not develop tumours; however, NF1+/− mice (genetically similar to individuals with NF1) with loss of NF1 expression in Schwann cell or astroglial cell precursors form neurofibromas and optic gliomas, respectively. Other studies have established that the mast cell is an important stromal cell type in mouse plexiform neurofibromas as they provide chemokines and growth factors critical for maintaining tumour growth. The identification of mast cells as microenvironmental drivers of plexiform neurofibroma growth led to the evaluation of imatinib, which inhibits c-kit, in preclinical NF1 mouse studies. These studies have now led to human clinical trials of imatinib for plexiform neurofibroma,69,113 rapamycin analogues and chemokine receptor inhibitors for NF1-associated glioma growth led to the evaluation of imatinib, which inhibits c-kit, in preclinical NF1 mouse studies. These studies have now led to human clinical trials of imatinib for plexiform neurofibroma,69,113 rapamycin analogues and chemokine receptor inhibitors for NF1-associated glioma.

### Table 2 | Genetically-engineered mouse models of NF-associated tumours

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Genetic strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1 optic glioma</td>
<td>Nf1flox/flox; GFAP-Cre</td>
<td>Bajenaru et al. (2003)102</td>
</tr>
<tr>
<td></td>
<td>Nf1flox/+; LSL-Kras; GFAP-Cre mice</td>
<td>Zhu et al. (2005)101</td>
</tr>
<tr>
<td>NF1 malignant glioma</td>
<td>Nf1flox/flox; p53flox/flox, Ptenflox/flox mice</td>
<td>Dasgupta et al. (2005)102</td>
</tr>
<tr>
<td></td>
<td>Nf1flox/flox; p53flox/flox mice</td>
<td>Kwon et al. (2008)103</td>
</tr>
<tr>
<td>NF1 cutaneous neurofibroma</td>
<td>Nf1flox/flox; PLP-Cre mice</td>
<td>Mayes et al. (2011)107</td>
</tr>
<tr>
<td>NF1 plexiform neurofibroma</td>
<td>Nf1flox/flox; Plp-Cre mice</td>
<td>Mayes et al. (2011)107</td>
</tr>
<tr>
<td></td>
<td>Nf1flox/flox; Dhh-Cre mice</td>
<td>Wu et al. (2008)98</td>
</tr>
<tr>
<td></td>
<td>Nf1flox/flox; Krox20-Cre mice</td>
<td>Zhu et al. (2002)99</td>
</tr>
<tr>
<td>NF1 MPNST</td>
<td>Nf1flox/+; p53flox/+ mice</td>
<td>Cichowski et al. (1999)103</td>
</tr>
<tr>
<td></td>
<td>Nf1flox/+; Mx1-Cre mice</td>
<td>Vogel et al. (1999)104</td>
</tr>
<tr>
<td>NF1 leukaemia</td>
<td>Nf1flox/flox; Mx1-Cre mice</td>
<td>Le et al. (2004)105</td>
</tr>
<tr>
<td>NF1 pheochromocytoma</td>
<td>Nf1flox/+ mice</td>
<td>Tischler et al. (1995)106</td>
</tr>
<tr>
<td>NF2 schwannoma</td>
<td>Nf2flox/flox mice +Ad-Cre</td>
<td>Giovannini et al. (2000)106</td>
</tr>
<tr>
<td></td>
<td>Nf2flox/flox; PO-Cre mice</td>
<td>Giovannini et al. (1999)107</td>
</tr>
<tr>
<td>NF2 meningioma</td>
<td>Nf2flox/flox; PGDS-Cre mice +Ad-Cre</td>
<td>Kalamardides et al. (2011)108</td>
</tr>
<tr>
<td></td>
<td>Nf2flox/flox mice +Ad-Cre</td>
<td>Kalamardides et al. (2002)109</td>
</tr>
<tr>
<td>NF2 mesothelioma</td>
<td>Nf2flox/flox; p16flox/+ mice +Ad-Cre</td>
<td>Jongsma et al. (2008)110</td>
</tr>
</tbody>
</table>

Abbreviations: MPNST, malignant peripheral nerve sheath tumour; NF1, neurofibromatosis type 1; NF2, neurofibromatosis type 2.

### Figure 4 | Targets for NF therapeutic drug design. a | Therapies that target either the tumour microenvironment (such as endothelial cells, mast cells, microglia) or the abnormal neurofibromin-controlled growth regulatory pathways in NF1-deficient cancer cells have been developed for evaluation in both preclinical mouse models and in human clinical trials. b | Similarly, therapies that target either the tumour microenvironment (endothelial cells) or the abnormal merlin-controlled growth regulator pathways in NF2-deficient cancer cells have been developed for evaluation in both preclinical mouse strains and in human clinical trials. Abbreviation: PEG-IFN-α-2b, pegylated interferon alpha-2b.
and MPNST,114–117 and the tyrosine kinase inhibitors nilotinib and lapatinib for NF2-associated VS.118,119
While GEM models have great potential for clinical translation, it is important to establish standards for the interpretation of results from mouse preclinical studies. Attention should be paid to the number of mice exhibiting radiographic responses, the durability of these responses, and the magnitude of tumour shrinkage. Pharmacokinetic and pharmacodynamic effects in these mouse models should also be taken into consideration when designing clinical trials.

A second, but equally critical, advance in NF clinical therapeutics was the establishment of the Department of Defense-sponsored Neurofibromatosis Clinical Trials Consortium (NFCTC).120 This consortium was developed to address the issues associated with conducting efficient therapeutic trials in NF where there is inherent clinical variability between patients and insufficient numbers of affected individuals at any one single institution. The mission of the NFCTC is to create an infrastructure to accelerate the development and completion of biologically-informed, statistically-sound translational clinical trials for adults and children with NF1 and NF2. To date, this consortium has initiated several clinical trials, including sorafenib for NF1-associated plexiform neurofibromas (NCT00727233),121 bevacizumab and everolimus for MPNST (NCT01661283),122 and everolimus for progressive NF1-associated glioma (NCT01158651).123 Future early phase studies will entail the critical evaluation of bevacizumab for NF2-associated VS and additional Ras effector targeted therapies for NF1-associated plexiform neurofibroma.124

Conclusions
The tumours that develop in individuals with NF1 and NF2 predisposition syndromes are molecularly and cellularly heterogeneous neoplasms, representing complex cancers in which distinct cell types and control pathways contribute to continued tumour growth (Figure 4). With the development of robust preclinical mouse models for many of the common tumour types in NF1 and NF2, coupled with the availability of a clinical trials infrastructure for rapid drug evaluation in people, unique opportunities for translational medicine have emerged, which will increasingly inform the future management of patients with NF.

Review criteria
Information for this Review was compiled by searching the PubMed database for articles published before March 2013. Search terms included “neurofibromatosis”, “NF1”, and “NF2”. Full articles were reviewed for additional material when appropriate, and articles that cited key references were also reviewed. Relevant clinical trials were identified by searching http://clinicaltrials.gov in the same manner.


Author contributions
Both authors researched the data for the article, made substantial contributions to the discussion of the content, wrote the article and reviewed and edited it prior to submission.
Eliminating barriers to personalized medicine
Learning from neurofibromatosis type 1

ABSTRACT

With the emergence of high-throughput discovery platforms, robust preclinical small-animal models, and efficient clinical trial pipelines, it is becoming possible to envision a time when the treatment of human neurologic diseases will become personalized. The emergence of precision medicine will require the identification of subgroups of patients most likely to respond to specific biologically based therapies. This stratification only becomes possible when the determinants that contribute to disease heterogeneity become more fully elucidated. This review discusses the defining factors that underlie disease heterogeneity relevant to the potential for individualized brain tumor (optic pathway glioma) treatments arising in the common single-gene cancer predisposition syndrome, neurofibromatosis type 1 (NF1). In this regard, NF1 is posited as a model genetic condition to establish a workable paradigm for actualizing precision therapeutics for other neurologic disorders.

GLOSSARY

cAMP = cyclic adenosine monophosphate; GEM = genetically engineered mouse; GWAS = genome-wide association studies; mTOR = mammalian target of rapamycin; NF1 = neurofibromatosis type 1; NSC = neural stem cell; OPG = optic pathway glioma; PA = pilocytic astrocytoma; RGC = retinal ganglion cell.

Neurofibromatosis type 1 (NF1) is one of the most common monogenic disorders in which affected individuals develop benign and malignant tumors.1 NF1 impacts 1:2,500 people worldwide, and individuals with NF1 are prone to the development of peripheral (neurofibromas, malignant peripheral nerve sheath tumors) and central (optic pathway glioma, malignant glioma) nervous system tumors.2,3 Similar to other autosomal dominant cancer predisposition syndromes,4,5 people with NF1 start life with a germline mutation in one copy of the NF1 tumor suppressor gene; however, tumors require somatic (acquired) inactivation of the remaining functional NF1 allele, leading to complete loss of NF1 expression in specific cell types.6,7 For example, complete Nf1 gene inactivation in neuroglial8,9 or Schwann cell10,11 progenitors is required for murine optic glioma or neurofibroma formation, respectively.

With the development of numerous accurate small-animal (genetically engineered mouse; GEM) models of NF1-associated nervous system tumors (table 1),8–18 the creation of the NF Clinical Trials Consortium,19 and the establishment of response criteria for NF1 clinical trials,20 the stage has been set for the discovery and validation of promising therapeutic strategies and their translation to people affected with NF1. However, despite these advances, there are currently no effective therapies, which likely reflects the striking biological and clinical heterogeneity inherent to this condition. This review uses NF1-associated brain tumors (optic glioma) as an illustrative platform to discuss the barriers and challenges to developing and implementing effective targeted therapies.

NF1-ASSOCIATED OPTIC PATHWAY GLIOMA

NF1-associated optic pathway gliomas (NF1-OPGs) are largely pediatric tumors typically arising in children younger than 7 years of age.21,22 As such, 15%–20% of children with NF1 will develop World Health Organization grade I pilocytic astrocytomas (PAs) anywhere along the optic pathway, from the retro-orbital optic nerve to the postchiasmatic optic tracts (figure 1). In addition to neoplastic glial cells, 30%–50% of the cells in these tumors are non-neoplastic cells (microglia).
Abbreviations: GEM = genetically engineered mouse; MPNST = malignant peripheral nerve sheath tumor.

Table 1  
Nf1 GEM nervous system tumor models

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Genetic strain</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Optic glioma</td>
<td>Nf1&lt;sup&gt;lox/lox&lt;/sup&gt;; GFAP-Cre</td>
<td>8,9</td>
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<tr>
<td>Malignant glioma</td>
<td>Nf1&lt;sup&gt;lox/lox&lt;/sup&gt;; p53&lt;sup&gt;-/-&lt;/sup&gt;</td>
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<td></td>
<td>Nf1&lt;sup&gt;lox/lox&lt;/sup&gt;; p53&lt;sup&gt;lox/lox&lt;/sup&gt;, nestin-Cre&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>15</td>
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<tr>
<td>Dermal neurofibroma</td>
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<td>Plexiform neurofibroma</td>
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<td>12</td>
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<td>10</td>
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<tr>
<td></td>
<td>Nf1&lt;sup&gt;lox/lox&lt;/sup&gt;; Dhh-Cre</td>
<td>13</td>
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<tr>
<td>MPNST</td>
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<td>16,17</td>
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<td></td>
<td>Nf1&lt;sup&gt;lox/lox&lt;/sup&gt;; Pten&lt;sup&gt;lox/lox&lt;/sup&gt;; Dhh-Cre</td>
<td>18</td>
</tr>
</tbody>
</table>

Abbreviations: GEM = genetically engineered mouse; MPNST = malignant peripheral nerve sheath tumor.

haboring one functional copy of the NFI gene and one nonfunctional NFI allele (germline NFI gene mutation).23

Since baseline MRI is not useful in predicting clinical outcome,24 it is currently not possible to identify children at greatest risk for developing NFI-OPG. In addition, following NFI-OPG identification, there are no reliable methods for determining which child is most likely to experience continued visual decline and require treatment, necessitating frequent MRI evaluations, which can be unreliable in preverbal children.25,26 At present, affected children typically receive chemotherapy routinely used for sporadic PA (carboplatin/vincristine), with varying success. Radiation therapy is not used because of the elevated risk of secondary cancer development in this cancer predisposition syndrome.27 Despite radiographic evidence of tumor stabilization or response following chemotherapy, only a minority of children with NFI-OPG experience improvements in vision.28,29 In addition, there is increasing concern that chemotherapy in this young age group may result in cognitive decline as a late secondary sequelae, negatively impacting long-term quality of life.30 Collectively, these issues highlight the pressing need to develop management strategies that reflect the unique biology of NFI-OPG.

USE OF NFI GEM STRAINS TO UNDERSTAND NFI-OPG PATHOGENESIS

There are numerous important unanswered questions relevant to the care of children with NFI-OPG that may lead to the improved management of these brain tumors, ranging from explaining the unique spatial and temporal distribution of these tumors to understanding the cellular and molecular constraints that drive glioma formation and progression. Ideally, these answers would emanate from discovery efforts using human tissue specimens; however, in the case of NFI-OPG, few patients undergo tumor biopsy or removal prior to or following treatment. Moreover, many of the available NFI brain tumor specimens represent tumors with unusual features, such as those arising in older children or in brain regions other than the optic pathway,15 and may not be representative of the more commonly encountered OPGs in children with NFI.

In light of the above challenges with human tissue specimens, NFI GEM strains have been developed that model the optic gliomas arising in children with NFI1,9,17,32 While not perfect representations of the human condition, NFI optic glioma mice have yielded several unanticipated observations critical to the future design and execution of clinical trials for these tumors. It should be appreciated that these findings were only made possible through the use of NFI GEM strains.

Understanding the unique temporal and spatial pattern of NFI-OPG. To address the question of why NFI gliomas display a propensity for the optic pathway and brainstem of young children, researchers used a series of NFI mutant mice in which somatic NFI gene inactivation could be experimentally manipulated. Murine optic gliomas require complete NFI gene inactivation in specific neural progenitors (neural stem cells; NSCs) during mid to late embryogenesis, thus creating a narrow developmental window in which tumor initiation must occur.52,53 The ability of these NSCs to generate gliomas following NFI loss during embryonic development, but not in more differentiated glial cell types after birth, likely accounts for the predilection for these tumors to arise in young children.

Second, NSCs from some brain regions, but not others, increase their growth following NFI gene inactivation. In these studies, NFI gene inactivation in NSCs residing in the third ventricle or brainstem results in stem cell expansion and increased glial differentiation, whereas no effect is observed following NFI loss in cortical or lateral ventricle NSCs.52,54 These findings partly explain the distinct brain region distribution of these tumors (optic pathway/brainstem but rarely in the cortex).

Identifying NFI-OPG targeted therapies. Further analysis of the molecular basis underlying the above brain region– and cell type–specific effects revealed that the impact of NFI gene inactivation is dependent on how the downstream RAS signaling pathway functions in specific cell types. The NFI gene codes for the protein neurofibromin, which contains a 300 amino acid domain similar to other proteins that function as negative regulators of the RAS proto-oncogene.55 While neurofibromin controls cell growth in numerous different cell types in a RAS-dependent manner,56,57
there are several distinct molecules that transmit the RAS growth signal, including MEK and AKT. Whereas RAS/MEK signaling is critical for murine Nf1 plexiform neurofibroma growth,38 Nf1 loss uniquely affects NSCs from the third ventricle and brainstem by activating AKT in a mammalian target of rapamycin (mTOR)–regulated manner,34 thus identifying the mTOR pathway as a key mediator of NF1-OPG growth. This cell type–specific growth dependence led to the evaluation of rapamycin, initially in preclinical mouse optic glioma models39 and then in a clinical trial for children with NF1-associated glioma (ClinicalTrials.gov identifier NCT01158651).

The key role of the microenvironment in tumor development and growth is further underscored by analogous observations involving mast cells and macrophages in Nf1 GEM plexiform neurofibromas.46 Similar to Nf1 murine optic gliomas, Nf1 plexiform neurofibroma formation requires that Nf1 loss in Schwann cell precursors occur in mice with a germline inactivating Nf1 gene mutation (Nf1+/− mice).10 Using bone marrow transplantation, Nf1+/− bone marrow–derived mast cells, but not those from normal mice, induce plexiform neurofibroma development in normal mice with Nf1 loss in Schwann cell precursors only.47 Moreover, the replacement of Nf1+/− bone marrow–derived mast cells with normal ones reduced plexiform neurofibroma growth in Nf1+/− mice with Nf1 loss in Schwann cell precursors. The dependence on mast cells led to the identification of the KIT receptor as a potential target for antitumoral therapy. In addition to promoting mast cell infiltration relevant to plexiform neurofibroma growth, KIT is also critical for tumor macrophage accumulation.48 As such, blocking KIT activation in Nf1 plexiform neurofibroma–bearing mice with imatinib resulted in attenuation of tumor growth, which is currently being explored further in human clinical trials.

Defining the cellular and molecular basis for NF1-OPG–associated visual loss. One of the major disappointments has been the limited visual recovery in children following treatment with chemotherapy for clinically progressive NF1-OPG. Using Nf1 optic glioma mice, visual impairment was shown to result from
Another genomic factor that might influence NF1-OPG is the sex of the patient. As such, one exciting observation to emerge from \( Nf1 \) GEM studies is the impact of sex on NF1-OPG-induced vision loss. In \( Nf1 \) optic glioma mice, reduced CAMP levels and higher levels of RGC apoptosis are observed in females, leading to reduced visual acuity in female mice only.\(^5\) Moreover, while the frequency of OPG development is similar in girls and boys with NF1, 3 times more females with NF1-OPG require treatment for visual decline.\(^5\) These findings support a role for sex as a critical factor that underlies clinical outcomes in children with NF1-OPG.

**TRANSLATING BASIC SCIENCE DISCOVERIES TO HUMAN THERAPEUTIC CLINICAL TRIALS** Despite these exciting advances in molecular/cellular biology and the ability to efficiently evaluate drugs in accurate preclinical models prior to human clinical trials, there are presently no effective treatments for most NF1-associated tumors. In part, this may reflect the manner in which \( Nf1 \) GEM preclinical drug study results are translated to the clinical workplace. As such, biologically based therapies frequently exhibit dramatic antitumoral responses in mice, but limited tumor shrinkage in human clinical trials. For example, imatinib was shown to be highly effective at attenuating \( Nf1 \) GEM plexiform neurofibroma growth,\(^47\) but it exhibited far less efficacy for treating human NF1-associated plexiform neurofibromas (<20% tumor response).\(^54\) Future preclinical studies will need to incorporate outcome expectations (e.g., number of mice exhibiting a response, percent of tumor reduction, and the durability of the effect) that parallel those employed in human clinical trials.

There are significant differences inherent in \( Nf1 \) GEM preclinical and NF1 patient clinical trials (table 2). Taking NF1-OPG as an illustrative example, human NF1 clinical trials do not involve the collection of pathologic specimens and they enroll patients of different ages, sexes, and tumor locations. As such, the genomic background, the timing of the somatic \( Nf1 \) gene inactivation event, and the specific germline \( Nf1 \) gene mutation vary from patient to patient. For this reason, the clinical outcomes reflect the fact that the patients enrolled in these trials constitute a diverse population of distinct disease subgroups, likely defined by the factors that influence NF1-OPG heterogeneity, with potentially different molecular drivers of tumor growth and druggable therapeutic targets. In addition, the lack of pathologic specimens prevents assessments of CNS drug penetration and target inhibition in the tumor, causing investigators to rely solely on changes in radiographic appearance (tumor volume) and visual improvement. Since tumor volume does not correlate with visual improvement and vision changes may require months

**Table 2**: Comparison of preclinical and clinical studies

<table>
<thead>
<tr>
<th>Factor</th>
<th>Human NF1-OPG</th>
<th>Mouse NF1 optic glioma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathologic specimens</td>
<td>Uncommonly acquired</td>
<td>Always acquired</td>
</tr>
<tr>
<td>Age and sex</td>
<td>Variable</td>
<td>Specific age or sex</td>
</tr>
<tr>
<td>Tumor locations</td>
<td>Variable</td>
<td>One location</td>
</tr>
<tr>
<td>Germline NF1 gene mutation</td>
<td>Variety of mutations</td>
<td>One specific targeted mutation</td>
</tr>
<tr>
<td>Genomic background</td>
<td>Heterogeneous</td>
<td>Inbred genetic background</td>
</tr>
<tr>
<td>Potential therapeutic targets</td>
<td>Many targets</td>
<td>Limited targets</td>
</tr>
</tbody>
</table>

Abbreviations: NF1 = neurofibromatosis type 1; OPG = optic pathway glioma.

Tumor-induced retinal ganglion cell (RGC) axonal dysfunction, leading to RGC death by apoptosis.\(^49\) The mechanism underlying this neuronal death involves neurofibromin regulation of cyclic adenosine monophosphate (cAMP) production, such that restoring normal cAMP homeostasis in \( Nf1 \) optic glioma mice partially ameliorates the RGC apoptosis.\(^50\) This finding raises intriguing questions about the role of neuroprotective approaches in reversing visual loss in children with NF1-OPG.

**Identifying genomic factors that influence NF1-OPG development and progression.** The low incidence of NF1-OPG could reflect the factors discussed above; however, it is also possible that genomic variation contributes to glioma development. As such, defining the genomic determinants that favor optic gliomagenesis has potential value for predictive risk assessment. In this regard, many \( Nf1 \) GEM studies employ mice maintained on a specific inbred genetic background, whereas others use mice on a mixed background. Humans by definition harbor a mixed genomic composition that reflects subtle chromosomal polymorphisms inherited from each of our parents. Studies have begun to unravel the genomic contributions to tumorigenesis in \( Nf1 \) GEM strains: whereas \( Nf1^{+/+} / -p53^{+/-} \) mice develop high-grade astrocytomas (gliomas) when maintained on a C57BL/6 background, \( Nf1^{+/+} / -p53^{+/-} \) mice maintained on a 129sv background exhibit a significantly reduced frequency of glioma formation.\(^51,52\) Similarly, \( >90\% \) of \( Nf1^{lox/lox}; \text{GFAP-Cre}\) mice maintained on a C57BL/6 background develop optic gliomas,\(^8\) compared to \(~20\% \) maintained on a mixed genetic background.\(^8\) While astrocytoma predisposition genomic modifiers have been identified in mice, their application to the human condition has not been fully explored.
(or even years) to fully manifest,\textsuperscript{26,29} the outcomes of current clinical trials may not be interpreted accurately.

In contrast, each \textit{Nf1} optic glioma GEM strain represents a homogeneous population of mice in which the genetic background, inherited \textit{Nf1} mutation, timing of somatic \textit{Nf1} loss, age, sex, and tumor location are identical. As such, these preclinical \textit{Nf1} GEM strains model only one disease subgroup with a more limited number of druggable targets. Moreover, optic glioma specimens are routinely acquired in these preclinical studies, enabling a demonstration of drug bioavailability (CNS penetration) and target inhibition. In this manner, while the use of a single homogeneous population of \textit{Nf1} optic glioma mice provides more interpretable experimental outcomes, the resulting findings may only be applicable to one subtype of \textit{Nf1}-OPG.

**NF1-OPG IS A DISEASE OF HETEROGENEITY** As outlined above, it is highly likely that NF1-OPG comprises multiple distinct diseases defined by specific factors, including patient age, sex, tumor location, \textit{NF1} germline mutation, genomic background, and other genetic changes (figure 2). The identification of the responsible risk factors will likely yield clinically relevant subgroups of patients who could be stratified for tailored therapeutic approaches that best match their unique subtype.

**Age.** In a large multicenter study, children younger than 2 years were more likely to experience visual decline secondary to NF1-OPG.\textsuperscript{55} While the etiology of this observation remains unknown, it may reflect the cell of origin and the developmental period during which \textit{NF1} inactivation occurs.

**Sex.** As mentioned above, female mice as well as female humans with NF1-OPG are more likely to experience visual decline and require treatment than their male counterparts.\textsuperscript{53} These observations raise the possibility that females have different epigenetic programming due to X-chromosome influences, which change the intracellular context in which changes in neurofibromin expression affect biological outcomes. Identifying these epigenetic determinants may reveal new genes for risk stratification and therapeutic targeting.

It is also possible that sex influences optic glioma outcomes through differential hormonal production. In this scenario, female hormones (e.g., estrogen) may directly act on RGC neurons to influence intracellular cAMP levels and cell survival. As such, reduced neurofibromin function in RGC neurons may have different consequences in females, in whom heterotrimeric G protein–induced cAMP production is uniquely modulated, relative to their male counterparts. By leveraging this potential hormonal mechanism, it may be possible to envision therapies that target this receptor by repurposing drugs used to treat hormonally responsive male and female cancers.

**Location.** Several studies have demonstrated that NF1-OPGs involving the postchiasmic optic tracts are more likely to cause visual decline and require treatment.\textsuperscript{55} Moreover, this effect is independent of sex, suggesting that it reflects the primary biology of these tumors.\textsuperscript{53} As more diverse GEM models of NF1-OPG are developed, it might be possible to mechanistically determine why tumors in this location have such different outcomes relevant to the design of targeted therapies.

**Germline NF1 gene mutation.** Emerging evidence from numerous laboratories has begun to reveal that the \textit{NF1} germline mutation in people with NF1 may have distinct consequences on the spectrum of the clinical features observed. As such, recognized genotype–phenotype correlations include individuals from different families harboring the c1756-1759 delACTA mutation, who, despite having the other features of NF1, do not develop dermal neurofibromas.\textsuperscript{56} Similarly, individuals with \textit{Nf1} gene frame-shift and premature truncation mutations are prone to developing optic gliomas.\textsuperscript{57,58}

In addition, patients with \textit{Nf1} locus microdeletions that delete the entire \textit{NF1} gene are prone to the development of malignancies (malignant peripheral nerve sheath tumor, high-grade glioma), which may reflect the co-deletion of other tumor suppressor genes (e.g., \textit{Suz12} gene) in the region.\textsuperscript{59,60} Together, these data suggest that not all germline \textit{NF1} gene mutations are equal in their biological effects.

In addition, it is important to recognize that the differential impact of these germline \textit{NF1} gene mutations on non-neoplastic cells (neurons and microglia) may be profound: mutations that mildly impair neurofibromin function in neurons would be predicted to have less deleterious effects on glioma-induced RGC survival and vision than mutations that completely abrogate neurofibromin expression from that allele. Similarly, microglia with \textit{NF1} germline mutations that result in significantly reduced neurofibromin expression may elaborate higher levels or a different spectrum of gliomagens (growth factors and chemo- kines) that promote glioma growth than those with relatively normal neurofibromin function. In this regard, correlating the germline \textit{NF1} mutation with tissue-specific neurofibromin expression levels may provide meaningful insights into NF1-OPG development and outcome as well as the design of future stroma-directed therapies and neuroprotective strategies. Studies are currently under way to generate \textit{Nf1} GEM strains harboring specific \textit{Nf1} patient-derived germline \textit{Nf1} gene mutations for such mechanistic studies.
Neurofibromatosis type 1–optic pathway glioma (NF1-OPG) heterogeneity is determined by a confluence of individual factors that individually affect cell biology and glioma risk. For example, the specific germline NF1 gene mutation creates differential effects on cyclic adenosine monophosphate (cAMP) levels and retinal ganglion cell (RGC) death in neurons (denoted R in the subgroup bar code), chemokine and growth factor production in microglia (denoted M in the subgroup bar code), and RAS pathway activation in neoplastic progenitors/glia (denoted S in the subgroup bar code). Similarly, other genetic alterations (KIAA1549:BRAF or PTEN mutation) alter the activity of the RAS pathway relevant to NF1-deficient tumor cell growth. In addition, patient sex leads to differences in cAMP levels (neurons) or RAS pathway activity (neoplastic progenitors/glia) to affect RGC survival or optic glioma growth. Likewise, the timing of and cell type with somatic NF1 gene inactivation influences NF1-OPG brain location (optic nerve vs postchiasmal tracts, denoted L in the subgroup bar code) or clinical features (clinical progression, denoted P in the subgroup bar code). Finally, the genomic background represents another strong determinant of tumor development and progression. Together, these factors (depicted as colored boxes to illustrate their relative effects) could be used to construct risk assessment algorithms that inform clinical practice.
Genomic factors. While genome-wide association studies (GWAS) have not yielded genomic predictors of glioma development in the general population owing to the diversity of initiating and cooperating genetic events required for malignant gliomagenesis, all patients with NF1-OPG share a similar genetic etiology (germline mutation in the NFI tumor suppressor gene). Recent studies have begun to reveal single nucleotide polymorphisms that may predict NF1-OPG development (Dr. Joshua Rubin, written communication, 2014). While still early in their validation and application to predictive testing, they may permit early glioma risk assessment in a population with a known propensity for brain tumor formation. Similarly, the use of twin studies may also facilitate the identification of these genomic factors.61 The preselection of at-risk children for intense monitoring changes the current anticipatory management paradigm to one of more directed medical monitoring.

Additional genetic mutations. While the vast majority of NF1-associated low-grade gliomas analyzed by next-generation sequencing harbor only NFI gene inactivation,23 recent data suggest that some NF1-OPGs may have monoallelic loss of PTEN gene expression or concurrent KIAA1549:BRAF alterations.62 Recent NFI GEM studies in our laboratory in which these genetic changes were introduced revealed differential effects on tumor volume and proliferation. Moreover, the extent and diversity of growth control signaling pathway activation (e.g., MEK or AKT activation) is also different, raising the possibility that tailored treatment regimens will be required to suppress the growth of these NF1-OPGs compared to their counterparts harboring only neoplastic cell NFI gene inactivation.

CHALLENGE FOR PRECISION MEDICINE The implementation of individualized therapies will require information not currently available that can only be derived from large collaborative studies that leverage resources not widely available in the NF community. First, large data sets for epidemiologic investigations aimed at identifying clinical associations using population science approaches are required to provide hypotheses for future mechanistic laboratory investigation. For example, with the launch of the NF1 Patient Registry Initiative, a Web-based, patient-driven registry,66 relationships between specific medical conditions and NF1 brain tumor risk have begun to emerge (Dr. Kimberly Johnson, written communication, 2014). Second, the availability of genomic DNA and NF1 germline mutation data may enable GWAS that reveal new genomic predictors of NF1-OPG development as well as novel genotype-phenotype correlations. The resulting data, when confirmed, may allow clinicians to more accurately predict which children with NF1 are likely to develop OPG as well as which at-risk children are most likely to require treatment following NF1-OPG formation.

Third, there is a paucity of renewable human biopspecimens for drug evaluation. The assembly of large induced pluripotent stem cell repositories for reprogramming into neurons, neuroglial progenitors, and microglia offers unprecedented opportunities for translational research. The use of human cells from patients with NF1 may set the stage for more personalized therapies. Fourth, future preclinical studies might consider the use of multiple GEM models of NF1-OPG that differ with respect to the germline NFI gene mutation, the timing of somatic NFI gene inactivation, the genetic background of the mouse, and the sex of the animal. The availability of a heterogeneous “clinic population” of NF1-OPG GEM strains may allow investigators to identify those distinct disease subgroups most likely to respond to specific targeted therapies.

With the successful deployment of the above strategies, it is possible to envision a future proactive model of NF1 clinical care in which young children with café-au-lait macules (initial presenting feature of NF1) are assessed for their risk of optic glioma development and then stratified into clinical subgroups based on predictive modeling for targeted therapies that reflect their individual underlying disease pathogenesis and biology. Of importance, the lessons learned from and paradigms created for NF1-OPG will likely apply to other neurologic disorders similarly characterized by clinical heterogeneity.

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51. Reilly KM, Tuskam RG, Christy E, et al. Susceptibility to astrocytoma in mice mutant for Nf1 and Tp53 is linked to chromosome 11 and subject to epigenetic effects. Proc Natl Acad Sci USA 2004;101:13008–13013.


Treatment Sequelae
Patients with malignant brain tumors are prone to complications that negatively impact their quality of life and sometimes their overall survival as well. Tumors may directly provoke seizures, hypercoagulable states with resultant venous thromboembolism, and mood and cognitive disorders. Antitumor treatments and supportive therapies also produce side effects. In this review, we discuss major aspects of supportive care for patients with malignant brain tumors, with particular attention to management of seizures, venous thromboembolism, corticosteroids and their complications, chemotherapy including bevacizumab, and fatigue, mood, and cognitive dysfunction.

Keywords: bevacizumab, brain tumor, chemotherapy, cognition, complications, corticosteroids, fatigue, mood, seizure, symptom management, vasogenic edema, venous thromboembolism.

Seizures in Brain Tumor Patients

Seizures are among the most frequent clinical manifestations of brain tumors. An overall estimate of seizure risk in brain tumor patients is misleading because the figure varies widely as a function of tumor histology, location, and growth rate. At one end of the spectrum are gangliogliomas and dysembryoplastic neuroepithelial tumors, which are associated with intractable epilepsy in at least 90% of patients.\(^1\) Surgical resection is often a highly effective treatment for these lesions, both in terms of recurrence-free survival\(^2\) and seizure control.\(^3\) Diffuse low-grade gliomas also provoke seizures in more than 80% of patients, often as the presenting symptom.\(^4\) In this cohort, seizures may be more common in patients with oligodendrogial tumors, which tend to involve the cortex,\(^5\) and in lesions of the temporal lobe and insula.\(^6\) In both adult and pediatric low-grade gliomas, gross total resection is a strong predictor of postoperative seizure freedom.\(^6,7\) Seizures are the presenting symptom in only \(\sim 20\%\) of patients with supratentorial high-grade gliomas, perhaps because of their rapid growth. Seizures occur at some stage of the illness in 30%–50% of high-grade glioma patients.\(^8,9\) Tumors isolated to white matter and the posterior fossa do not often cause seizures, although deep tumors are frequently multifocal and thus are potentially epileptogenic. Brain metastases cause seizures in 20%–40% of patients, particularly when they are hemorrhagic, multifocal, or involve the temporal lobe.\(^10\)

Retrospective data suggest that antitumor therapy may have a favorable impact on seizure control in brain tumor patients.\(^11\) In one study, 39 low-grade glioma patients treated with temozolomide had a higher rate of reduction in seizure frequency compared with a matched cohort that was not treated with temozolomide (59% vs 13%, \(P < .001\)).\(^12\) This observation was independent of changes in the antiepileptic drug (AED) regimen. Other reports suggested a similar therapeutic benefit for patients treated with radiation therapy.\(^13,14\) However, a retrospective series of 1509 patients with low-grade gliomas showed no significant improvement in seizure control for patients treated with chemotherapy or radiation therapy.\(^4\) This issue will remain controversial until it is addressed definitively in prospective fashion.
Use of Antiepileptic Drugs

The standard of care for brain tumor patients who present with seizures includes the administration of AEDs. Conversely, there is no consensus in daily clinical practice regarding the administration of prophylactic AEDs to patients with supratentorial tumors who have not had seizures. In a 1996 survey of practice patterns, 33% of radiation oncologists, 50% of oncologists, 53% of neurologists, and 81% of neurosurgeons reported administering prophylactic AEDs. The overall rate of prophylactic AED administration was 55%. A retrospective study showed that 27% of 164 brain tumor patients treated in Canada between 2003 and 2005 received phenytoin despite a negative history of seizures.

Several studies have evaluated the usefulness of AED therapy for brain tumor patients with no history of seizures and have produced conflicting results (Table 1). Most of these have included patients with gliomas, brain metastases, and meningiomas, in varying proportions. Many brain tumor patients are treated with AEDs because they have had a craniotomy. It is unclear, however, whether prolonged prophylactic AED therapy reduces the frequency of seizures after craniotomy. In a prospective trial involving 276 consecutive supratentorial craniotomy patients (including 50 with meningiomas) who were randomized postoperatively to receive an AED or no treatment, there was no difference in the incidence of seizures (37%) or death between the 2 groups, suggesting that prophylactic AED therapy may not be routinely necessary after craniotomy. A meta-analysis of 6 controlled studies determined that prophylactic AEDs tended to prevent postoperative seizures, but the effect was not statistically significant. A recent Cochrane systematic review found insufficient high-quality evidence to draw any definitive conclusion about the effectiveness of prophylactic AEDs in this setting. A randomized trial, published after the Cochrane review, assigned patients undergoing craniotomy for glioma or metastases to either 7 days of phenytoin or no seizure prophylaxis. Although the study was likely underpowered, the incidence of seizures in the 30 days following surgery was 10% in the phenytoin group and 8% in the group that did not receive prophylaxis (P = .99). This finding also calls into question the potential benefit of AED prophylaxis for patients undergoing craniotomy.

In 2000, the Quality Standards Subcommittee of the American Academy of Neurology reviewed the evidence concerning the efficacy of prophylactic AEDs in patients with all brain tumor types. Because the numbers of patients in the studies reviewed were small, they performed a meta-analysis of the 4 available randomized studies that addressed this issue. They concluded that the evidence did not show a benefit from prophylactic AED use and recommended that these drugs not be administered as a standard practice. More contemporary systematic reviews of the published literature have reached the same conclusion. An ongoing, randomized, double-blind, placebo-controlled trial is expected to provide definitive data regarding the benefit of prophylactic AED administration for patients with newly diagnosed glioblastoma (NCT01432171). Following surgical resection, patients who have not experienced seizures are randomly assigned to lacosamide or placebo and then observed for up to 1 year. The primary endpoint is time to first seizure. Results are expected in 2017.

Side Effects and Drug Interactions

AED use has been traditionally associated with many unpleasant adverse effects. Approximately 20%–25% of glioma patients treated with phenytoin who undergo cranial irradiation develop rash and, rarely, Stevens-Johnson syndrome. Stevens-Johnson

<table>
<thead>
<tr>
<th>Study</th>
<th>Total No. Patients</th>
<th>No. Patients on AEDs</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Retrospective studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boarini et al. 199</td>
<td>71</td>
<td>33</td>
<td>Odds ratio for seizure 0.41 (95% CI, 0.14–1.19). No patients with therapeutic AED levels had seizures; 18% of untreated patients did.</td>
<td>None</td>
</tr>
<tr>
<td>Moots et al. 200</td>
<td>36</td>
<td>4</td>
<td>No seizures in AED group compared with 31% in untreated patients (P = .60).</td>
<td>None</td>
</tr>
<tr>
<td>Mahaley and Dudka 201</td>
<td>59</td>
<td>Unreported</td>
<td>Odds ratio for seizure 1.63 (95% CI, 0.52–5.14).</td>
<td>None</td>
</tr>
<tr>
<td><strong>Prospective studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Franceschetti et al. 202</td>
<td>63</td>
<td>41</td>
<td>Odds ratio for seizure in the AED group 0.36 (95% CI, 0.07–1.76).</td>
<td>AEDs included phenytoin and phenobarbital.</td>
</tr>
<tr>
<td>Forsyth et al. 203</td>
<td>100</td>
<td>46</td>
<td>Odds ratio for seizure in the AED group was 0.82 (95% CI, 0.33–2.01).</td>
<td>Median follow-up period of 5.4 months. This study had a high noncompliance rate (45% of patients had low AED levels).</td>
</tr>
<tr>
<td>Glantz et al. 16</td>
<td>74</td>
<td>37</td>
<td>Odds ratio for seizure in the AED group was 1.7 (95% CI, 0.6–4.6).</td>
<td>This was a prospective, placebo-controlled, randomized study of valproic acid.</td>
</tr>
<tr>
<td>North et al. 204</td>
<td>81</td>
<td>42</td>
<td>Odds ratio for seizure in the AED group was 1.85 (95% CI, 0.56–6.12).</td>
<td>This was a prospective, non–placebo-controlled, randomized study of phenytoin.</td>
</tr>
</tbody>
</table>

Abbreviation: AED, antiepileptic drug.
syndrome has also been described in glioma patients receiving carbamazepine, and patients receiving phenobarbital have an increased incidence of shoulder-hand syndrome. Additional AED side effects include sedation, dizziness, nausea, vertigo, ataxia, cognitive impairment, myelosuppression, and liver dysfunction, many of which appear to be more common in brain tumor patients. Overall, 24% of brain tumor patients on AED therapy experience side effects severe enough to warrant a change or discontinuation of AED therapy. Although carefully controlled studies are lacking, newer AEDs such as levetiracetam, lamotrigine, pregabalin, and lacosamide have more favorable adverse effect profiles, as noted below.

The majority of older AEDs also have clinically significant interactions with other drugs commonly used for brain tumor patients. Phenytoin induces hepatic metabolism and significantly reduces the half-life and bioavailability of dexamethasone. Conversely, dexamethasone may also reduce phenytoin levels. Some chemotherapy agents commonly used in brain tumor patients, including carmustine (BCNU), reduce phenytoin levels. Additionally, some AEDs induce the cytochrome P450 (CYP450) enzyme system and markedly accelerate the metabolism of several chemotherapy agents including nitrosoureas, irinotecan, and erlotinib. Consequently, the optimal doses of these chemotherapeutic agents for patients taking enzyme-inducing AEDs are frequently higher and less predictable than in patients not taking AEDs.

### Selecting an Antiepileptic Drug

No published data suggest differential efficacy of one AED over another in the brain tumor population. Hence, AED selection should be based on side effects, drug interactions, convenience, availability, and cost. In current neuro-oncological practice in the United States, older enzyme-inducing AEDs such as phenytoin, carbamazepine, and phenobarbital are rarely used. One of the most frequently prescribed AEDs is levetiracetam, which has no known drug-drug interactions, may be initiated at a therapeutic dose, does not require blood level monitoring, has oral and intravenous formulations, is well tolerated by most patients, and is affordable because of its generic status. Lacosamide shares many of levetiracetam’s favorable properties and is gaining popularity as a result. Levetiracetam-lacosamide combination therapy is also safe and feasible for brain tumor patients with refractory seizures. Other agents that are often prescribed include valproic acid and lamotrigine. Valproic acid is an inhibitor of the CYP450 system and thus may increase chemotherapy toxicity. The benefits of lamotrigine are limited by the need to slowly escalate the dose in an effort to minimize the risk of severe skin toxicity. Table 2 summarizes AEDs used in brain tumor patients.

### Antiepileptic Drugs and Possible Antitumor Activity

Recent data suggest that valproic acid has antiglioma effects distinct from its anticonvulsant properties. A histone deacetylase inhibitor, valproic acid may function as a radiosensitizer. A retrospective report found that the addition of valproic acid to standard therapy with radiation and temozolomide may prolong survival for patients with newly diagnosed glioblastoma. Similar findings were reported in a post hoc analysis from the definitive clinical trial that established temozolomide as a standard-of-care for glioblastoma. However, the published studies suffer from several limitations, and the benefit from valproic acid is not consistently demonstrated. A randomized trial may be needed to settle this question. Valproic acid decreases temozolomide clearance by 5%, but the clinical relevance of this finding is unknown.

### Corticosteroids: Use and Complications

Almost all patients with brain tumors receive corticosteroids at some point in the course of their disease. Steroids help control peritumoral vasogenic edema and alleviate accompanying signs and symptoms. They also have antiemetic and analgesic effects and improve appetite and mood. In lymphoma and leukemia, steroids exert oncolytic effects and are utilized as part of the treatment regimen. The effects of steroids on neuroimaging are relevant to response criteria in high-grade gliomas; both RANO and Macdonald criteria require patients to be off steroids or on stable doses for response evaluation. There are no standardized guidelines for the timing, dose, duration, and taper schedule of steroids despite their widespread use in neuro-oncology. An individual patient’s steroid

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**Table 2. Selected non–enzyme-inducing antiepileptic drugs that are frequently used in brain tumor patients**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose Frequency</th>
<th>Route</th>
<th>Notable Side Effects</th>
<th>Primary Metabolism</th>
<th>Need for Level Monitoring?</th>
<th>Titration Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gabapentin</td>
<td>TID</td>
<td>p.o.</td>
<td>Sedation with rapid titration, ataxia, weight gain</td>
<td>Renal</td>
<td>No</td>
<td>Slow</td>
</tr>
<tr>
<td>Lacosamide</td>
<td>BID</td>
<td>p.o./i.v.</td>
<td>Dizziness</td>
<td>Mixed</td>
<td>No</td>
<td>Slow</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>BID</td>
<td>p.o.</td>
<td>Drug rash, Stevens-Johnson syndrome</td>
<td>Hepatic</td>
<td>Not routinely</td>
<td>Extremely slow</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>BID</td>
<td>p.o./i.v.</td>
<td>Agitation, aggression, psychosis</td>
<td>Unknown</td>
<td>No</td>
<td>Rapid</td>
</tr>
<tr>
<td>Pregabalin</td>
<td>BID-TID</td>
<td>p.o.</td>
<td>Sedation, weight gain, thrombocytopenia</td>
<td>Renal</td>
<td>No</td>
<td>Slow</td>
</tr>
<tr>
<td>Topiramate</td>
<td>BID</td>
<td>p.o.</td>
<td>Weight loss, cognitive impairment, paresthesias, metabolic acidosis, renal calculi</td>
<td>Mixed</td>
<td>No</td>
<td>Slow</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>TID</td>
<td>p.o./i.v.</td>
<td>Hair loss, easy bruising, thrombocytopenia, weight gain, hyperammonemia, tremor, pancreatitis, Parkinsonism</td>
<td>Hepatic</td>
<td>Yes</td>
<td>Rapid</td>
</tr>
</tbody>
</table>

**Abbreviations:** BID, 2 times daily; TID, 3 times daily.
requirements may differ depending on lesion size and location, mass effect, and symptoms. Patients are often started on steroids at diagnosis and continue to receive them through surgery and chemoradiation and sometimes even after treatment because of the symptomatic benefit.

Dexamethasone is often preferred due to its lack of mineralocorticoid activity, although prednisone and methylprednisolone have also been used. Dexamethasone has a biological T1/2 of 36–54 hours and thus provides symptomatic benefit for a prolonged period. As such, despite the tendency for administration every 6 hours or 4 times daily, it can generally be given in more convenient twice-daily administration. The conventional starting dose is 16 mg/day. Recent studies have indicated that lower starting doses suffice in selected patients. Vecht et al evaluated patients with brain metastases who were randomized to receive daily doses of 4, 8, or 16 mg of dexamethasone. After 1 week of treatment, there was no difference in improvement between 4 and 16 mg as long as there was no evidence of impending brain herniation.

The duration of steroid use and taper schedule in clinical practice is arbitrary and often clinician or institution dependent and symptom dependent. In one prospective study, 29% of high-grade glioma patients were able to taper off steroids 3 months post radiation. In another study, only 21% of patients tolerated steroid taper. In both studies, 55%–58% of patients required an increase in dose during radiation. Headache was the most common symptom requiring steroid increase (34%–41%). Better performance status was associated with successful early taper. Patients with primary brain tumors tend to remain on steroids for a longer time (23 weeks) than those with secondary brain tumors (7 weeks). In the aforementioned Vecht study, patients who were on 4 mg required a slower taper and often needed reinstatement of steroids after discontinuation. Twice-daily dexamethasone taper during radiation for brain metastases was found to be effective in one study, in which 13 of 14 patients remained off steroids at 30 days post radiation. Analyses of various cooperative group trials have all indicated that baseline corticosteroid use in glioblastoma is negatively associated with survival. In general, every effort should be made to start steroids at low doses and taper as quickly as possible.

A recent review and clinical practice guideline for brain metastases suggested that dexamethasone be started at 4–8 mg/day for mild symptoms or 16 mg/day for severe symptoms from mass effect, with an attempt to taper slowly over 2 weeks or longer in symptomatic patients.

Unfortunately, the side effects of corticosteroids limit their long-term use. The incidence of toxicity is related to cumulative dose and duration of treatment. Most studies have shown that steroid-related side effects occur frequently in patients using dexamethasone 16 mg/day for more than 2–3 weeks. Corticosteroid side effects may be neurological or nonneurological. Myopathy is a common neurological side effect and typically produces proximal extremity weakness (particularly in the legs) and, in severe cases, neck flexor and respiratory muscle weakness. In a paucity of supporting literature, many patients receiving corticosteroids are prescribed histamine receptor (H2) blockers or proton pump inhibitors. High-dose steroids are also associated with a risk of colonic perforation, which usually affects the sigmoid colon. Patients may present with an acute abdomen or have an insidious course due to masking of signs and symptoms by the anti-inflammatory effects of steroids. Endocrine side effects include Cushing's syndrome and hyperglycemia, which are usually reversible after steroid discontinuation. In patients with pre-existing diabetes, the insulin requirement may increase. Adrenal insufficiency or steroid withdrawal syndrome may occur when patients on long-term steroids undergo a rapid taper. Patients may present with headache, nausea, anorexia, malaise, myalgia, arthralgias (pseudorheumatism), and low-grade fever.

Metabolic effects of corticosteroids on bone are another cause of steroid morbidity. Osteoporosis, leading to fractures of the spine and hip, is not rare. Bone loss is likely related to reduced calcium absorption, secondary hyperparathyroidism, and decreased sex hormones. Calcium and vitamin D supplements in standard doses are recommended for prevention. Oral bisphosphonates may be used, but there is a risk of increased peptic ulcer disease, especially in conjunction with corticosteroids. Kyphoplasty may be helpful for compression fractures. Avascular necrosis of the hip should be considered in a patient with hip pain on steroids.

A medication that controls vasogenic edema without corticosteroid side effects would be of great value. Bevacizumab has substantial steroid-sparing effects; a majority of patients in the BRAIN trial were able to lower their steroid doses, and the reductions were often substantial. Tyrosine kinase inhibitors (TKIs) potently targeting vascular endothelial growth factor receptor 2 (VEGFR-2), such as cediranib and cabozantinib, have also shown steroid-sparing effects in clinical trials but are not utilized clinically for this purpose. Corticorein acetate, a synthetic formulation of human corticotropin-releasing factor, is also under study with promising steroid-sparing effects on edema.
Venous Thromboembolism

Brain tumors confer a high risk for venous thromboembolic (VTE) disease, both during and beyond the perioperative period. This has been best studied in high-grade glioma, where it is estimated that 3%–20% of patients develop perioperative deep venous thrombosis (DVT) or pulmonary embolism (PE), depending upon prophylaxis and type of screening. In fact, radiolabeled fibrinogen scans have shown DVTs in 60% of postoperative glioblastoma patients. An elevated risk persists beyond the perioperative period; cumulative incidence at 6 months is 17% and ~20% at 1 year.

Table 3 summarizes risk factors for VTE development. The high incidence of VTE should translate into a correspondingly low threshold for pursuing lower extremity Doppler studies or CT pulmonary angiogram in patients with lower extremity edema, calf discomfort, dyspnea, chest pain, or other cardiopulmonary symptoms. Upregulation of tissue factor and its downstream effectors appears to play a key role both in activation of clotting pathways and oncogenic signaling mechanisms important for cancer progression. The interested reader is referred elsewhere for in-depth discussion of the pathophysiology of hypercoagulability in neuro-oncology patients.

Management of VTE in neuro-oncology patients is influenced by concerns of precipitating intratumoral hemorrhage with anticoagulant administration. Large case series have shown anticoagulation to be effective and acceptably safe in high-grade gliomas as well as brain metastases. Metastases from lung and breast tumors have a relatively low incidence of spontaneous hemorrhage and should not be seen as a strong contraindication to anticoagulation. Anticoagulation is often avoided in tumors with a particularly strong tendency towards hemorrhage such as melanoma and renal cell carcinoma, although selected patients with brain metastases from melanoma have been safely anticoagulated for VTE. Thus, the presence of a nonhemorrhagic brain tumor is not a strong contraindication to anticoagulation. Noncontrast head CT, to exclude more than petechial hemorrhage, may serve as a useful risk stratification approach.

The alternative to anticoagulation is placement of an inferior vena cava (IVC) filter. No prospective studies have compared IVC filters to anticoagulation in any patient population. However, case series have reported unacceptable outcomes with IVC filters in a mixed population of brain tumor patients, with a 12% incidence of recurrent PE along with a 57% incidence of postphlebitic syndrome, recurrent DVT, or IVC/filter thrombosis. Fatal PE despite IVC filter is well documented. Consequently, we restrict their use to patients with VTE and strong contraindications to anticoagulation, such as recent intracranial surgery or hemorrhage. Little evidence supports combined therapy with anticoagulation and IVC filter.

Several options for anticoagulation exist (Table 4). Choices for initial therapy include low molecular weight heparin (LMWHs) or unfractionated heparin; LMWHs are generally preferred, with unfractionated heparin being reserved for symptomatic PE, renal insufficiency, or patients at high risk for bleeding. For chronic therapy, FDA-approved agents include warfarin and LMWH. While both are effective, LMWHs avoid the need for frequent laboratory monitoring and the potential drug-drug interactions with warfarin that phenytoin, trimethoprim/sulfamethoxazole, omeprazole, and other commonly prescribed medications pose. LMWH was markedly superior to warfarin at preventing recurrent VTE in cancer patients in general, although no study restricted to neuro-oncology patients has been performed. The newer oral agents that inhibit thrombin or factor Xa are not well studied to date in cancer patients. Duration of anticoagulation should be individualized based on the patient’s risk factors. Three to 6 months represent a minimum duration for anticoagulation, and patients with active malignancy or ongoing chemotherapy should be considered for prolonged therapy. Thus, lifelong anticoagulation is a consideration for the glioblastoma patient.

The high incidence of VTE has led to interest in prophylaxis. The benefits of VTE prophylaxis in the perioperative setting have been clearly demonstrated; a large study, which randomized more than 300 patients (almost all of whom had brain tumors) to...
compression stockings + enoxaparin 40 mg daily on postoperative day 1, halved the rate of VTE without increasing bleeding. Long-term primary prophylaxis outside the perioperative period has been studied in high-grade glioma. The PRODIGE study randomized patients to dalteparin versus placebo. The study was terminated early because of drug supply issues. While a trend towards reduced VTE was seen in the dalteparin arm, intracranial hemorrhage was more frequent (5% vs 1%). Thus, primary prophylaxis is not advised at present. A biomarker-based scale to predict risk of VTE with newly diagnosed high-grade glioma has been proposed and warrants validation.

**Adverse Events with VEGF/VEGFR Targeting Agents**

Angiogenesis is a characteristic feature of aggressive malignancies, including many brain tumors. We will focus on the use of inhibitors of VEGF signaling, as this is the most prominent mediator of tumor-associated angiogenesis.

Over the past several years, evaluation of antiangiogenic agents has been a highly active area of clinical research in neuro-oncology and culminated in the FDA’s accelerated approval of bevacizumab, a humanized, recombinant in recurrent glioblastoma patients was further heightened by a recent phase II study demonstrating significantly improved outcome when bevacizumab was combined with lomustine compared with either agent alone. However, the role of bevacizumab in newly diagnosed glioblastoma patients remains unclear following data from 2 recently reported randomized, placebo-controlled phase III trials that demonstrated improved progression-free survival but failure to improve overall survival and mixed results in quality-of-life evaluations.

Interest in the use of bevacizumab for indications other than glioblastoma has expanded in the past few years. Although randomized phase III trials have not been performed, single arm phase II studies have supported the use of bevacizumab for recurrent grade III malignant glioma patients. Retrospective series have also demonstrated encouraging benefit associated with bevacizumab therapy in patients with vestibular schwannoma and neurofibromatosis type 2. In meningioma, there are reports of response in ependymoma, hemangioblastoma, and some metastatic CNS tumors. Moreover, it is a potent agent against symptomatic radiation necrosis.

In addition to bevacizumab, a variety of other angiogenics agents has been investigated for malignant glioma patients including TKIs targeting VEGFR (monoclonal antibodies that block VEGF binding to VEGFR) and a soluble decoy VEGFR. The use of these agents has become widespread in oncology because many are approved for a variety of cancer indications. The spectrum of toxicities associated with agents that block VEGF/VEGFR signaling is thus now well established. Therapeutics with additional targets, such as many VEGFR TKIs, are typically associated with broader toxicity profiles. We will summarize the aggregate adverse event experience associated with bevacizumab as the prototypical inhibitor of VEGF/VEGFR signaling. Two main categories of adverse events emerge from this experience: those that are common and typically mild and those that are uncommon and often severe.

**Common/Often Mild Adverse Events**

Fatigue, hypertension, and proteinuria occur frequently in bevacizumab recipients, although the severity is generally mild. Fatigue, the most common adverse event associated with VEGF/VEGFR inhibitors, is low grade and manageable in most cases. Among recurrent glioblastoma patients on the AVG3708g study, 45% of patients experienced fatigue of any grade, while grade ≥ 3 fatigue was reported in 3.6% and 8.9% of those treated with bevacizumab and bevacizumab plus irinotecan, respectively. Adding bevacizumab to adjuvant temozolomide in newly diagnosed glioblastoma patients increased grade ≥ 3 fatigue frequency modestly compared with placebo (13.1% and 9.0% on Radiation Therapy Oncology Group [RTOG] 0825 and 7.4% and 4.7% for AVAglio).

Hypertension with VEGF/VEGFR therapy is linked with both patient-related factors (eg, age, comorbidities, lifestyle factors) and concurrent medications as well as drug-related factors including agent, dose, and schedule. A recent meta-analysis demonstrated that 55% of bevacizumab recipients developed a > 10 mmHg increase in systolic blood pressure (SBP) or > 5 mm Hg increase in diastolic blood pressure (DBP); 7.6% developed either a > 40 mmHg SBP increase or > 20 mmHg DBP increase, and 0.12% developed hypertensive crisis. Hypertension of any grade has been reported in 36.5%–39% of glioblastoma patients, while grade ≥ 3 hypertension affects 4%–11% of patients. Recent reviews provide guidance on monitoring and treatment of hypertension in patients treated with VEGF/VEGFR inhibitors.

Proteinuria develops due to inhibition of VEGF-mediated maintenance of podocyte-endothelial cell integrity of normal glomerular capillaries and the subsequent development of a thrombotic microangiopathy. Hypertension increases the risk of proteinuria. Up to 63% of cancer patients treated with bevacizumab develop grade 1–2 proteinuria, while grade 3–4 proteinuria has been reported in 1%–15%. Proteinuria of any grade has been noted in up to 16% of glioblastoma patients, with 1%–3% developing grade ≥ 3 proteinuria. Current management guidelines include regular prospective urine analysis monitoring, early referral of patients with more severe proteinuria for nephrology consultation, and interruption of dosing. Angiotensin-converting enzyme inhibitors and angiotensin 2-receptor antagonists can provide a renoprotective effect that may reduce proteinuria and help control blood pressure.

Dysphonia or hoarseness affects up to 37% of patients treated with VEGF/VEGFR inhibitor therapy. Management considerations include fiberoptic laryngeal examination and discontinuation of antiangiogenic therapy.

**Less Common/Often Severe Adverse Events**

Anti-VEGF/VEGFR therapy has been reported to increase the risk of cancer-associated hypercoagulability. A recent meta-analysis demonstrated a relative risk of 1.33 for VTE in oncology patients treated with bevacizumab compared with controls, although other such studies have been negative. Among general oncology patients with VTE, systemic anticoagulation administered with ongoing bevacizumab therapy has been associated with a low (<1%) hemorrhage risk; however, a recent retrospective analysis noted an 11% rate of intracranial hemorrhage in...
bevacizumab patients receiving concurrent anticoagulation compared with only 3% of those on bevacizumab without anticoagulation. 

Grade ≥3 VTE and arterial thromboembolism (ATE) occurred in 3.6% and 2.4% of recurrent glioblastoma patients, respectively. In newly diagnosed glioblastoma patients, grade ≥3 VTE occurred within similar frequency (7%–10%) in bevacizumab and placebo recipients. In contrast, the frequency of grade ≥3 ATEs was clearly higher in bevacizumab recipients (5.0% vs 1.3%). Additional bevacizumab administration is contraindicated following ATEs, while bevacizumab may be continued with care monitoring for patients with VTEs who are appropriately anticoagulated.

Bevacizumab increases the risk of hemorrhage in oncology patients. Grade 3 bleeding occurred in 3.5% of 12 617 cancer patients treated across 20 randomized trials, with a 2.48 relative risk for bevacizumab recipients compared with controls. Approximately 35% of glioblastoma patients experience bleeding of any grade, with grade ≥3 hemorrhage limited to 1%–2%. The overall intracranial hemorrhage (ICH) rate among cancer patients treated with bevacizumab is 0.3%–0.9%, and increases to 0.9%–1.5% in those with known primary or metastatic brain tumors. ICH of any grade affects 1%–3% of glioblastoma patients treated with bevacizumab, while grade ≥3 ICH occurs in 0.6%–2%. Of note, spontaneous ICH of any grade and grade ≥3 without bevacizumab occur in 2% and 0.9%, respectively. Further bevacizumab dosing is contraindicated for oncology patients who develop ICH.

Bevacizumab appears to increase the risk of ischemic stroke above a baseline spontaneous level that occurs in high-grade glioma patients. A recent meta-analysis of glioma patients noted a 1.8% rate of ischemic stroke that increased to 6.2% for bevacizumab recipients. Another recent series noted a 1.9% rate of ischemic stroke in glioblastoma patients treated with bevacizumab, while grade ≥3 ICH occurs in 0.6%–2%. Of note, spontaneous ICH of any grade and grade ≥3 without bevacizumab occur in 2% and 0.9%, respectively.

Hematological Toxicity from Chemotherapy

While hematological toxicity of standard adult brain tumor therapy is generally less common and milder than for other malignancies, it remains a highly pertinent issue for clinicians. Profound anemia is rare with temozolomide but is slightly more common with nitrosourea-based regimens. High-grade lymphopenia is very common with dose-dense temozolomide regimens and corticosteroid use and predisposes to pneumocystis and other infections (vide infra). Neutropenia is relatively uncommon with temozolomide but fairly common with nitrosoureas. With temozolomide, myelosuppression in general is approximately twice as common in women than in men. The addition of bevacizumab to temozolomide increases the risk of grade 3+ neutropenia from 3.7% to 7.2% and grade 3+ thrombocytopenia from 7.7% to 10.2%. Table 5 summarizes the frequency of high-grade hematological toxicity with common regimens.

The management of acute hematological toxicity in brain tumor patients is divided from medical oncology, with standard guidelines applicable for decisions regarding use of red blood cell transfusions and colony-stimulating factors. The possible exception is in the prophylactic management of thrombocytopenia. ASCO guidelines recommend a threshold of 10 000 platelets for prophylactic transfusion in solid tumors. However, these guidelines note it may be appropriate to raise the threshold to 20 000 for patients with necrotic tumors that are at increased risk of hemorrhage, and that in some patients a risk of major bleeding of 2%–5% might suffice to use a trigger of 20 000. There is an absence of data derived from the brain tumor population. It would seem reasonable to set an even higher threshold for a patient with an already-hemorrhagic brain tumor. Fever, sepsis, and the rapidity of platelet count drop should also be considered when a threshold is set for an individual patient. For neurosurgical intervention, it is recommended that patients have at least 100 000 platelets.

The use of alkylating agents has also been associated with long-term hematological toxicity. The risk of aplastic anemia is estimated at 1 per 10 000 patients exposed to temozolomide; 17 cases of leukemia and 7 cases of myelodysplastic syndrome were reported between 1999 and 2008. How temozolomide compares with other alkylating agents regarding the risk of secondary leukemias and prolonged or permanent bone marrow failure remains unknown.

Infections

Multiple factors conspire to predispose neuro-oncology patients to CNS and systemic infections. Neurosurgical procedures...
Table 5. Hematological toxicities of commonly utilized brain tumor chemotherapies

<table>
<thead>
<tr>
<th>Grade 3 (ANC 500–1000; Platelets 25 000–50 000; Hemoglobin &lt; 8.0)</th>
<th>Grade 4 (ANC &lt; 500; Platelets &lt; 25 000)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temozolomide</strong></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>3% (concomitant phase), 11% (adjuvant phase)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>4% (concomitant phase), 4% (adjuvant phase)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>12% (adjuvant), 3% (adjuvant)</td>
</tr>
<tr>
<td>Anemia</td>
<td>1% (concomitant), 1% (adjuvant)</td>
</tr>
<tr>
<td><strong>BCNU</strong></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>14%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>24%</td>
</tr>
<tr>
<td>Anemia</td>
<td>6%</td>
</tr>
<tr>
<td><strong>CCNU</strong></td>
<td>32%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>26%</td>
</tr>
<tr>
<td><strong>Thrombocytopenia</strong></td>
<td>25%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>20%</td>
</tr>
</tbody>
</table>

Abbreviations: ANC, absolute neutrophil count; PCV, procarbazine/CCNU/vincristine.

create barrier disruption, while chemotherapy and corticosteroids contribute to impairment of cell-mediated immunity and occasional neutropenia. Poor nutritional status is another likely contributor. The risk of a surgical site infection following craniotomy is 2%–3%. Several groups have found that using carmustine wafers increases this risk,143 but a recent case-control study reported an odds ratio of 6.7 for surgical site infection with their use,144 although others have disputed this point.145

Immunosuppression is an important cause of infections, particularly outside the perioperative period. Neutropenic fever is uncommon with standard neuro-oncology regimens and will not be discussed further because its management does not differ from other populations. Impairment of cell-mediated immunity, in contrast, is particularly germane to brain tumor patients.

Even prior to the use of temozolomide, neuro-oncologists recognized that corticosteroid use predisposes to Pneumocystis jirovecii pneumonia (PCP) and that lymphopenia is a key risk factor.146 The phase II trial that served as the forerunner to the “Stupp regimen” (incorporating daily temozolomide with radiation) reported that 79% of patients developed grade 3+ lymphopenia and 2 of their first 15 patients (both lymphopenic) developed PCP; PCP prophylaxis was subsequently given to all patients.144 A study in melanoma patients found that 60% developed lymphopenia with CD4 counts preferentially affected while on a dose-dense schedule akin to the Stupp regimen; since these patients were generally not on corticosteroids, dose-dense temozolomide was clearly the culprit.144 The recommendation to give PCP prophylaxis to patients being treated with radiation and temozolomide is part of the package insert. Clear guidelines for when prophylaxis may be safely discontinued are lacking; a recent publication suggested giving prophylaxis to patients on chronic steroids and patients receiving temozolomide with lymphocyte counts ≤500, although this approach has not been prospectively validated.147 Because PCP is not rare in other brain tumors treated with chemotherapy and corticosteroids (eg, primary CNS lymphoma), it is prudent to follow lymphocyte counts and consider prophylaxis as well.148 The clinician has a choice of several regimens.149

Reactivation of herpesviruses has been seen with temozolomide, and disseminated zoster and CMV have also been reported.149 CMV pulmonary, colonic, and hepatic infections have been reported150 and are treatable with antiviral therapy. Prophylaxis with acyclovir may prevent zoster.151 Other rare infections associated with temozolomide and dexamethasone include aspergillosis,152 disseminated strongyloides,153 bronchopulmonary infection with Bordatella bronchiseptica (a cause of “kennel cough”),154 cryptococcal meningitis,155 disseminated tuberculosis,156 and hepatitis B reactivation.157–159

Rituximab, a monoclonal antibody targeting the protein CD20 on the surface of B lymphocytes, is commonly incorporated into CNS lymphoma therapy. Rituximab has been linked to reactivation of hepatitis B virus,160 and antiviral prophylaxis may be indicated.161 Hepatitis C virus reactivation has also been reported. Numerous cases of progressive multifocal leukoencephalopathy have been seen following rituximab use in other disorders,162 and it is likely only a matter of time until this is reported in primary central nervous system lymphoma.

**Endocrine and Fertility Issues**

The incidence of radiation-induced damage to the hypothalamic-pituitary axis in adults is uncertain but may exceed 30% when the hypothalamus and pituitary are in the radiation field.163 The hypothalamus is more sensitive than the pituitary gland. Risk factors include increasing total dose and dose per fraction and age (children and young adults are the most vulnerable). Endocrine dysfunction typically starts within a few years of radiation. In adults, the growth hormone axis is most sensitive to radiation; manifestations of growth hormone deficiency include fatigue, altered body composition, decreased bone mineral density, and increased cardiovascular mortality. Gonadotropin deficiency may manifest as oligomenorrhea/amenorrhea or low testosterone. Adrenocorticotropic hormone deficiency is less common and may require hydrocortisone replacement therapy. Mild hyperprolactinemia may also be a consequence. This topic is comprehensively reviewed elsewhere.163

Preservation of fertility represents an important concern for brain tumor patients and is especially complex in young women. Alkylating agents are the most gonadotoxic chemotherapy drugs.164 The incidence of infertility in brain tumor patients is poorly studied. Alkylating drugs cause follicular depletion and destruction of oocytes, commonly resulting in premature ovarian failure. Hormonal abnormalities and alterations in menstrual cycles (amenorrhea or oligomenorrhea) are commonly seen in women treated with radiation and alkylator-based chemotherapy for gliomas in another pilot study.165 Small pilot studies confirm an at least transient deleterious effect of temozolomide on sperm count, motility, and density in some men.166

Although there are case reports of preservation of male167 and female168 fertility after temozolomide exposure, discussion of possible fertility preservation must precede initiation of chemotherapy. Cryopreservation of sperm is widely available for men.
Techniques to preserve female fertility include in vitro fertilization, embryo cryopreservation, cryopreservation of unfertilized ova, cryopreservation and transplantation of thawed ovarian tissue, and use of GnRH-a to simulate a prepubertal hormonal environment and decrease the risk of ovarian failure. Preventing conception is recommended in the first 2 years after chemotherapy in women.

Fatigue and Mood

Fatigue is a common symptom in primary brain tumor patients, with 40%–70% reporting fatigue during the course of their illness. The prevalence is even higher in primary brain tumor patients undergoing cranial irradiation, with more than 80% reporting fatigue during treatment. The pathophysiology underlying fatigue is not well understood.

It is often underreported, underdiagnosed, and undertreated. Fatigue is typically assessed through patient self-reporting. History and physical examination, laboratory data, and family members’ descriptions of patient behaviors can help supplement patient self-reporting. For use in clinical research, there are well-established questionnaires for fatigue assessment validated in brain tumor patients including the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC-QLC-C30) fatigue subscale. For patients with moderate to severe fatigue, the NCCN guidelines recommend evaluation for treatable contributing factors including pain, medications (eg, anticonvulsants and opioids), emotional distress (eg, depression or anxiety), sleep disturbance, anemia, nutritional deficiencies, decreased functional status, and comorbidities (eg, alcohol/substance abuse, endocrine dysfunction, and infection).

Mood disorders are common in brain tumor patients and may be a treatable cause of fatigue. In glioma, depression can be associated with physical functional impairment, cognitive impairment, higher mortality, increased frequency of medical complications, and reduced work productivity. One longitudinal twin-center study showed that 20% of glioma patients developed major depressive disorder (MDD) in the first 6 months after starting radiotherapy and that MDD was 3–4 times more likely to occur in patients with prior depression or significant functional impairment. However, antidepressants may lower the seizure threshold, impair memory, or cause fatigue. A recent Cochrane meta-analysis found no eligible randomized controlled trials, controlled trials, cohort studies, or case-control studies of the pharmacological treatment for depression in primary brain tumor patients. While antidepressants are effective treatments for depression in a variety of other patient populations and may be indicated in brain tumor patients with MDD, it is unclear which pharmacological intervention is optimal.

Few studies have evaluated pharmacological and/or nonpharmacological interventions for fatigue in brain tumor patients (Table 6), but the literature in the general cancer population is more extensive. Favorable effects on fatigue have been reported with exercise, psychoeducation on self-management of fatigue, and corticosteroids. A meta-analysis found aerobic exercise to be more effective than the control intervention for fatigue during and following tumor-directed therapy, especially in solid tumor patients. However, the optimal type, intensity, and timing of exercise are not known. A recent double-blind, randomized, placebo-controlled study of dexamethasone in 84 patients with advanced cancer revealed a significant improvement in the Functional Assessment of Chronic Illness Therapy—Fatigue (FACIT-F) subscale with dexamethasone 4 mg orally twice daily for 14 days. Since many brain tumor patients are already on dexamethasone for management of cerebral edema, it is unclear if increasing the dose of dexamethasone is a meaningful intervention for fatigue.

Several other pharmacological interventions have been studied for fatigue, but there is no class I evidence to support their routine use. Drugs to improve anemia, including erythropoietin and darbepoetin, improve fatigue but cannot be recommended because they are also associated with increased mortality in advanced cancer patients and more adverse events compared with placebo. Studies of psychostimulants in cancer patients, including primary brain tumor patients, have yielded mixed results. A randomized study of modafinil in primary brain tumor patients did not significantly reduce fatigue compared with placebo. Fatigue scores significantly declined compared with baseline assessment in both the modafinil and placebo groups, demonstrating the difficulty of interpreting single-arm studies. Preliminary results from 2 double-blinded, placebo-controlled studies of armodafinil (the R-enantiomer of modafinil) for primary brain tumor patients receiving brain irradiation suggest no statistically significant reduction in fatigue. However, subgroup analysis of the Shaw et al study suggests that those patients with more baseline fatigue (defined as a fatigue subscale less than median) may experience less fatigue when treated with armodafinil versus placebo.

Sleep disturbances, both insomnia and hypersomnia, can also exacerbate fatigue. Sleep interventions designed to enhance sleep quality are simple interventions that may help patients with fatigue. Examples include stimulus control (getting out of bed after 20 min if unable to fall asleep), sleep restriction (avoiding long or late afternoon naps, limiting total time in bed), and good sleep hygiene (avoiding caffeine after noon, establishing an environment conducive to sleep). Medications such as corticosteroids may also contribute to insomnia. Pharmacological interventions for insomnia have not been tested in a randomized fashion in brain tumor patients, although basic principles for pharmacological management of insomnia have been advocated, including starting with low doses and avoiding long-term use of benzodiazepines.

Neurocognitive Impairment

Impairment of neurocognitive function is very common in brain tumors patients, both as a result of the direct effects of the tumor and its surrounding edema and the sequelae of therapy. As treatments for brain tumors improve and patients live longer, it is likely that these complications will increase in importance, similar to the situation encountered with childhood survivors of brain tumors.

Neurocognitive impairment is very common after radiation therapy. In some studies, more than 90% of patients who survive more than 6 months after receiving whole brain radiation therapy (WBRT) have evidence of neurocognitive impairment. Radiation therapy can cause functional deficits in memory, attention, and executive function and thereby affect the patient’s quality of life.
of life (QOL). Although the underlying mechanisms remain ill-defined, there is increasing evidence that radiation, in addition to its well-known damage to microvessels, induces neuroinflammation with increased infiltration of activated microglia, decreased hippocampal neurogenesis, and altered neuronal function. These changes can be partially abrogated by administration of

### Table 6. Clinical trials of interventions for fatigue in brain tumor patients

<table>
<thead>
<tr>
<th>Reference</th>
<th>Intervention</th>
<th>Patient Population</th>
<th>Study Design</th>
<th>Assessment of Outcome Measures</th>
<th>Fatigue Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boele, et al. Neuro Oncol 2013&lt;sup&gt;180&lt;/sup&gt;</td>
<td>Modafinil (up to 400 mg/day) vs placebo for 6 weeks, with cross-over after 1-week washout period</td>
<td>37 patients with primary brain tumors and no evidence of tumor recurrence in previous 6 months</td>
<td>Randomized, double-blinded, placebo-controlled study with cross-over</td>
<td>Self-reported questionnaires including CIS for fatigue at baseline, immediately after first treatment period (6 weeks) and immediately after cross-over treatment period (12 weeks)</td>
<td>No significant difference in CIS score for fatigue severity for modafinil vs placebo</td>
</tr>
<tr>
<td>Butler, et al. Int J Radiat Oncol Biol Phys 2007&lt;sup&gt;209&lt;/sup&gt;</td>
<td>d-three-MPH (5 – 15 mg BID) vs placebo</td>
<td>68 patients with primary or metastatic brain tumors undergoing brain irradiation</td>
<td>Randomized, double-blinded, placebo-controlled study</td>
<td>Fatigue assessed by FACIT-F subscales at baseline, end of radiation, and 4, 8, 12 weeks after radiation</td>
<td>No significant difference in FACIT-F subscales between groups at 8 weeks after radiation</td>
</tr>
<tr>
<td>Gehring, et al. J Clin Oncol 2009&lt;sup&gt;210&lt;/sup&gt;</td>
<td>7-week cognitive rehabilitation program vs waiting-list control group</td>
<td>140 patients with low-grade and anaplastic gliomas who were clinically stable (no evidence of disease progression)</td>
<td>Randomized, non-blinded study</td>
<td>Battery of neuropsychological tests and self-reported questionnaires at baseline, after 7-week intervention, 6-month follow up. Mental fatigue evaluated by MFI</td>
<td>Statistically significant difference between groups for self-reported measures of mental fatigue (P = .049)</td>
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<td>Gehring, et al. J Neurooncolog 2012&lt;sup&gt;196&lt;/sup&gt;</td>
<td>IR-MPH 10 mg BID vs SR-MPH 18 mg daily vs modafinil 200 mg daily for 4 weeks</td>
<td>34 patients with primary brain tumors: 11 IR-MPH, 13 SR-MPH, 10 modafinil (planned sample size was 75 total, 25 per group)</td>
<td>Open-label, randomized, pilot study</td>
<td>Cognitive testing and self-reported measures of fatigue including BFI Total, POMS-Fat, POMS-Vig to evaluate fatigue at baseline and after treatment (median = day 30)</td>
<td>Study terminated early due to slow accrual. Patient reported improvements in fatigue but no statistically significant difference between MPH groups and modafinil group Preliminary analysis suggests no statistically significant difference between armodafinil and placebo at any time point Preliminary analysis suggests no statistically significant difference in the 42-day change (baseline vs day 43) between armodafinil and placebo</td>
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<tr>
<td>Shaw, et al. J Clin Oncol (abstract 9505)&lt;sup&gt;181&lt;/sup&gt;</td>
<td>Armodafinil 150 mg/day vs placebo during radiation and 4 weeks after radiation</td>
<td>54 patients with primary brain tumors undergoing brain irradiation</td>
<td>Phase II, double-blinded, placebo-controlled, randomized study</td>
<td>Fatigue assessed by BFI, ESS, FACT, FACT-BR, FACIT-F subscales at baseline, end of radiation, 4 weeks after radiation</td>
<td>Preliminary analysis suggests no statistically significant difference between armodafinil and placebo at any time point Preliminary analysis suggests no statistically significant difference in the 42-day change (baseline vs day 43) between armodafinil and placebo</td>
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<td>Lee, et al. J Clin Oncol (abstract 2004)&lt;sup&gt;182&lt;/sup&gt;</td>
<td>Armodafinil 150 mg/day vs placebo during radiation and 2 weeks after radiation</td>
<td>80 patients with glioma undergoing brain irradiation</td>
<td>Randomized, placebo-controlled pilot trial</td>
<td>Fatigue assessment by FACIT-F subscale, BFI, CSF at baseline, day 22, day 43, day 56</td>
<td></td>
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</table>

Abbreviations: BFI Total, Brief Fatigue Inventory; CFS, Cancer Fatigue Scale; CIS, Checklist Individual Strength; ESS, Epworth Sleep Scale; FACIT, Functional Assessment of Chronic Illness Therapy; FACT-Br, Functional Assessment of Cancer Therapy, Brain cancer; IR-MPH, Immediate release methylphenidate; MFI, multidimensional fatigue inventory; MPH, methylphenidate; POMS-Fat, Profile of Mood States Fatigue-Inertia; POMS-Vig, Profile of Mood States Vigor-Activity; SR-MPH, Sustained release methylphenidate.
indomethacin, although the precise mechanisms involved remain to be clarified. More recent studies suggest a potential role of peroxisomal proliferator-activated receptor (PPAR) α and γ agonists as well as renin-angiotensin system (RAS) blockers for preventing radiation-induced neuroinflammation and neurocognitive impairment independent of improved neurogenesis. Other strategies to reduce neurocognitive impairment from radiation therapy involve more conformal approaches such as the use of intensity-modulated radiation therapy or proton-beam therapy as well as strategies to spare the hippocampus.

There is also emerging evidence that chemotherapeutic agents (possibly including temozolomide) may affect neurocognitive function by a variety of mechanisms including inhibition of hippocampal neurogenesis, oxidative damage, white matter damage, decreased hypothalamic-pituitary-adrenal axis activity, and reduced brain vascularization and blood flow.

Patients with neurocognitive impairment can benefit from a detailed evaluation, sometimes including neuropsychological testing. It is important to determine if fatigue and depression are contributing factors and to treat them optimally when they are present. Many medications can contribute to neurocognitive impairment including AEDs, antidepressants, psychotropics, and even corticosteroids. These should be eliminated, if possible, or used at the lowest possible doses. Laboratory tests should be performed to exclude metabolic abnormalities, anemia, primary hypothyroidism, and vitamin B12 deficiency.

Several agents have been evaluated for potential beneficial effects on neurocognitive function. The RTOG conducted a large, placebo-controlled randomized trial evaluating the benefit of memantine, a N-Methyl-D-aspartate inhibitor, in patients with brain metastases receiving WBRT (RTOG 0614, NCT00566852). Memantine was started within 3 days of radiotherapy and continued for 24 weeks, and side effects were limited. There was less decline in the primary endpoint of delayed recall in the memantine arm at 24 weeks, although it did not reach statistical significance (P = 0.059). The lack of statistical significance may be partially due to the fact that only 149 of the 508 initially eligible patients were analyzable at 24 weeks because the majority of patients had progressed and died, which resulted in only 35% statistical power. However, the memantine arm had a significantly reduced rate of decline in memory, executive function, and processing speed in patients receiving WBRT, suggesting that it may be of benefit. Whether patients receiving other forms for radiation therapy for different types of brain tumors will have similar benefits remains to be determined in future studies.

Donepezil, an acetylcholinesterase inhibitor, has also been evaluated in brain tumor patients. In an early open-label phase II study of donepezil in irradiated brain tumor patients, neurocognitive functioning, mood, and health-related QOL were significantly improved following a 24-week course of treatment with minimal toxicities. A double blind, placebo-controlled phase III trial of donepezil (5–10 mg/day) was conducted in long-term brain tumor survivors to confirm these favorable results. Although the neurocognitive composite score was not improved in the donepezil arm, there was improvement in verbal memory, working memory, visuomotor and psychomotor performance, and executive functioning, especially in patients with more severe baseline neurocognitive impairment. These results suggest that some long-term brain tumor survivors may benefit from treatment with donepezil, especially if they have severe neurocognitive impairment.

Other agents, such as methylphenidate and modafinil, have been evaluated in small pilot studies of brain tumor patients with a suggestion of benefit, but definitive studies have not yet been performed. Given the relatively limited toxicities of these agents, it may be reasonable to consider brief trials of these medications in selected patients. Cognitive rehabilitation and exercise may also be beneficial.

Conclusion

Therapeutic advances against malignant brain tumors (eg, concurrent chemoradiation with temozolomide and the use of bevacizumab) have added complexity and new complications for neuro-oncologists to master. Because optimal supportive care management in these patients promises to improve QOL and perhaps overall survival itself, understanding of these issues is mandatory. Further studies targeting fatigue, mood, and cognitive dysfunction are also of vital importance for improving our patients’ well-being.

Ultimately, many patients with malignant brain tumors reach a point in their illness when further antineoplastic treatment is futile and symptom management and end of life care become the priority. The transition to this phase is often gradual and requires sensitive and empathetic conveyance of the tumor and patient’s status at each encounter to ease this passage for the patient and family and to foster maintenance of autonomy and dignity.

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References


Introduction

Treatment-induced neurotoxicity represents a vast chapter of neuro-oncology because of its frequency, polymorphic clinical presentation, and because the side effects of treatment may severely affect the quality of life in patients who may otherwise be cured of their primary tumour. In any cancer patient suffering neurological dysfunction, consideration should be given to iatrogenic damage to the nervous system [58]. All antineoplastic treatments (surgery, radiotherapy, chemotherapy) can lead to severe neurotoxicity, which often simulates other complications of cancer such as recurrence or metastases, but has to be distinguished from them.

Nowadays, many patients receive multimodality therapy, raising the question of additive or synergistic toxicity and making identification of the cause or causes very difficult. The neurotoxicity of radiotherapy, chemotherapy and combined treatment will be discussed briefly. More detailed reviews have been published [23, 34, 42, 58, 77, 78].

Neurological complications of radiotherapy

The main reason why the neurotoxicity of radiotherapy remains a significant problem is the low therapeutic index. Therapeutic ionizing radiation may affect the neural structures directly or indirectly when radiation therapy damages large blood vessels supplying the brain or endocrine organs, or when it produces secondary tumours. The neurological complications of radiotherapy are classified as acute, early-delayed or delayed. The most important are radionecrosis and cognitive dysfunction/leukoencephalopathy. Neurotoxicity of chemotherapy is frequent and depends upon dose, type of drugs (especially cisplatin and methotrexate) and their combination with radiotherapy.

Key words Radiotherapy · Chemotherapy · Neurotoxicity

Abstract Neurological complications of radiotherapy and chemotherapy can affect the central or peripheral nervous system. Most are dose-dependent and constitute a limiting factor in the administration of treatments. Radiation-induced neurological complications are classified as acute, early-delayed or delayed. The most important are radionecrosis and cognitive dysfunction/leukoencephalopathy. Neurotoxicity of chemotherapy is frequent and depends upon dose, type of drugs (especially cisplatin and methotrexate) and their combination with radiotherapy.

Key words Radiotherapy · Chemotherapy · Neurotoxicity

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Neurological complications of radiotherapy and chemotherapy

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Corticosteroids are useful in relieving the acute complications of radiotherapy. In patients with threatened herniation, high-dose steroids (at least 16 mg dexamethasone daily) should be administered over 2–3 days before the onset of radiotherapy, and large doses per fraction should be avoided.

Early-delayed complications

Early-delayed complications occur between 2 weeks and 3–4 months after the completion of radiotherapy and may take several forms: (1) The somnolence syndrome develops in many patients (particularly children) who have received whole brain or large volume irradiation [25]. It is mainly characterized by hypersomnia, drowsiness, irritability and sometimes headache and fever. At this stage, neuropsychological evaluation often demonstrates attention deficits and alteration of recent memory functions. Improvement occurs spontaneously over a few weeks to months. Steroids reduce the duration of the syndrome and may prevent its development. (2) In about 15% of patients, early-delayed complications simulate local tumour recurrence. Patients may complain of recurrent focal symptoms. Computed tomography (CT) of the brain or magnetic resonance imaging (MRI) also suggest recurrent tumour with an increase in the size of a low-density lesion and the appearance of contrast enhancement not previously present. Improvement occurs spontaneously, but steroids accelerate its resolution [38]. (3) A severe leukencephalopathy with cognitive dysfunction and pseudobulbar syndrome is a very rare early-delayed complication of cerebral irradiation, which may be transient or persistent. Most of the patients who develop this syndrome are elderly or have received concurrent chemotherapy. (4) A rare but serious early-delayed syndrome is brain stem encephalopathy following irradiation of the posterior fossa. Most patients recover spontaneously, but the symptoms may progress to stupor, coma and death [48]. (5) In the spinal cord, a transient Lhermitte’s sign is the classic presentation of early-delayed complication [43].

The pathogenesis of early-delayed complications is unknown, although radiation-induced demyelination resulting from transient damage to oligodendroglia is suspected.

Delayed complications

Delayed complications occur 4 months to many years after completion of radiotherapy. The likelihood that the irradiation will induce delayed damage to the nervous system depends on many factors including the total dose delivered to the nervous system, the dose delivered with each treatment, and the total volume of nervous system irradiated. On the brain, a dose of 60 Gy delivered with 1.8–2 Gy fractions represents the upper limit of the “safe dose”. Other factors that influence tolerance of the nervous system include the length of survival after completion of radiation therapy, the presence of other systemic diseases that enhance the side effects of irradiation (e.g., diabetes, hypertension), concomitant chemotherapy and other unidentifiable host factors [74]. The brain, spinal cord and peripheral nerves may be affected.

### Table 1 Neurological complications of radiotherapy

<table>
<thead>
<tr>
<th></th>
<th>Acute (minutes–1 week)</th>
<th>Early-delayed (4–16 weeks)</th>
<th>Late-delayed (4 months–years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Acute Encephalopathy</td>
<td>Radiation necrosis</td>
<td>Dementia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late cognitive dysfunction</td>
<td>Endocrinopathy</td>
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<tr>
<td></td>
<td></td>
<td>Transient cognitive function</td>
<td>Radiation arteriopathy</td>
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<td></td>
<td></td>
<td>Radiation-induced tumour</td>
<td></td>
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<tr>
<td>Spinal cord</td>
<td>Lhermitte’s sign</td>
<td>Transverse myelopathy</td>
<td>Haemorrhagic myelopathy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Motor neuron syndrome</td>
<td></td>
</tr>
<tr>
<td>Cranial nerves</td>
<td>Anosmia</td>
<td>Visual loss</td>
<td>Hearing loss, hair cell damage</td>
</tr>
<tr>
<td></td>
<td>Ageusia</td>
<td>Hearing loss</td>
<td>Lower cranial nerve paralysis</td>
</tr>
<tr>
<td>Peripheral nerves</td>
<td>Brachial or lumbosacral reversible plexopathy</td>
<td>Brachial or lumbosacral late plexopathy</td>
<td>Radiation-induced tumours</td>
</tr>
</tbody>
</table>

* Italics indicate those that are irreversible
signs simulating a tumour de novo. CT may show increased hypodensity affecting predominantly the white matter, occasionally with contrast enhancement. The white matter lesions are seen better on MRI. It may be extremely difficult to differentiate necrosis from recurrent tumour. Positron emission tomography (PET) with glucose, single photon emission computed tomography (SPECT) with thallium or methoxy-isobutyl-isonitrile (MIBI) or angiogram can help, but in fact the only way to confirm the diagnosis of radionecrosis is a pathological examination of a surgical specimen.

Histologically, the typical lesion is an area of coagulative necrosis in the white matter, with relative sparing of the overlying cortex. The most striking abnormalities are found in blood vessels, with hyalinized thickening and fibrinoid necrosis of the walls [7].

These patients do best when the area of radiation necrosis is resected. Most patients respond transiently to corticosteroids, and there are reports of prolonged responses after corticosteroid therapy without surgery at the price of a frequent dependence on corticosteroids. A few studies suggest that anticoagulants may be of help [30].

There are two hypotheses concerning the pathogenesis of this disorder. The first is that the vascular changes (particularly of the microcirculation) lead to infarction and necrosis. The second is that radiation therapy directly damages glial cells, both astrocytes and oligodendrocytes, leading to destruction of tissue.

Cognitive dysfunction/leukoencephalopathy. Radiation-induced cognitive dysfunction and leukoencephalopathy without necrosis is becoming the most frequent complication in long-term survivors [14]. This clinical “entity”, also called “diffuse radiation injury” or “radiation-induced leukoencephalopathy” differs from radionecrosis in clinical-radiological aspects as well as in pathology. The most dramatic complication is dementia, but there is also evidence that radiotherapy can induce a less severe encephalopathy leading to subtle neuropsychological impairment [2, 77].

Mild or moderate neuropsychological impairment: This complication may occur in children (after prophylactic treatment of acute leukaemia or irradiation for primary brain tumour) and in adults (after prophylactic irradiation for small cell lung cancer or in long-term survivors of primary or secondary brain tumours). The symptoms generally occur within 4 years of irradiation and are mainly characterized by attention deficits, memory dysfunction and immediate problem solving ability. CT changes consist in ventricular enlargement and periventricular hypodensities with an increase in the normal interface between grey and white matter. On MRI, which is more sensitive than CT, the characteristic abnormality is a bilateral increase in T2 signal throughout the white matter with a gross correlation between MRI lesions and neuropsychological status.

The clinical course is usually characterized by slow decline of neuropsychological scores without decrease in performance status, but spontaneous stabilization may also occur [77].

Radiation-induced dementia: Progressive “subcortical dementia” represents the main clinical characteristic of this disorder. At a late stage, severe cognitive deterioration is typically characterized by a severe intellectual loss, with predominant fixative memory impairment, difficulties in focusing attention, emotional lability and apathy. Productive phenomena such as delirium or hallucination are typically absent. Signs of cortical involvement, like aphasia, apraxia or agnosia, are unusual. As a consequence of preserved insight, depression is frequent, but antidepressants do not improve intellectual performance. Gait disturbances, ranging from mild retropulsion to severe ataxia, are constant features, as is incontinence at later stages [16]. CT and MRI are invariably abnormal and show severe white matter abnormalities, ventricular enlargement and cortical atrophy. A diagnosis of radiation-induced dementia requires that all other possible causes of organic dementia have been carefully excluded. A normal-pressure hydrocephalus should also be ruled out, particularly when CT or MRI show gradual ventricular enlargement in the absence of cortical atrophy and when temporal horn enlargement is prominent.

The course is characterized by progressive deterioration (80% of cases), more rarely by stabilization and exceptionally by a lasting improvement. Patients become bedridden over a few weeks to months and usually die 1–48 months after the onset of symptoms. There is no effective therapy.

There are at least three factors that affect the risk of developing cognitive dysfunction/dementia: (1) radiation schedule: the risks of cognitive dysfunction are very low with “safe” doses of whole brain irradiation and are virtually absent for patients undergoing focal conventional radiotherapy alone (i.e. without concurrent chemotherapy); (2) concurrent chemotherapy: the frequency of cognitive dysfunction/dementia is increased in patients treated with radiotherapy and concurrent chemotherapy, at least when methotrexate (MTX) is used (see below); (3) age: elderly patients appear to be much more sensitive to the diffuse neurotoxicity of radiotherapy.

The pathological substrate for intellectual decline has not been clearly identified, but all authors found predominant involvement of the white matter, in agreement with neuropsychological and radiological findings. Diffuse white matter spongiosis, multiple miliary foci of necrosis, and demyelination with severe loss of oligodendrocytes have been reported.

Radiogenic tumours. Radiation-induced tumours, including meningiomas, sarcomas and, less frequently, gliomas and malignant schwannomas, may appear years to decades
after cranial irradiation and follow even low doses of radiation therapy. Malignant or atypical nerve sheath tumours may follow irradiation of the brachial, cervical and lumbar plexuses [22, 85]. Some patients may be able to tolerate additional surgery, radiation therapy or chemotherapy [17].

**Vascular abnormalities.** Lesions of large blood vessels, whether intra- or extracranial, generally follow radiation therapy by many years. Patients develop transient ischaemic attacks or strokes. Arteriography reveals stenosis or occlusion of the radiation portal, sometimes associated with moyamoya disease. The pathological changes in radiation-induced vascular occlusion are similar to those of severe atherosclerosis [37, 51, 58].

**Endocrinopathies.** After irradiation, endocrinopathies may result from direct damage to the glands themselves or more frequently from a hypothalamic dysfunction. Primary hypothyroidism or hyperparathyroidism may appear after irradiation for Hodgkin’s disease, head and neck tumours, or after craniospinal irradiation [71].

Hypothalamic-pituitary dysfunction is a frequent delayed complication of irradiation for head and neck tumours, brain tumours, or after prophylactic irradiation for acute leukaemia, especially in children [52]. The vulnerability of the hypothalamus could be owing to a greater radiosensitivity.

In children, the most frequent endocrinopathy is growth hormone deficiency, which should be differentiated from growth failure resulting from spine irradiation. Gonadotropin deficiency and secondary or tertiary hypothyroidism are less frequent endocrinopathies.

In adults, radiation-induced progressive endocrine dysfunction of hypothalamic origin has been reported in as many as 80% of adults treated for head and neck cancer and in one-third of the patients treated for a supratentorial glioma. It is not clear how often these endocrine abnormalities are clinically relevant. In addition to hyperprolactinaemia, the main abnormalities are hypogonadism and hypothryoidism. Adrenal failure is rare. No patient has had diabetes insipidus.

**Delayed complications of spinal cord irradiation**

Late-delayed radiation myelopathy appears in two forms. The first and more common is characterized by progressive myelopathy, often beginning as a Brown-Séquard syndrome and progressing over weeks or months to paraparesis or quadriplegiasis [41, 62]. The myelopathy sometimes stabilizes, leaving the patient with only a moderate paraparesis. The myelogram is usually normal. MRI may show spinal cord swelling and a hypersignal within the cord, as well as contrast enhancement. Pathologically, the lesions are characterized by confluent areas of necrosis with a predilection for the white matter. There is no effective treatment, although corticosteroids sometimes delay progression of the lesion.

A second form of late-delayed radiation myelopathy involves anterior horn cells and occurs mainly after pelvic irradiation. Months to years following irradiation, patients develop a subacute flaccid, often asymmetrical paraparesis that affects both distal and proximal muscles, accompanied by atrophy, fasciculations, and areflexia. There is no sensory disturbance or sphincter dysfunction. The myelogram and MRI are normal. On electromyography (EMG) there are varying degrees of denervation, but sensory and motor conduction velocities are normal. The deficit usually stabilizes after a few months, often while the patient is still able to walk [47, 68]. Pathological reports describe degeneration of cauda equina roots with anterior horn cell central chromatolysis.

**Delayed complications of peripheral nerve irradiation**

An early-delayed brachial plexus reaction is characterized by paraesthesias in the hand and forearm, sometimes associated with pain and accompanied by weakness and atrophy in a C6–T1 distribution. Nerve conduction studies reveal segmental slowing, and the course is characterized by recovery over a few weeks or months. This disorder is particularly common when carcinoma of the breast is being treated [70]. Late-delayed radiation plexopathy has been reported after irradiation of either the brachial or lumbosacral plexus [44, 59]. The disorder usually occurs a year or more after radiation therapy with doses of 60 Gy or more when conventional fractions are used. Brachial plexopathy is characterized by paraesthesias, loss of sensation and weakness of muscles in the upper or lower plexus. This disorder is frequently accompanied by lymphoedema and palpable induration in the supraclavicular fossa. Clinical or electrical myokymic discharges in the territory of affected nerves is a useful criterion for differentiating radiation damage from tumour infiltration of the plexus [35]. The course is unpredictable. The disorder may stabilize after many months to years of slow deterioration or progress rapidly to a panplexopathy, rendering the entire arm useless, although usually without severe pain. The important differential diagnosis is between radiation damage and recurrent tumour affecting the plexus. The most important differentiating clinical feature is pain. Severe pain is almost invariably present if tumour is the culprit and is rare with radiation fibrosis. CT or MRI is important to exclude a tumour mass, but often they simply reveal a diffuse loss of tissue planes that is nonspecific and can be seen with either plexopathy or tumour infiltration. The need for diagnostic certainty rarely requires surgical exploration of the plexus.

Lumbosacral plexopathy causes a slowly progressive weakness of one or both legs. Pain is usually absent [75].
Neurological complications of chemotherapy

Neurotoxicity of antineoplastic drugs is frequent (Table 2). As for radiation-induced complications, a low therapeutic index is an important dose-limiting factor for these agents. Chemotherapy neurotoxicity produces a limited number of nonspecific clinical pictures [58].

Table 2 Neurotoxicity of chemotherapeutic agents in humans (modified from [17])

<table>
<thead>
<tr>
<th>Agents</th>
<th>Drug</th>
<th>Neurotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormonal agents (except adrenocorticosteroids):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex hormones</td>
<td>Diethylstilbestrol</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ethinyl estradiol</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Stillbestrol</td>
<td>+</td>
</tr>
<tr>
<td>Antioestrogens (receptor-binding-agents)</td>
<td>Tamoxifen</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Megestrol</td>
<td>-</td>
</tr>
<tr>
<td>Progestins</td>
<td>Hydroxyprogesterone capraote</td>
<td>-</td>
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<tr>
<td>Nonhormonal agents:</td>
<td></td>
<td></td>
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<tr>
<td>Plant alkaloids (mitotic inhibitors)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Periwinkle derivatives</td>
<td>Vincristine</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Vinblastine</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Desacetyl vinblastine amide</td>
<td>-</td>
</tr>
<tr>
<td>– Podophyllotoxins</td>
<td>Epipodophyllotoxin VM-26</td>
<td>?+</td>
</tr>
<tr>
<td></td>
<td>Epipodophyllotoxin VP-16</td>
<td>?+</td>
</tr>
<tr>
<td>– Other plant alkaloids</td>
<td>Paclitaxel</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Docetaxel</td>
<td>+</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Bleomycin</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Actinomycin D</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adriamycin</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Daunomycin</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mithramycin</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mitomycin C</td>
<td>-</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>Methotrexate</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5-Fluorouracil</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>6-Mercaptopurine</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6-Thioguanine</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Cytarabine</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5-Azacytidine</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hydroxyurea</td>
<td>-</td>
</tr>
<tr>
<td>Alkylating agents</td>
<td>Cyclophosphamide</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Melphalan</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Busulfan</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CCNU</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>BCNU</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Thiopeta</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chlorambucil</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Aziridinylbenzoquinone</td>
<td>-</td>
</tr>
</tbody>
</table>

Clinical pictures

Acute encephalopathy

This is one of the most frequent syndromes occurring after administering many different agents: MTX [high dose intravenous (IV), intrathecal (IT)], cisplatin, vincristine, asparaginase, procarbazine, 5-fluorouracil, cytosine arabinoside, nitrosoureas [high dose or intra-arterial (IA), ifosfamide/mesna, tamoxifen, etoposide (high dose), paclitaxel (?). It generally begins with insomnia, rapidly fol-
lowed by a state of confusion that may be associated either with stupor or, more often, with agitation. Generalized seizures and myoclonus may occur.

**Stroke-like episodes**

These occur after treatment with high-dose MTX or, more rarely, cisplatin. They are typically characterized by the acute onset of encephalopathy with fluctuating motor deficit that resolves spontaneously.

**Chronic encephalopathy**

The most characteristic is a “subcortical dementia” of variable severity developing progressively, months to years after treatment that often but not always included a combination of cranial radiotherapy and chemotherapy. The syndrome is characterized by apathy, intellectual and memory loss, frontal syndrome, sleep disorders and often incontinence and gait disorders. Seizures may be present [58]. Spontaneous improvement may occur but, in many cases, progressive deterioration is the rule. The main agents incriminated in the syndrome are indicated in Table 3.

**Cerebellar syndrome**

A cerebellar syndrome, ranging from a simple gait ataxia to a pancerebellar syndrome, has been reported in patients treated with 5-fluorouracil and high-dose cytarabine (Ara-C). Recovery after discontinuation of the offending agent is variable.

**Transverse myelopathy**

This is a rare syndrome that is encountered essentially after IT treatment, especially with MTX, aracytine and more rarely thiotepa.

**Neuropathy**

Chemotherapy may induce different types of peripheral nervous system disorders: (1) An acute or subacute Guillain-Barré-like syndrome, probably owing to selective demyelination. Neuropathy is reversible after drug discontinuation. This syndrome is seen with suramin, an agent used for prostate cancer [20], and sometimes after Ara-C. (2) A distal sensorimotor axonal neuropathy is the most frequent type of neuropathy. It generally starts with distal paraesthesia. On examination, all sensory modalities are affected. Deep tendon reflexes disappear early, followed by distal extremity weakness. Vincristine is one of the most characteristic causes. Drug discontinuation usually permits recovery. (3) A purely sensory neuropathy or neuronopathy involving predominantly large fibres (cisplatin), but sometimes both large and small fibres (taxanes), is a classic complication. Loss of position sense and ataxia are frequent in cisplatin-induced neuropathy, whereas taxane-induced neuropathy affects preferentially pinprick and tact. Recovery is generally delayed and very slow. (4) Autonomic dysfunction consisting in constipation, orthostatic hypotension, urinary retention is sometimes associated with sensory motor neuropathy, especially with vincristine treatment.

### Table 3. Agents causing chemotherapy-induced chronic encephalopathy (modified from [32]) (IV intravenous, IT intrathecal, IA intra-arterial, IVT intraventricular, Ara-C cytarabine)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>IVT, IT, IV</td>
</tr>
<tr>
<td>BCNU</td>
<td>IV, IA</td>
</tr>
<tr>
<td>Ara-C</td>
<td>IV, IT</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>IV</td>
</tr>
<tr>
<td>Carmofur</td>
<td>IV</td>
</tr>
</tbody>
</table>

### Table 4. Cisplatin neurotoxicity

<table>
<thead>
<tr>
<th>Localization</th>
<th>Pathogenesis</th>
<th>Clinical findings</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>Axonal loss with demyelination in dorsal root ganglia</td>
<td>– Cramps</td>
<td>Recovery</td>
</tr>
<tr>
<td>Peripheral nerves</td>
<td>Hair cell loss</td>
<td>– Sensitive neuronopathy</td>
<td>Variable</td>
</tr>
<tr>
<td>Cranial nerves</td>
<td>Retinal cone dysfunction</td>
<td>– Proprioceptive loss</td>
<td>Permanent</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>Demyelinating lesion in the posterior columns</td>
<td>– Abolition of deep tendon reflex</td>
<td>Recovery</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td>– Ototoxicity</td>
<td>Permanent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Vestibular syndrome</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Visual loss (retinopathy, papilloedema, retrobulbar neuritis)</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Lhermitte’s sign</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Encephalopathy (seizures, cortical blindness)</td>
<td>Recovery</td>
</tr>
</tbody>
</table>
Cisplatin

Cisplatin is responsible for several neurological complications affecting the peripheral and central nervous systems (Table 4).

Peripheral neuropathy

This disorder follows doses of cisplatin of more than 400 mg/m² and is characterized by numbness and tingling in the extremities, which are occasionally painful. It affects predominantly large sensory fibres; the deep tendon reflexes disappear, and patients lose proprioception, sometimes to the point where they cannot walk. However, pin-prick and temperature appreciation are relatively spared, and motor power may be entirely normal. Nerve conduction studies are compatible with a primarily sensory axonopathy. If the patient survives the cancer, the neuropathy may improve and even return to normal after many months [58, 64]. Clinical studies testing protective agents are under way [11].

Ototoxicity

This is sometimes associated with a vestibulopathy and is another frequent complication of cisplatin. Hearing loss, resulting from hair cell damage, is often subclinical, detected only by serial audiograms; most loss occurs in the high-frequency range. Rarely, cisplatin produces acute deafness [33].

Cranial nerve neuropathies

These have occasionally been reported after infusion of cisplatin into the internal carotid artery [1].

Lhermitte’s sign

A transient Lhermitte’s sign during or shortly after treatment with cisplatin has been reported, suggesting the presence of transient demyelinating lesions in the posterior columns [19].

Encephalopathy

Encephalopathy with seizures and diffuse or focal brain dysfunction (in particular cortical blindness) may occur after IV or IA infusion.

Nitrosoureas

The nitrosoureas include lomustine (CCNU), carmustine (BCNU), methyl-CCNU, ACNU, HeCNU, streptozocin, and chlorozotocin. All of these drugs cross the blood-brain barrier easily.

Nitrosoureas in usual doses do not cause neurological toxicity. However, in patients with primary central nervous system (CNS) tumours who have been treated with high-dose IV BCNU or infusions of BCNU into the carotid artery, ocular toxicity and encephalopathy have been reported [8, 65]. After intracarotid treatment, the disorder is sometimes heralded by seizures and generally characterized by slowly progressive neurological dysfunction. The exact signs vary according to the area infused by the IA injection. White matter hypodensity is often apparent on CT at a site distant from the tumour being treated; with time, the area of white matter hypodensity may develop calcification. The pathology is that of a necrotizing encephalopathy, giving an appearance similar to that of radiation damage, but strictly confined to the vascular territories perfused by the drug.

Ifosfamide

Ifosfamide is an analogue of cyclophosphamide with substantial neurotoxicity. The most common neurological side effect occurring in 10–40% of cases is a diffuse encephalopathy with somnolence, which may rarely progress to coma and even death [84]. Methylene blue may reverse the encephalopathy [46].

Thiotepa

This drug is not neurotoxic when it is given systemically at conventional doses. At high doses prior to bone marrow transplantation, severe neurotoxicity may occur (somnolence, seizures, coma and even death) [49]. IT thiotepa (10 mg/m²) can induce a severe radiculomyelopathy.

Procarbazine

Procarbazine is now always given orally; it may produce an encephalopathy ranging from mild drowsiness to stupor or, rarely, a manic psychosis. A peripheral neuropathy occurs in some patients after several weeks of treatment; it is reversible after discontinuation of the drug [81].

Vincristine

Vincristine and, to a lesser degree, vinblastine and vindesine cause peripheral neuropathy. Most patients receiving vincristine develop paraesthesias in the fingertips and feet, and absent ankle jerks. In a few patients, there may be loss of all sensory modalities as well as motor weakness and, in particular, bilateral footdrop. The weakness
can begin several weeks after completion of the course of vincristine. The drug should be given with care to patients with any preexisting peripheral neuropathy. Nerve conduction velocities and EMG suggest a “dying back” axonal neuropathy. Histological studies confirm the presence of a primary axonal neuropathy. Vincristine probably causes its effects on the peripheral nervous system by its reaction with microtubules within axons, interfering with axonal transport [17, 69, 73].

Rarely, individual cranial nerves, including the oculomotor nerves, recurrent laryngeal nerve and optic nerve, are involved. One-third of the patients suffer autonomic symptoms including ileus with cramping abdominal pain followed by severe constipation, at times associated with urinary hesitancy, impotence or orthostatic hypotension [58, 61]. A few patients develop seizures following IV administration of Vinca alkaloids.

Paclitaxel (taxol) and docetaxel (taxotere)

Approximately 60% of patients receiving taxol at a dose of 250 mg/m² develop paraesthesias of the hand and feet. In some, the symptoms do not progress, but sensory or sensorimotor peripheral neuropathy may be a dose-limiting effect [24, 29, 66], especially when taxol is combined with cisplatin [13, 80]. Taxotere can also induce a sensory neuropathy affecting all sensory modalities [36, 55]. Pathological study of the peripheral nerves reveals axonal damage with secondary demyelination. The possible preventive value of nerve growth factor is being tested. Rarely, taxol and taxotere cause proximal motor weakness [26].

5-Fluorouracil

At usual doses, 5-fluorouracil (5-FU) rarely causes neurological toxicity, although high-dose 5-FU sometimes causes a florid cerebellar syndrome. On rare occasions, the drug has been reported to produce encephalopathy, optic neuropathy or an oculomotor disturbance [63]. Combining 5-FU with levamisole can induce an inflammatory multifocal leukoencephalopathy [39].

Methotrexate

MTX causes both acute and delayed neurotoxicity after IT administration or after IV high-dose MTX (Table 5).

Acute toxicity

*Aseptic meningitis.* This begins 2–4 h after IT injection and generally lasts 12–72 h [53]. The symptoms consist in headache, stiff neck, nausea, vomiting, fever and lethargy. Cerebrospinal fluid (CSF) pleocytosis is often present. The symptoms are self-limited, and there is no specific treatment. Rarely, sudden death has been reported during or shortly after intrathecal instillation.

*Transverse myelopathy.* This is a rare complication whose clinical presentation consists in pain in the legs followed by rapidly developing sensory changes, paraplegia and bladder dysfunction. The symptoms usually begin 30 min–48 h after IT treatment, but may be delayed. Pathologically, there is a necrotic myelopathy without striking inflammation or vascular abnormalities. The pathogenesis is believed to be an idiosyncratic reaction to the drug [28, 32].

*Stroke-like syndrome.* In both adults and children, a stroke-like syndrome occasionally follows systemic high-dose MTX infusion given in weekly treatments. The disorder characteristically follows the treatment by 5 or 6 days and is characterized by alternating hemiparesis associated with aphasia and sometimes encephalopathy or coma. Unequivocal seizure activity is rare. The electroencephalogram is slow. Patients generally recover spontaneously within 48–72 h. The syndrome does not usually recur after subsequent treatments. The pathogenesis of the disorder is unknown [79].

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Methotrexate neurotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Route</strong></td>
<td><strong>Dose</strong></td>
</tr>
<tr>
<td>IT</td>
<td>Conventional</td>
</tr>
<tr>
<td>IT</td>
<td>Several treatments</td>
</tr>
<tr>
<td>IV</td>
<td>High dose</td>
</tr>
<tr>
<td>IV or IT</td>
<td>High dose</td>
</tr>
<tr>
<td>IV or IT</td>
<td>High dose</td>
</tr>
</tbody>
</table>
Delayed toxicity

Although MTX alone can induce delayed toxicity, it is much more frequent when this agent is combined with radiotherapy, and this issue is discussed below.

Cytosine arabinoside

Cytosine arabinoside is used both systemically and intrathecally. Intrathecally, it can cause aseptic meningitis or, on rare occasions, myelopathy. High doses can cause an acute pan-cerebellar dysfunction, particularly in patients who have received more than 36 g/m² of the drug and especially in those over the age of 40 years [40, 54]. Pathological changes include widespread dropout of Purkinje cells. In some patients, the disorder resolves spontaneously. There is no treatment. High-dose cytosine arabinoside has also been reported to produce a peripheral neuropathy mimicking the Guillain-Barré syndrome [5]. A brachial plexus neuropathy has been described. Encephalopathy has occasionally been reported, as has an extrapyramidal syndrome.

Fludarabine

In lower doses, the drug causes little neurotoxicity except for transient somnolence. At doses greater than 40 mg/m² per day, a severe encephalopathy may occur with cortical blindness, dementia and sometimes coma. MRI and pathological examination show a necrotizing leukencephalopathy, most severe in the occipital lobes [12].

Asparaginase

This agent may interfere with liver function and may cause hepatic encephalopathy. However, L-asparaginase affects coagulation, depletes plasma proteins involved in coagulation and fibrinolysis, and may cause haemorrhagic or thrombotic complications. If patients receiving the drug develop sudden neurological abnormalities, in particular seizures and focal neurological signs, one should suspect thrombosis of the sagittal sinus [21].

Multiple chemotherapy

Most patients being treated for cancer receive multiple drugs rather than a single agent, and it is sometimes impossible to determine whether one agent is responsible for neurological dysfunction. An example are the vascular syndromes that follow multiple agent chemotherapy for testicular tumour. The vascular syndromes that may develop months to years after chemotherapy include Raynaud’s phenomenon, coronary occlusion and vascular occlusion of medium-sized vessels of the brain, leading to ischaemic infarction. Cisplatin may be the responsible agent, but this has not yet been determined with certainty [18, 72].

Neurological complications of combined treatment with radiotherapy and chemotherapy

The study of combined radiation-chemotherapy-induced neurotoxicity is difficult, because the lesions are often the same whatever the responsible agent (radiotherapy, chemotherapy or both) making identification of the culprit(s) difficult or even impossible. In addition, the incidence and clinical picture of combined neurotoxicity may vary greatly depending upon the schedule of administration (e.g. chemotherapy before, during or after radiotherapy), the precise doses of each modality and host factors.

Nevertheless, clinical experience indicates that “dangerous liaisons” between radiotherapy and chemotherapy sometimes exist. To explain these interactions, several hypotheses have been suggested: (1) chemotherapeutic agents may attack the same cellular structures as irradiation, resulting mainly in an additive effect; (2) the chemotherapeutic agent may act as radiosensitizer, increasing the sensitivity of the normal tissue to radiation (e.g. adriamycin); (3) radiotherapy may alter the distribution kinetics of chemotherapeutic agents in the CNS by increasing permeability of the blood-brain barrier or decreasing clearance of the drug. The main neurotoxicity of combined treatment is briefly described below [58] (Table 6).

Cisplatin

Cisplatin is a radiosensitizer in vitro. In vivo, cisplatin has been shown to be synergistic with cranial irradiation.

In humans, previous or concomitant cranial irradiation substantially increases the risk of severe audiotoxicity, at least in children [31, 45]. Cisplatin should be used with caution in patients with intracranial tumours who have received cranial irradiation, and the value of therapy must be weighed against the risk of significant hearing loss.

There is no clinical evidence that cisplatin increases the risk of radiation-induced delayed necrosis or myelitis, but we have seen a few cases of severe leukencephalopathy or myelitis in young patients who had received low or moderate doses of cerebral irradiation in combination with cisplatin.

Nitrosoureas

Experimental data on a radiosensitizing effect of nitrosoureas are contradictory. There is no evidence that previous or concomitant irradiation increase the neurotoxicity of high-dose IV or IA nitrosoureas.
Conventional systemic doses of BCNU or CCNU have been used in combination with cerebral irradiation in malignant gliomas for more than 25 years, including many phase III studies comparing radiotherapy alone and radiotherapy plus nitrosoureas. An increased neurotoxicity with the combined treatment has never been formally proved, but some observations suggest that the neurotoxic risk of combined treatment may be higher than treatment with radiotherapy alone [57, 76].

Methotrexate

The best example of combined radiation and chemotherapy neurotoxicity is provided by the association of cranial irradiation and IV or IT MTX [4].

Although concurrent radiation may reduce the incidence of acute aseptic meningitis, the risk of delayed complication is by far the most important issue. Three types of delayed complication have been reported: necrotizing leukencephalopathy, cognitive dysfunction and mineralizing microangiopathy.

Necrotizing leukencephalopathy/dementia

Necrotizing leukencephalopathy begins within a year of treatment. This syndrome may rarely follow repeated doses of high-dose IV MTX alone or IT MTX alone in patients with defective CSF dynamics. Much more commonly, it occurs in patients receiving the standard dose of IV MTX and cranial irradiation for acute lymphocytic leukaemia or sometimes primary brain tumours [15].

The clinical picture consists of a multifocal encephalopathy with change of personality followed by dementia, seizures, ataxia, sphincter disorders, hemi- or quadriparesis and pseudobulbar palsy. CT of the brain or MRI show bilateral white matter lesions located predominantly in the periventricular areas (hypodensity on CT, hypersignal on T2-weighted images on MRI) as well as atrophy, ventricular dilatation and sometimes cortical calcification. The CSF examination typically shows increased protein and an elevated myelin basic protein indicating myelin breakdown. The course may be progressive, but most patients develop a stable neurological disability or, rarely, improve. There is no recognized treatment. Pathologically, the lesions are characterized by multifocal noninflammatory areas of coagulative necrosis located in the deep white matter. Axonal swellings and mineralization are characteristic findings [67].

The incidence and the potential risk of the development of leukencephalopathy are directly related to the total dose of cranial irradiation, systemic and IT MTX, and the sequence of their administration [15].

<table>
<thead>
<tr>
<th>Agent</th>
<th>Combined toxicity</th>
<th>Definite</th>
<th>Possible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>Ototoxicity (children)</td>
<td>Ootoxicity, leukencephalopathy, migraine-like episodes, myelopathy</td>
<td></td>
</tr>
<tr>
<td>Carboplatin</td>
<td></td>
<td></td>
<td>Ootoxicity, Leukoencephalopathy/necrosis, cognitive dysfunction, myelopathy</td>
</tr>
<tr>
<td>Nitrosoureas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Reduced incidence of aseptic meningitis, necrotizing leukencephalopathy, cognitive function with or without leukencephalopathy, mineralizing microangiopathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ara-C</td>
<td></td>
<td>Leukoencephalopathy, cognitive dysfunction, myelopathy</td>
<td></td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td></td>
<td>Myelopathy</td>
<td></td>
</tr>
<tr>
<td>Vincristine</td>
<td></td>
<td>Myelopathy, peripheral Neuropathy</td>
<td></td>
</tr>
<tr>
<td>Etoposide</td>
<td></td>
<td>Encephalopathy</td>
<td></td>
</tr>
<tr>
<td>Actinomycin D</td>
<td></td>
<td>Leukoencephalopathy/necrosis, cognitive dysfunction</td>
<td></td>
</tr>
<tr>
<td>Multiagent chemotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preparative regimen for bone marrow transplantation</td>
<td>Acute encephalopathy, leukoencephalopathy/cognitive dysfunction, stroke</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6 Neurotoxicity of combined radiation/chemotherapy (modified from [78])
The timing of irradiation and chemotherapy is important. The risk of leukoencephalopathy is lower when MTX is given before radiotherapy, while the risk is maximal when MTX and cranial irradiation are given concurrently or when MTX is given after radiotherapy. Other risk factors for the development of a leukoencephalopathy include young age, renal or liver failure, neoplastic meningitis and abnormalities of CSF flow or hydrocephalus as well as malposition of a catheter tip of an Omaya reservoir.

Isolated neuropsychological decline

Isolated neuropsychological decline without dementia is much more common than overt leukoencephalopathy with dementia.

In children, cognitive dysfunction has been reported mainly in long-term survivors of acute lymphocytic leukaemias (ALL) treated with cranial irradiation and parenteral MTX [9]. The clinical picture consists mainly of learning disability affecting school performances and academic achievement, which may require (in up to 40% of cases) placement of the children in special education classes.

The radiological expression and pathological substratum of this syndrome have not been clearly delineated as the respective responsibilities of cranial irradiation and of parenteral MTX.

Reduction of the dose of prophylactic radiotherapy from 24 to 18 Gy did not substantially lower the risk of cognitive dysfunction. Nowadays, most patients with standard-risk ALL do not receive cranial irradiation, and hopefully the incidence of this complication will be significantly reduced.

In adults, recent evidence suggests that the use of IV and IT MTX-based chemotherapy prior to cranial irradiation for primary CNS lymphoma [27] produces a high risk of progressive cognitive dysfunction, which may culminate in severe dementia/leukoencephalopathy. Clearly, the administration of MTX before irradiation is not sufficient to prevent the risk of combined toxicity in this setting. Age over 60 years is apparently a major predisposing factor for this complication.

Mineralizing microangiopathy

Mineralizing microangiopathy is a pathological entity attributed to the combined toxicity of cranial irradiation and MTX. It is characterized by the presence of mineralized deposits in the small vessels of the grey matter. Patients may be asymptomatic or present a variety of symptoms including headache, ataxia, seizures or cognitive dysfunction [15]. On CT, calcification of the basal ganglia and vascular border zones of the cerebral cortex are found. Young age and a long delay (at least 10 months) after the completion of treatment are predisposing factors for this complication.

The mechanisms underlying the increased toxicity of combined treatment with MTX and radiotherapy are poorly known. MTX has radiosensitizing properties. In addition, radiation induces a breakdown of the blood-brain barrier, which facilitates the entry of MTX into the parenchyma. However, the radiation-induced opening of the blood-brain barrier is relatively small, and it is not clear whether this effect is sufficient to substantially increase penetration of MTX.

Finally, both cranial irradiation and MTX could cause cerebral damage through a direct toxic effect on the same target cells, including neurons and glial cells.

Cytosine arabinoside (cytarabine)

Experimental studies suggest that parenteral Ara-C enhances the effect of radiation on the CNS when administered shortly before irradiation. While the incidence of severe cerebellar dysfunction is apparently not increased by previous cranial irradiation, a few reports suggest enhanced brain (cognitive dysfunction/leukoencephalopathy) and spinal cord neurotoxicity from combined treatment in humans.

5-Fluorouracil

5-FU has radiosensitizing properties, but neurotoxicity is apparently not increased by cranial irradiation.

Vincristine

Isolated case reports suggest that combined treatment with vincristine and radiotherapy increases the risk of myelitis and peripheral neuropathy [10].

Etoposide (VP-16)

Etoposide has no neurotoxicity in standard doses. Severe encephalopathy with confusion, increased hemiparesis and seizures has been observed after high-dose therapy in patients with recurrent malignant gliomas [50]. These complications were not found in patients who received the same regimen for lung carcinoma, suggesting that previous irradiation played a role in this toxic reaction.

Actinomycin D

Actinomycin D is a well-known radiosensitizer that reduces the repair of sublethal radiation damage. This agent
can also induce a recall phenomenon when it is used after irradiation. Very early and severe cases of radionecrosis have been reported after combined treatment with radiotherapy [60].

Bone marrow transplantation

A combination of high-dose chemotherapy (most often with cyclophosphamide) and total body irradiation is the standard regimen prior to bone marrow transplantation. In this setting, neurological complications are frequent, affecting up to 60–70% of patients. The nature of these complications is multifactorial, related to the primary disease (CNS involvement by tumor), to the deleterious effects of myelosuppression (CNS infection or hemorrhage) or to a nonspecific “metabolic” encephalopathy or to a nonspecific “metabolic” encephalopathy [56, 82]. Toxicity of the preparative regimen is difficult to identify, further complicated by the fact that many patients have been previously treated with neurotoxic chemotherapy (MTX) and sometimes cranial irradiation. In one study, the frequency of regimen-related neurotoxicity was 14% for mild/moderate toxicity, while 1.5% had severe toxicity [3].

Acute complications include transient drowsiness and occasionally seizures or a severe encephalopathy. Delayed complications generally consist in mild/moderate cognitive dysfunction with cerebral atrophy.

Multiagent chemotherapy

A syndrome of multifocal pontine lesions sometimes occurs after combined treatment with cranial irradiation and various chemotherapeutic agents (MTX, nitrosoureas) [6]. The pathological hallmark of this syndrome consists in multifocal necrotic lesions in the basis pontis without inflammation or vascular abnormalities.

References


Abstract | Study of brain tumors (BT) has revealed the importance of cognitive and behavioral assessment to clinical care and prognosis. This paper overviews recent literature, focusing on the main points of interest and current methods, providing recommendations for advancing research. Histological aspects, disease progression, treatment-related neurotoxicity, and co-morbidities determine the cognitive patterns of BT. Mental slowing with prominent executive and memory compromise usually mark the advanced phases of disease, whereas normal cognitive performance or subtle behavioral symptoms characterize the early disease course, irrespective of tumor location. Neurocognitive assessment may indicate brain damage in otherwise neurologically normal patients, explain pathological behavior, and provide reliable measures of outcome, contributing to improving the management of patients. Scarce attention has been devoted to social cognitive deficits which are expected to impair autonomy and relationships. Interest in non-pharmacological treatment of cognitive impairment is a growing area although methodological difficulties persist. Homogeneous patient populations, longitudinal study designs including baseline evaluations, and measurement of the lowest and highest levels of cognitive performance seem indispensable to advancing the study of the cognitive and behavioral changes provoked by BT. Future investigations are also expected to clarify the clinical significance of such changes, their effect on quality of life, and the efficacy of specific rehabilitation treatments.

Keywords | Cognitive function · Behavior · Brain tumor · Glioma · Radiotherapy · Surgery · Cognitive rehabilitation

Introduction | Impairment of cognitive function, for example language, memory, visuospatial perception, time–space orientation, attention, and executive function, occurs in nearly all patients with brain tumors (BT) and eventually compromises their independence [1, 2]. Therefore, assessment of cognitive impairments has become increasingly important to diagnosis and follow-up for clinical and research purposes [3, 4]. Neuropsychological testing is often required to implement the results of neurologic examination and neurophysiological or imaging studies that cannot explain the nature of behavioral alterations. Particular indications, for example the monitoring of multiple treatment, and the psycho-physical limitations of BT patients (e.g., fatigability, depression) place special demands on cognitive assessment. A few questions may guide the choice and planning of measurements: What are the mechanisms of impairment? Which cognitive patterns characterize BT? Who needs detailed evaluation? Which batteries respond to these questions? This paper overviews recent literature, focusing on current points of interest, open questions, and directions of research.

Causes and mechanisms of cognitive impairment | Histology, disease progression, treatment-related neurotoxicity, neural reorganization, individual psycho-physical conditions, and co-morbidity, for example epilepsy and
cardiovascular failures, contribute to determining the type and severity of cognitive impairment in BT patients [5–8]. According to recent findings, BT cause not only focal neuron disruption and mass effects but also alterations of brain connectivity [9]. Pathological changes in amplitude and synchronization of low-frequency connectivity, involving different neural networks, have been related to learning and memory deficits [10]. Together with toxic and metabolic insults, such alterations explain the non-focal cognitive patterns of BT, suggesting whole brain dysfunction.

The histology and location of BT are major determinants of onset, progression, and severity of impairment. In low-grade gliomas (LGG), clinical experience indicates mild cognitive deficits at disease onset, usually marked by epileptic seizures [11, 12]. In a pre-surgical investigation, Tucha et al. [13] found impaired executive functions, memory, and attention in 91% of patients, reporting specific tumor-related effects. However, neuropsychological tests are rarely conducted before treatment, preventing definite conclusions about the effect of the tumor. Radiotherapy (RT) may provoke different cognitive changes [14, 15], usually many months after disease onset [6]. According to Klein et al. [16], 1–22 years (a mean of six years) after diagnosis, memory, attention, perception, psychomotor speed, and executive ability were worse for 195 LGG patients than for healthy subjects and for patients with low-grade hematological illnesses. Cognitive decline was worse for irradiated than for non-irradiated patients; RT was associated with cognitive impairment, irrespective of disease duration and total radiation dose, but higher fraction doses (greater than 2 Gy) resulted in more severe deficits. This study also demonstrated that attention and loss of executive function was, in part, related to hemispheric location and antiepileptic drugs (AED) but not to surgery. In 65 out of 195 patients, 6–28 years (a mean of 12 years) after diagnosis, cognitive decline was significantly worse in the irradiated than in the non-irradiated patients, irrespective of fraction dose; the decline of executive function and information processing was related to brain atrophy and white matter changes [6]. By contrast, a study of 20 patients by the North Central Cancer Treatment Group, using a wide range of neuropsychological tests, reported no evidence of cognitive deterioration after RT during three-year follow-up [17]. Another study, in which LGG patients were screened for cognitive performance by the mini mental state examination (MMSE) before and after a mean of 7 years after RT [18], also showed that few patients had cognitive deterioration irrespective of the doses and modalities of radiation. This suggests that the tumor itself is of major importance in determining cognitive deficits, and that RT has additional deleterious effects or leaves the cognitive pattern unchanged [3, 6, 14–16]. It is difficult to disentangle the effects of LGG and RT, because tumor growth may outweigh the benefits of RT, and neural reorganization may compensate the effects of BT and RT [19], and concomitant therapy or co-morbidity may induce additional deficits. Worthy of note is that most neuropsychological studies of LGG are retrospective, patient groups and RT schedules are heterogeneous, and baseline evaluations are not performed [3, 5, 20–23], preventing precise deductions about RT.

Given increased survival, cognitive assessment has become increasingly important in high-grade glioma (HGG) patients. Steinbach et al. [24] and Hottinger et al. [2] reported the significant prevalence of cognitive deficits in long-term survivors of glioblastoma in Germany and USA. Bosma et al. [3] evaluated, prospectively, the course of cognitive functioning in 32 HGG patients: compared with baseline assessment performed between surgery and RT, the 8 and 16 month follow-ups revealed deterioration in attention, information processing, and psychomotor speed; cognitive decline was more pronounced in patients with tumor recurrence or under medication with corticosteroids and AED. Corn et al. [25] also reported progressive deterioration of cognitive functioning in glioblastoma patients. Brown et al. [26] showed that six, 12, 18, and 24 months after surgery the percentage of long-term survivors with cognitive impairment was stable in the absence of recurrence; a clinically significant decrease in the MMSE scores preceded the radiographic changes of tumor progression. Likewise, Armstrong et al. [27] showed that serial neuropsychological evaluations predicted tumor recurrence. Longitudinal studies suggest that the rate of tumor growth and multiple treatment determine the severity of cognitive impairment, although specific cognitive deficits may relate to tumor location [3, 26–28]. Further, in 80 patients with recurrent HGG, Meyers et al. [29] demonstrated that a verbal memory test score was independently and strongly associated with survival, when adjustments were made for age, Karnofsky performance status, and the number and extent of surgical resections.

Metastatic BT treated with whole brain RT or yet untreated may be associated with deficits in motor speed, manual dexterity, memory, and executive functions [30, 31]. In brain lymphoma, attention, executive functions, memory, and motor speed deficits represent prominent clinical manifestations, before and after whole brain RT or methotrexate chemotherapy (CT) [32, 33]. However, after CT, cognitive functions may also significantly improve or remain stable [34].

Surgery may provoke cognitive deficits related to the location and extent of tumor removal [11, 35]. Surgical resection of LGG may cause transient specific deficits [36], with improvement of memory functions supported by the
opposite hemisphere [11]; long-term impairments are not usually related to the type of surgery [19]. As for HGG, surgery is rarely a significant cause of cognitive deficits. Actually, reduction of mass effects may be beneficial to cognitive functioning, or prevent further deterioration. In a recent study of patients with high-grade tumors Talacchi et al. [37] observed presurgical deficits in memory and word fluency; although these functions improved after surgery, executive function worsened in relation to tumor size, irrespective of the extent of surgical removal. This suggests that, although BT affect cognitive function, surgery may contribute to improving or maintaining the stability of some abilities.

The cognitive effects of CT, steroids, and AED have not been systematically investigated [38]. The negative effects of CT cannot be easily distinguished from those provoked by RT, although CT usually causes transient symptoms [39]. Higher neurotoxicity has been reported with intra-arterial and intra-thecal CT, cisplatin, or methotrexate, but there is scarce neuropsychological documentation of its severity, typology, and duration [8]. AED mainly affect attention and executive function, although some deficits are counterbalanced by improvement secondary to seizure reduction [11] or they vary independently of the number of AED [40].

**Cognitive patterns**

The effect of BT location on specific cognitive patterns can vary. In this regard, the disorders caused by BT are usually less severe and localizing than stroke-related impairments [41]. Before surgery, RT, and CT, precise neuroanatomical correlations are inconstant: the functions of the area affected by the tumor may be preserved whereas those supported by the opposite hemisphere may be impaired by mass effects and epileptic discharges [11]. In slow-growing tumors, compensation and substitution neural mechanisms tend to mask focal deficits. In high-grade tumors, focal deficits may be surpassed by confusion, headache, and physical symptoms. After treatment, neuropsychological assessment rarely yields focal deficits. Therefore, clinical-pathological variables, treatment, and emotional-behavioral distress may provoke non-localizing cognitive patterns, manifested as mental slowing, poor psychomotor coordination, “frontal” behavior, personality changes, and memory failures [7–9, 12].

In the absence of confusion and psychological-behavioral changes, some cognitive patterns may be related to particular brain areas. Frontal tumors are associated with deficits in working memory, the inhibition of interference on ongoing actions, social cognition, risk assessment, decision making, use of external feedback, initiative, abstraction, flexibility, and expression. Temporal tumors may affect naming, verbal fluency, comprehension, memory, semantic competence, and social cognition. Tumors of the diencephalon and corpus callosum may provoke memory failure. Occipital-parietal tumors may impair visuospatial recognition, semantic competence, and social cognition. Tumors of the cerebellum may compromise the capacity to modulate and check the mental operations implicated in a variety of activity (executive function, prosody, grammar, theory of mind, spatial memory) [7, 11, 40, 42, 43]. Patients with malignant BT in the language-dominant hemisphere have more problems with memory, attention, word fluency, and verbal learning than patients with non-dominant hemisphere tumors [4], and have little chance of improving after surgery [44].

**Objectives of neuropsychological assessment**

Basic objectives, common to different neurological disorders, include identification of individual strengths and deficits and neuroanatomical correlation [37, 40, 45]. For patients with severe disease burden and short life expectancy, time-consuming assessment may uselessly increase fatigue and distress, whereas a few tests may contribute to determining the level of awareness and the reliability of informed consent before investigation or treatment. In single cases or groups, the objectives of neuropsychological testing vary in relation to the phase of disease and treatment. For instance, in the early course of the disease, tailored assessment may characterize the effects of BT in patients with otherwise normal neurology [7]; after diagnosis, the neuropsychological results provide criteria for clinical care and decision making [27, 46] and yield indicators for monitoring postsurgical changes and the effects of treatment that requires specific monitoring.

Some functions, for example autobiographical memory and verbal comprehension, affect patients’ ability to report their quality of life (QOL). Further, cognitive deficits are expected to impair QOL, by reducing the capacity to live flexibly in one’s own environment and to adopt a satisfactory lifestyle [33, 47]. Therefore, neuropsychological testing may contribute to QOL assessment, although a direct association between cognitive deficits and QOL has rarely been investigated [46]. Serial neuropsychological evaluations also support prognosis; test scores may predict survival in patients with HGG [29] or brain metastases [31] and may anticipate tumor recurrence by weeks or months [26, 27, 47]. When assessing QOL and prognosis, study of cognitive abilities may improve the management of patients and the results of clinical trials. Finally, neuropsychological testing may yield information for planning non-pharmacological treatment, for example cognitive rehabilitation or psychological support.
Neuropsychological testing

There is large variability in the prevalence of the cognitive deficits associated with BT, ranging from 29% in LGG to 90% in different tumor types [13, 17, 31]. Differences in neuropsychological measurements, and differences in patients populations, tumor types, and treatments may explain this variability. Table 1 summarizes the criteria commonly used to choose neuropsychological tests and batteries. Multidimensional testing enables comprehensive characterization of the cognitive pattern. One test, even if sensitive to a particular problem and with other functions, cannot yield exhaustive information. Pre-structured tests, for example WAIS and MMSE [17, 18], are inconstantly sensitive to specific changes in patients with BT, whereas tests tailored according to precise clinical or research purposes can provide accurate results [8, 17, 18, 29, 46, 47]. One neuropsychological index cannot reflect multiple cognitive changes and the MMSE is not generally regarded as a comprehensive tool for detection of BT or RT related deficits.

Neuropsychological testing should follow standardized procedures, have adequate psychometric properties (content, structure, convergent, and divergent validity; inter-rater and inter-test reliability), and have alternative forms. For BT patients the test measures should also be sensitive to the highest and lowest levels of performance, in different phases of disease and treatment, and be able to detect clinically significant changes (those with practical consequences or effects on everyday activity) that may be different from statistically significant changes (observed at the group level). The neuropsychological results should be contrasted with neurologists’ and patients’ reports of autonomy and QOL, with extrapolation of measurements with practical implications [16, 29, 43, 47].

Given the complexity of the pathogenic mechanisms and the variability of patients’ collaboration, stepwise evaluation, from brief testing to detailed examination, may flexibly satisfy clinical indications, patients’ fatigability, and staff burden. Screening should not surpass 30 min. Test timing, in particular in clinical trials, depends on the study design. In any case, baseline presurgical testing is indispensable to the determination of cognitive changes.

Table 2 summarizes the most sensitive tests used in multidimensional studies [6–8, 11, 18, 27, 29, 42, 43, 46–48].

According to Taphoorn and Klein [8], tests for patients with LGG or HGG, adopting a hierarchical model, assessing perception, information processing, attention, executive, memory, and intellectual abilities, would take approximately one hour. By use of a 40-min set of tests assessing cognitive impairment, disability, and QOL, Meyers and Hess [47] showed that tests for working memory, verbal learning, word fluency, attention, and manual dexterity predicted the radiological progression of malignant BT. Inclusion of a few neuropsychological test scores in a regression analysis with disease-related variables also enabled prediction of survival of patients with HGG [29]. Costello et al. [43] showed that specific testing of memory, visuospatial perception, executive, and intellectual abilities distinguished patients with frontal low-grade or non-malignant tumors from patients with frontal high-grade tumors who did not improve after RT. Douw et al. [6] adopted a small group of tests of executive function, mental control, attention, learning, and memory, documenting only attention deficits in long-term survivors of LGG treated by RT. Vigliani et al. [48], using a group of tests for memory, learning, abstract reasoning, attention, and executive function, demonstrated that a reaction time test was able to detect attention deficits 6 months after RT and to monitor long-term tumor progression. In adult patients with high-grade, low-grade, or non-malignant BT, lowest performance was associated with left hemisphere location and tests assessing memory and executive function were most accurate in detecting cognitive deficits (Giovagnoli et al., personal communication). Brown et al. [18]

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<th>Table 2</th>
<th>Cognitive tests sensitive to brain tumor and treatment effects [6–8, 11, 18, 27, 29, 42, 43, 46–48]</th>
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<tr>
<td>Trail making test, digit-symbol association, Corsi blocks span (visuomotor coordination speed, set shifting, working memory)</td>
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<td>Stroop color-word test, attentive matrices (divided attention, interference control)</td>
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<td>Grooved pegboard (motor speed and dexterity)</td>
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<td>Raven colored progressive matrices, Wisconsin card sorting test (abstraction, set shifting)</td>
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<td>Word fluency on letter and semantic cues, design fluency (initiative, fluency)</td>
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<td>Rey complex figure recall, short story recall (episodic memory)</td>
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<td>California verbal learning test, Rey auditory learning test (learning)</td>
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<td>Mini mental state examination</td>
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demonstrated that better MMSE scores in HGG patients were associated with longer time to tumor progression and suggested that even generic neuropsychological indices may yield practical information. Fliessbach et al. [49] described computer-based assessment of memory, verbal fluency, attention, and inhibitory control; testing was fast, valid, and reliable, and therefore useful for clinical trials, although executive functions were under-represented. Study of primary central nervous system lymphoma indicated that, as in glioma, attention, executive function, memory, and motor dexterity tests were sensitive measurements of impairment [32, 34]. In lymphoma patients treated by RT, Harder et al. [33] also demonstrated that psychomotor speed, attention, executive functions, memory, and learning were related to white matter changes caused by RT.

According to these studies, tests for attention, executive function, and memory can detect the main BT-related cognitive deficits and, among these, some measures also have clinical and prognostic significance. Recent findings in patients with focal brain damage and epilepsy [40, 50, 51] suggest that frontal and temporal lobe lesions impair the ability to understand others’ mental states, an important prerequisite to social behavior based on complex neural circuits [52]. Patients with tumors in different brain areas are expected to show social cognitive deficits as a consequence of the lesion itself, seizures, or altered brain connectivity [42], suggesting a screening evaluation of such domain.

Non-pharmacological treatment of cognitive deficits

Improved prognosis and attention to QOL have elicited increasing interest in non-pharmacological treatment of the long-term cognitive sequelae of BT. The effects of cognitive rehabilitation using either retraining or compensation strategies have been reported for single cases, small patients groups, or patient–caregiver dyads, with improvement in attention and memory for patients with low or high-grade BT [53–55]. Hassler et al. [55] documented much variability in the efficacy of cognitive training based on “holistic” memory empowerment for 11 patients with HGG, although mean group improvement was observed after 12 weeks. Gehring et al. [53] reported improvement of subjectively perceived cognitive function and scores in verbal attention tests for 140 patients with LGG or HGG. There is also some evidence that non-pharmacological treatment is feasible and well-accepted by patients and may have immediate or short-term benefits. However, a lack of control groups, randomized study designs, and neuropsychological baseline or follow-up evaluation prevent precise assessment of the reliability and efficacy of cognitive rehabilitation for BT patients.

Considering that cognitive impairment is a major issue for patients with a favorable prognosis, careful neuropsychological measurement of deficits and reserves are substantial prerequisites for non-pharmacological treatment of these patients.

Conclusions

Careful assessment of cognitive function may contribute to clarifying clinical status, providing reliable indicators for clinical care and treatment. According to the literature, recommendations for clinical care may be: to use sensitive not time-consuming tests, to adopt a stepwise evaluation strategy (screening tests followed by detailed evaluation of specific cognitive–behavioral disorders), and to compare the cognitive pattern with neurologic signs and patients’ reports. Cognitive testing should be routinely associated with QOL assessment, with the objective of verifying the reliability of self-reports and extrapolating the impact of cognitive deficits on everyday life. The neuropsychological results, with evaluation of behavior, mood, and QOL, may yield indications for non-pharmacological treatment. Recommendations for advancing research may be: to select homogeneous patient populations according to histology, lesion location, disease duration, and clinical burden, to plan perspective investigations including baseline pre-surgical assessment, to follow adequate test timing, to construct tests sensitive to the lowest and highest levels of performance, and to assess social cognitive ability.

Conflict of interest The authors declare they have no conflict of interest.

References

Palliative Care
Neuro-oncology and palliative care: a challenging interface

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Patients with high-grade gliomas almost invariably develop disease progression despite recent advances in anticancer therapy. Increasingly, the value of formal palliative care consultation and management has been recognized in both cancer and noncancer medicine. However, there is a paucity of data to definitively guide the provision of palliative care for neuro-oncology patients.

This paper aims to review the existing evidence for and describe the interface between palliative care and neuro-oncology, with a particular focus on glioblastoma multiforme (GBM). We also discuss the role of palliative care in nonmalignant neurologic disease where parallels with neuro-oncology might be drawn.

Glioblastoma

GBM remains an uncommon diagnosis. However, for those patients and carers affected, it is life-changing from first diagnosis. The median overall survival for patients receiving GBM therapy is 14 months, and fewer than 10% of patients are alive at 5 years following diagnosis. Further, patients older than 70 years and/or those with poor KPS may not benefit from current palliative anticancer therapies and can have significantly worse outcomes. Indeed, population-based management surveys document median survivals of <10 months and negligible 5-year survival rates. Many clinicians view GBM as having the worst of all cancer prognoses; however, median and long-term survival of GBM patients compare favorably with those of patients with other incurable cancers, such as metastatic pancreatic and metastatic lung.

GBM results in significant morbidity, which can be challenging for patients and their carers, as well as for health professionals. Physical symptoms include headache, nausea, vomiting, easy fatigue, and excessive somnolence. Local tumor effects, depending on site, may result in focal or generalized neurologic problems such as seizure, motor weakness, aphasia, and impaired vision. Mood and cognitive disturbance are common.

A multidisciplinary approach including neurosurgery, radiation oncology, neuro-oncology, and allied health is recommended to optimize treatment outcomes. However, few of these multidisciplinary teams would routinely include palliative care, despite the lack of curative treatment options and significant symptom burden. The palliative care needs of patients with GBM can be complex, with a paucity of published data in this area. Parallels do exist, however, between the needs of patients with GBM and those of patients with other, more common cancer diagnoses and nonmalignant chronic neurologic illness.

Palliative Care and Cancer Patients

Palliative care is defined by the World Health Organization as “an approach that improves the quality of life of patients and their families facing the problems associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems, physical, psychosocial and spiritual.” Palliative care encompasses symptom control and provision of practical support to patients and their carers—from first referral through terminal care and death and into bereavement.

The goal of palliative care is to maximize quality of life, drawing on the skills of a multidisciplinary team to help the patient live as actively as possible whilst neither hastening nor postponing death. Palliative care can play an important role in the management of malignant and nonmalignant conditions, in both inpatient and outpatient settings.

There is increasing recognition that early integration models of palliative care are beneficial, particularly...
when it is provided concurrently with anticancer therapy. A recent landmark study by Temel and colleagues\cite{15} is the first to definitively demonstrate a range of benefits to patients with metastatic non-small-cell lung cancer. Patients were randomized to early palliative care integrated with standard oncologic care or to standard oncologic care alone. Perhaps not surprisingly, there was objective demonstration that early intervention with palliative care services resulted in improved quality of life, improved symptom management, and a reduction in “aggressive therapies” at end-of-life.

Perhaps more surprising was the observation that patients referred at diagnosis for early palliative care intervention had an improved survival outcome of nearly 3 months. This was considered clinically significant as well as statistically significant. The basis for this improvement in median survival is the subject of much ongoing debate.\cite{16,17} With randomly allocated intervention groups evenly matched for performance status, gender, age, and disease stage, the observation is likely due to improvements in symptom control, quality of life, and mood, as for cancer patients there is evidence of an association between increased symptoms, in particular dyspnea and drowsiness, and shorter survival.\cite{18}

A new review\cite{19} of the effectiveness of palliative care consultation within a cancer center highlighted the prevalence of symptoms within a referred general cancer population. Six hundred eleven general cancer patients seen over 3 years had an average of 6 uncontrolled symptoms, including 80% presenting with fatigue and 70% with pain (14% classified as severe pain). Of note, review by the palliative care service resulted in significant improvements in pain, somnolence, and symptom distress scores and overall well-being, in most instances observed within the first few days of consultation.

Two recent systematic reviews have analyzed the effectiveness of palliative care involvement in cancer patients.\cite{20,21} Despite methodological limitations associated with much of the research in this population, these reviews add to accumulating evidence supporting the benefits that palliative care can offer. Improvements were demonstrated across a range of domains, including quality of life, patient and family satisfaction, and end-of-life care. Higginson and Evans,\cite{20} incorporating randomized controlled trials and observational/quasi-experimental studies, reported that specialist palliative care input significantly improved pain and symptom management and reduced hospital admissions. Improvement was demonstrated in a variety of care settings, including home-based, hospital consultation, and specialized inpatient palliative care.

Palliative Care and Nonmalignant Neurologic Disease

There is increasing recognition of the efficacy of palliative care for nonmalignant disease, in particular for management of symptoms of respiratory, renal, and cardiac failure.\cite{15,16,22,23} Several studies have highlighted the symptom burden of end-stage organ failure as being similar if not more pronounced than that of advanced malignancy, as well as the possible utility of palliative interventions for symptom control.\cite{23,24}

The literature also reflects increasing recognition of the efficacy of palliative care for progressive nonmalignant neurologic disease. In cases of stroke, motor neuron disease, and amyotrophic lateral sclerosis (ALS), there is marked historical recognition and evidence outlining efficacy of palliative care, due in large part to an absence of meaningful disease-modifying therapies.\cite{26} Additionally, the literature supports palliative care for advanced Parkinson’s disease, multiple sclerosis, and Huntington’s disease.\cite{27} The role of palliative care in these specific patient populations focuses not only on assistance with patients’ physical care, but on communication among treatment teams and formulation of advanced care plans for mechanical ventilation and artificial feeding.\cite{28}

Progressive neurodegenerative disorders also confer significant physical, social, and emotional burdens on carers. A recent review of palliative care for ALS documented the difficulties faced by sufferers and their families and highlighted the important role that palliative care can play in the multidisciplinary treatment team in maximizing quality of life.\cite{29} In stroke, there is increasing recommendation within established guidelines for the early involvement of palliative care services for those with poor-prognosis intracerebral hemorrhage and/or cerebral infarction.\cite{30,31}

Despite widely held clinician fears that patients, particularly those without a cancer diagnosis, may be unduly disturbed by referral to palliative care, the evidence suggests that a sensitive discussion and introduction to palliative care need not cause distress.\cite{32} A prospective study found that the cohort of 40 patients, half of whom had nonmalignant conditions, were able to independently estimate their life expectancy and did not object to questions about end-of-life care.\cite{32,33}

Palliative Care and GBM

There are limited data on the symptoms experienced by patients with GBM and their palliative care needs. In a series of 169 patients, 82% of whom had primary brain or CNS tumors (with the remainder having metastatic disease to brain), the most commonly encountered symptoms in the last month of life were dysphagia (85%), drowsiness (85%), headache (36%), seizures (30%), and agitation/delirium (15%).\cite{9}

For patients with end-stage GBM, a palliative care cost-effectiveness study of 141 patients who died during an observation period demonstrated a reduction in need for rehospitalization prior to death for those receiving home-based palliative care of 8.3% compared with 26.8% of those not receiving support, despite a similar median survival time in both groups of 13 and 11 months, respectively. Costs associated with hospitalization per patient dying were found to be substantially different, with those for home-based palliative care...
Palliative Care and Other Neuro-oncologic Diagnoses

Palliative care data regarding neuro-oncologic diagnoses other than GBM are even more scant. There is little, if any, study into the role of palliative care in lower-grade gliomas and rarer tumors such as medulloblastomas, ependymomas, and malignant meningiomas, among others. No doubt, the problems associated with the care of patients with GBM apply to other tumor diagnoses, which may bring their own issues. The palliative care approach to adolescents and young adults will likely require particular skills and a heightened level of awareness, as must the approach to infants and children with terminal illness.

The Interface between Neuro-oncology and Palliative Care

Models of palliative care service delivery in developed countries differ, particularly in regard to availability of palliative care services in combination with disease-modifying therapies such as chemotherapy, radiotherapy, and surgery.41

Australia is regarded as having a palliative care service delivery system with well-established quality and benchmarking measures and outcomes among the world’s best.41 The delivery of palliative care is funded by state health departments, in accordance with national guidelines, and no funding or eligibility restrictions are placed on patients regarding anticipated prognosis, diagnosis, age, or concurrent therapies being delivered.42 It is provided by multidisciplinary teams including specialist physicians, nurses, allied health and pastoral care services in hospitals, palliative care units, patients’ homes, and aged care facilities.

Victoria is the most densely populated state in Australia, with 5.5 million inhabitants, over 75% of whom live in the state capital, Melbourne. There are 6 major tertiary referral hospitals in Melbourne, with 11 designated palliative care units, each ranging in size from 10 to 30 beds, and 7 community-based palliative care providers,43 who service distinct population areas providing specialist nursing, medical, and allied health care, predominantly with the aim of supporting patients and carers at home until death.

The Royal Melbourne Hospital (RMH) is the main provider of surgical and medical neuro-oncology services in the state.44 The RMH palliative care service provides both a dedicated 10-bed inpatient unit, on-site within the acute care hospital, and a consultancy service that receives referrals from all departments within the hospital for management advice on symptom control, terminal care, and complex discharge planning. Care in the home after hospital discharge is provided in collaboration with Melbourne Citymission Palliative Care, a nongovernmental organization funded by the state health program.

The referral service sees more than 1100 patients per year, with 64% referred with cancer diagnoses, of which ~20% are primary brain tumors. The inpatient unit receives 450 admissions per year, the average length of stay being 13 days. Fewer than 2% of these admissions have a diagnosis of a primary brain tumor.

The RMH palliative care service works in close collaboration with the neuro-oncology and wider medical oncology service to deliver seamless care to patients from diagnosis, surgery, and anticancer therapy to death, with shared clinical meetings, symptom management protocols, and, importantly, clinical research (including clinical trials).45

Conclusion

While the available data are limited regarding the symptoms experienced by GBM patients at end-of-life, and therefore their palliative care needs, there exists
increasing literature supporting the efficacy of palliative care in other cancers and in progressive nonmalignant illnesses, including a range of neurologic disorders.

Palliative care teams bring complementary expertise in symptom management, communication skills, and practical physical and psychosocial support, both within and outside the hospital environment. With the known consequences of GBM diagnosis, it follows that palliative care should and must become an integrated standard part of best practice neuro-oncologic care.

Further research is essential to define this role, particularly for those who may remain reluctant to involve palliative care due to concerns about raising alarm in patients and removing hope. This research, in a robust way, needs to further delineate the symptoms experienced and document the palliative interventions that can provide meaningful improvements, as has been done in other areas of cancer treatment.

From existing knowledge, patients with GBM experience a range of uncontrolled symptoms throughout their journey, including pain, seizures, and fatigue, that affect quality of life and may be amenable to improvement by palliative care services. Given the demonstrated benefits of early palliative care integration with anticancer therapies in other cancer diagnoses, the time for robust investigation into palliative care for patients with GBM is now.

References


Development of an in-home standardized end-of-life treatment program for pediatric patients dying of brain tumors

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Abstract

Purpose. To evaluate an end-of-life (EOL) program related to specific outcomes (i.e., number of hospitalizations and place of death) for children with brain tumors.

Design and Methods. From 1990 to 2005, a retrospective chart review was performed related to specified outcomes for 166 children with admission for pediatric brain tumors.

Results. Patients who received the EOL program were hospitalized less often (\(n = 114; \chi^2 = 5.001, df = 1, p < .05\)) than patients who did not receive the program.

Practice Implications. An EOL program may improve symptom management and decrease required hospital admissions for children with brain tumors.

Prevalence of children dying of brain tumors

Cancer is the leading cause of non-accidental death in children and adolescents (Gupta, Banerjee, & Hass-Kogan, 2004; Klopfenstein, Hutchison, Clark, Young, & Ruymann, 2001; Ries, 1975–2002; Ullrich & Pomeroy, 2003). Brain tumors compare second in frequency to leukemia, the most common type of cancer occurring during childhood (Pui et al., 2012). Unfortunately, brain tumors generally do not have a good prognosis compared to other types of childhood cancer. In fact, a higher mortality exists among children with a brain tumor compared to children with leukemia (Gupta et al., 2004; Ries, 1975–2002; Ullrich & Pomeroy, 2003). The 5-year survival for pediatric patients with central nervous system (CNS) tumors treated between the years 1995 and 2001 was about 75% (Ries, 1975–2002). Hence, nearly one quarter of all children with a brain tumor will not be cured, and many will die within 2 years of receiving treatments.

Symptoms of children with a progressive brain tumor

According to Goldman, Hewitt, Collins, Childs, and Hain (2006), children with a progressive brain tumor (i.e., poor prognosis) commonly experience one or more of the following symptoms during treatments: speech problems (76.3%), excessive oral secretions (69.5%), headache pain (64.4%), swallowing difficulties (62.7%), vision/hearing problems (62.7%), and dizziness symptoms (33.9%). In one pilot study, symptoms associated with the suffering
of children with a brain tumor during the last month of life included weakness (93.4%), limited mobility (89.8%), speech problems (76.3%), weight loss (57.6%), and changes in breathing (34–40%) (Hendricks-Ferguson, 2008).

With the exception of headache pain, general physical pain is only slightly less common among children with progressive brain tumors (81.4%) compared to children with leukemia (91.4%) during the last month of life (Goldman et al., 2006). However, research has shown mixed findings regarding the suffering of children from pain when being treated for all types of cancer (Wolfe et al., 2008). For instance, 56% of children with advanced cancer have reported a higher level of pain than the pain ratings documented by healthcare providers (Van Cleve, Munoz, Riggs, Bava, & Savedra, 2012).

Convulsions (or seizures) may occur among children with progressive brain tumors during treatments and at end of life (EOL; Carter & Levetown, 2004; Shuper et al., 2003). Approximately 39% of affected children will experience unpredictable and uncontrollable seizures during the last week of life (Goldman et al., 2006), which can also trigger significant emotional distress for their observing parents (Kang et al., 2005). Hence, successful control of a child’s seizures with antiseizure medications is essential to decrease the child’s symptom distress and parental emotional distress.

Nausea and vomiting are two unpleasant symptoms commonly experienced by all children with cancer during treatments. However, these symptoms can usually be controlled through administration of one or more antiemetic therapies (Kang et al., 2005; Wolfe, Friebert, & Hilden, 2002). Effective antiemetic therapies include: benzodiazepines and selective 5-hydroxytryptamine (5-HT3) antagonists (e.g., ondansetron, prochlorperazine, scopolamine, and metoclopramide). Still research has shown that 62 to 67% of children with a brain tumor experience nausea and vomiting during the last month of life (Goldman et al., 2006).

Psychosocial needs of children with a progressive brain tumor

The psychological adjustment of pediatric patients with a brain tumor and their parents is a concern of healthcare providers. Research has shown that affected children may experience more psychological adjustment problems (e.g., anxiety) than children with leukemia (Harris, 2004). Anxiety has also been reported as a common complaint among adolescents during EOL care (Cohen-Gogo et al., 2011). Additionally, research has shown that parents of children with a brain tumor often report having a high level of emotional distress compared to parents of children with leukemia (Hoven, Anclair, Samuelsson, Kogner, & Boman, 2008), worrying that their children will suffer uncontrollable pain during the last month of life (Dussel et al., 2010), and suffering anxiety related to the children’s loss of physical functions (Pritchard et al., 2008).

Palliative and end-of-life (EOL) care

There is a lack of clarity in recognized definitions for both palliative care (PC) and EOL care (Becker, 2009; NIH State-of-the-Science Conference Statement on improving end-of-life care, 2004). It is recognized that PC is focused on reducing and preventing suffering and improving the quality of life (QOL) regardless of the patient’s diagnosis (Harris, 2004; Klick & Hauer, 2010). In contrast, EOL is recognized as part of the continuum of PC with the major focus on symptom management and prevention of suffering for patients with a life-threatening diagnosis that is expected to lead to death (Harris, 2004; NIH State-of-the-Science Conference Statement on improving end-of-life care, 2004). The common goal of PC and EOL care is achievement of the best QOL for all affected patients. Thus, pediatric healthcare providers focus on prevention and treatment of all symptoms experienced by children with cancer to ensure delivery of effective and quality PC/EOL care.

Studies of pediatric hospices and EOL care programs are few, and factors related to provision of pediatric EOL care may be related to membership in professional groups to enhance pediatric expertise (Lindley et al., 2012). In one study, less than 10% of dying children received the care and benefits of these specialized programs (Carter & Levetown, 2004). In another study that compared two cohorts of children who had died between 1990 to 1997 and 1997 to 2004, the parents in the latter group reported feeling more support from their children’s healthcare providers and also had the perception that their children died with less suffering (Wolfe et al., 2008). Also, in a recent qualitative study, parents of children dying of cancer revealed the common themes of patient–provider communication, extending time, and understanding prognosis (Heinze & Nolan, 2012).
Location of EOL care

Multiple factors and variables may influence the location of EOL care, such as the child’s diagnosis, age at death, socioeconomic status, and the geographic location in which the child’s family resides (Feudtner, Silveira, & Christakis, 2002). An estimated 45% of children are dying of cancer at home, while 47% to 49% of children are still dying in the hospital setting (Klopfenstein et al., 2001; Shah et al., 2011; Wolfe et al., 2000). Children with leukemia and those with treatment-related complications are more likely to die in the hospital (Shah et al., 2011; Wolfe et al., 2008). However, children with cancer with a poor prognosis who are offered comprehensive hospice services (e.g., home delivery of chemotherapy and blood transfusions) are more likely to die in their homes (Bradshaw, Hinds, Lensing, Gattuso, & Razzouk, 2005; Fowler et al., 2006; Klopfenstein et al., 2001).

Conflicting evidence exists regarding whether the place of death affects the psychosocial adjustment of the child’s family. The majority of parents of children with cancer have shared their preferences to care for their dying children in their homes (Sirkia, Hovi, Pouttu, & Saarinen-Pikhal, 1998; Wolfe et al., 2002). Research has also shown that the number of children who died in the hospital or intensive care unit (ICU) has decreased when comparing two groups from the early to the late 1990s (Wolfe et al., 2008). Also, findings from one pilot study provided evidence that most parents would have preferred to receive earlier discussions (i.e., at diagnosis or at the time of reoccurrence) about EOL options for their children with cancer rather than near death (Hendricks-Ferguson, 2007).

In one unpublished, institutional review board (IRB)-approved focus group study, parents shared perspectives about the benefits of their children receiving EOL care in their homes (Pearson, 2005). The focus group data showed parents perceived that the major benefits of their children receiving EOL care in their homes included the children’s desires to be at home, the children being happier in a familiar environment, family and friends having easier access to visit the children; the family enjoying the freedom from negative perceptions of hospitalization (e.g., less disruption in the family’s normal routine); and the ability to provide the children with their preferred food choices. Additional advantages to home-based EOL care in that study included decreased cost, increased involvement of siblings, increased parental time to attend to their other children, and increased protected time for personal matters. The parents in Pearson’s (2005) study also reported that having their children receive EOL care in their homes allowed them to have increased protected time with their ill children, to be present at the time of the children’s deaths, and to be able to grieve in an unhurried and private manner.

Research has shown that providing EOL care for a dying child in the home can be stressful for some healthcare providers. In one study, investigators reported that the most difficult aspect of providing home EOL care for the dying child was having the family continuously witnessing the child’s physical decline and the healthcare providers’ stress related to monitoring the child’s care during the nighttime (Hooke, Hellsten, Stutzer, & Forte, 2002). Other reported difficulties of healthcare providers included fears related to what may happen at the time of the child’s death and unexpected physical symptoms and nursing responsibilities in caring for the dying child and his/her siblings (Collins, Stevens, & Cousens, 1998; Theunissen et al., 2007). Caregiver fatigue may also occur if the child’s dying process is prolonged, the demands of caring for the dying child are perceived as overwhelming, and if social and home medical support is lacking (Feudtner et al., 2002). Also stressful are the necessary decisions and emotions related to initiating discussions with parents about the need to transition a child from the home environment back into the hospital setting when the parents and siblings require a physical and emotional break from caring for the dying child.

EOL medications

The use of narcotics for pain control for children dying of brain tumors is considered standard of care. Benzodiazepines are also commonly used for EOL care. Midazolam is a short acting, water-soluble benzodiazepine that exhibits dose-related hypnotic, anxiolytic, amnestic, and anticonvulsant actions (Cohen, Gallagher, & Pohlman, 2002). Benefits of administering midazolam during EOL care can include: (a) a reduction in fear and anxiety if the child is experiencing a decrease in respiratory function; (b) effective management of delirium symptoms; (c) effective control of motor spasticity symptoms while protecting normal motor function; and (d) enhanced pain control when used in addition to other pain medications, such as morphine and clonidine (Campbell, 2004; Elsayem et al., 2009; Yaksh & Allen, 2004).
Healthcare providers are often concerned about over-sedation and respiratory depression when administering midazolam during EOL care. However, research has shown that hypotension is expected to be minimal when midazolam is administered as a continuous infusion (Cohen et al., 2002). Also, no evidence has been found to support the risk of developing a tolerance to the sedative effects of midazolam when it is used for extended periods of time; instead this tolerance is actually a benefit for children with a brain tumor because the patient will not remain sedated unless it is caused by progression of tumor growth (Morita, Tei, & Inoue, 2003). Still, there is no published evidence on the occurrence of patients’ tolerance to receiving midazolam related to its anxiolytic, anticonvulsant, or antiemetic properties.

Theoretical framework

This study was guided by a theoretical framework developed by Desbiens, Gagnon, and Fillion (2011) that is also based on a shared theory for the field of palliative care nursing. The development of this theoretical framework was based on tenets from Bandura’s (1989) social cognitive theory and Orem’s conceptual model (Biggs, 2008; Clarke, Allison, Berbiglia, & Taylor, 2009). Also, a major goal of the theory developed by Desbiens and colleagues (2011) is to provide a framework for healthcare providers to evaluate the quality of life among patients with a life-threatening illness.

Study objective

The primary objective of this study was to examine if a relationship existed between specific patient outcomes (i.e., symptoms, hospitalizations, and location of death) before and after implementation of a standardized EOL program among pediatric patients with brain tumors.

OVERVIEW OF OUR STANDARDIZED EOL PROGRAM

Our standard EOL program encompassed four components: comprehensive EOL discussions, medications for symptom control, primary family liaison, and home visits. Prior to the activation of our standard EOL program, the home care of children dying of brain tumors was generally managed by individual hospice programs among geographical areas surrounding our institution. Great variation in the use of medications and the anecdotal experience by the families was noted between these hospice programs. In an attempt to standardize this care, an organized treatment strategy for EOL care of neuro-oncology patients was initiated. The primary goal of the EOL program was to standardize and simplify care that the child would receive at home at EOL, thereby decreasing the need for the child to be admitted to the hospital and improving the experience for the family.

EOL discussion

The first step of our standard EOL program encompassed comprehensive EOL care discussions delivered by the child’s healthcare providers (e.g., physician, nurse, and/or social worker) to the child’s family. Two healthcare providers collaboratively reviewed planned topics with the child’s family. Specifically, one provider (the child’s physician) delivered the information, while the other provider (nurse or social worker) confirmed that each topic had been reviewed sufficiently. The child’s neuro-oncologist led all discussions with family members. The second healthcare provider was a team member (e.g., bedside nurse, pediatric nurse practitioner (PNP), or social worker) that the family knew well. Collaborative team delivery of the selected topics was planned to prevent exclusion of topics because of the potential emotional distress experienced by the child’s family. Our standard EOL program included review of the following topics: purpose of do not resuscitate (DNR) orders, alternative brain tumor medical-treatment options, anticipated loss of physical functions, hospice and home-care options, medical equipment needs, comprehensive medication needs (e.g., control of pain and seizures), make-a-wish requests, respite needs, and postmortem care needs (Appendix 1; Goldman et al., 2006).

Medications for symptom control

Medication guidelines for our standard EOL program were established for the pediatric patients with starting doses based on prior narcotic usage (Table 1). Standardized medication orders (inpatient, outpatient clinic, home care, and hospice services) for starting dosages and guidelines for escalation of medication dosages (Appendix 2) were developed for patients receiving care from the EOL program. A central component of the treatment plan was a standard order that included use of a pump-controlled continuous infusion of two medications.
Table 1. Palliative Medications (End-of-Life Care Dosing Suggestions ONLY, Please Refer to Individual Institution Guidelines)

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Directions</th>
<th>Age-appropriate dosing</th>
<th>Dosage forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids</td>
<td>Dexamethasone</td>
<td>5-day course for breakthrough pain</td>
<td>&lt; 5 years: 2 mg TID; 5 years: 4 mg TID</td>
<td>Elixir—12 mg/5 ml; Tabs—10 mg; solution</td>
</tr>
<tr>
<td>Oral pain control</td>
<td>Acetaminophen with codeine</td>
<td></td>
<td>&lt; 8 years: 1 mg codeine/kg Q 4 hr PRN; &gt; 8 years: 10 mg codeine Q 2 hr PRN; 3–8 years: 3–5 mg PO Q 2–4 hr PRN</td>
<td>Tabs; immediate or sustained release; 15, 30 mg; 20 mg/ml; 10 mg/5 ml; 50 mg/ml</td>
</tr>
<tr>
<td>Morphone</td>
<td>Morphone (continuous infusion)</td>
<td>If not effective, double the dose. If still not effective, increase × 3 by 20%. If still not effective, contact MD.</td>
<td>&lt; 3 years: 50 mcg/hr; 3–8 years: 250 mcg/hr; &gt; 8 years: 500 mcg/hr</td>
<td>Dispense 10,000 mg; Some hospice programs have difficulty providing small doses on pump, may require MD consultation.</td>
</tr>
<tr>
<td>Intravenous: Start IV medications early for raised intracranial pressure</td>
<td>Morphone (continuous infusion)</td>
<td>If not effective, double the dose. If still not effective, increase × 3 by 20%. If still not effective, contact MD.</td>
<td>&lt; 3 years: .5 mg/hr; 3–8 years: 1 mg/hr; &gt; 8 years: 2.5 mg/hr on oral morphine for at least 1 week;</td>
<td>Dispense 10,000 mg</td>
</tr>
<tr>
<td>Narcotics and midazolam are compatible to be Y’d in together</td>
<td>Midazolam (continuous infusion)</td>
<td>Indicated for supratentorial tumors or metastatic disease. Also used for intractable vomiting.</td>
<td>&lt; 3 years: 3 mg/hr; 3–8 years: 2.5 mg/hr; &gt; 8 years: 5 mg/hr.</td>
<td>Dispense 10,000 mg</td>
</tr>
<tr>
<td>Transdermal</td>
<td>Scopolamine Patch</td>
<td>For oral secretions</td>
<td>1 disc (1.5%) behind ear or shoulder every 72 hr for secretions</td>
<td>Apply patch to intact skin to cover painful area. Change patch every 12 hr.</td>
</tr>
<tr>
<td></td>
<td>Lidocaine Patch (5%)</td>
<td>For numbness or tingling</td>
<td>Do not apply to broken skin. Remove from skin if burning occurs.</td>
<td></td>
</tr>
</tbody>
</table>

Note: TID, three times daily; Q, every; PRN, as needed; PO, by mouth; MD, physician.
(e.g., hydromorphone or midazolam) for treatment of pain, intractable vomiting, anxiety, and seizures. A PNP with prescriptive authority prepared the standardized medication orders based on the recommended starting dose. The PNP was also responsible to review these medication orders with the child’s physician. The PNP and the primary outpatient clinic nurse assigned to care for the child coordinated the outpatient hospice referral and communication with the hospice nursing team members. Often, the PNP needed to make multiple phone calls to the hospice nurse and hospice pharmacy advocating for the use of intravenous medications and educating the hospice nurse regarding the compatibility of selected medications.

**Primary liaison**

Another key component of our standard EOL program was assignment of a specific healthcare provider to be the contact person for the family and for the hospice or home-care agency. The PNP and outpatient clinic nurse were the designated primary liaisons in our EOL program. Giving the hospice and the family a liaison contact allowed for close supervision by a team member and afforded the child and family the ability to ask questions of a team member who knew them well. The PNP or clinic nurse would call the family at least weekly and serve as a resource for both the family and the hospice nurse. Because we serve a large urban and rural geographic area including eight surrounding states, the primary liaison is a key consultant for hospice nurses who may have little or no clinical experience in caring for pediatric patients.

**Home visits**

Also, the standard EOL program included home visits to assess the patient’s symptoms by one or two healthcare providers from the team. The ideal combination of providers was a physician and PNP or a PNP and the child’s primary outpatient clinic nurse. The hospice nurse often received an update about the child’s care and status from the neuro-oncology providers in the child’s home. The home visits enhanced the ability of the healthcare providers: (a) to deliver more accurate healthcare information about the child based on current assessments of the child’s physical needs and (b) have sufficient time to provide reassurance to both the family and the hospice nurse, minimizing the need to transport the dying child to the clinic or hospital.

**METHODOLOGY**

**Research design and setting**

A retrospective chart review was conducted for pediatric patients with a brain tumor before death who received care from the Children’s Hospital Colorado between 1995 and 2005. This hospital provided health care to children from the surrounding eight states.

**Sample**

The target sample for this chart review encompassed all pediatric patients dying of a brain tumor at the Children’s Hospital Colorado during the previous 16-year period. The pediatric patients for this chart review were divided into two groups. Group 1 \((n = 22)\) was a historical comparison group of patients who died between 1990 and 1995 before the implementation of the current EOL program. Group 2 \((n = 92)\) included patients that died between 1996 and 2005 after initiation of the EOL program. Because no formal neuro-oncology programs existed at our center prior to 1995, our ability to collect similar data for the control group was limited by the quality of the recorded data prior to 1995. Therefore, a decision was made to limit the historical comparison group to 5 years in order to obtain the most accurate data possible \((n = 22)\).

The inclusion criteria for the target population were: (a) deceased pediatric patients diagnosed with a brain tumor, (b) an age between 1 month and up to 19 years, (c) documentation of the child’s place of death, and (d) documentation of reasons for the child’s hospitalization. EOL care for Group 1 patients was designated as starting when one of the following criteria were documented in the child’s chart: (a) a notation in the chart stating that the child had entered EOL care, (b) referral of the child to hospice care, or (c) a completed DNR order. Several of the patients in Group 1 did not have a designated date in their charts to indicate the start date of receiving EOL care. Therefore, radiology reports were reviewed to determine the date of disease recurrence or progression, both of which are often seen as a sign that the cancer is incurable. EOL care for Group 2 patients was defined as when one of the three following criteria were documented in the child’s chart: (a) parental and/or child participation in an EOL discussion, (b) referral of the patient to hospice care, or (c) completed DNR order. The exclusion criteria included having any gaps in chart docu-
Data collection procedure
IRB approval was obtained prior to initiating data collection. The data were collected by a retrospective review of the pediatric patients’ medical records (i.e., inpatient and outpatient charts of all pediatric brain tumor patients who died during the study period). Variables of interest were abstracted from the patients’ medical records. Abstracted data for each patient’s record received a research code number, and no personal identifiers were collected. The investigators extracted demographic and disease-related information for the eligible patients including: age at diagnosis, type of malignancy, date of initial EOL care discussion, date of disease progression, place of death, the number and duration of hospitalizations during the terminal period, and the reason for each hospital admission. In addition, accessible hospice or home-care charts were reviewed for data for a subset of patients whose clinic or inpatient charts were lacking needed information.

Data analysis
Data were only considered complete for pediatric patients who received all treatment from our center and whose records covered the entire time frame of the terminal portion of their illness. Descriptive statistics were compiled for all data retrieved from the charts of eligible pediatric patients. Chi-square statistical analyses were conducted to compare: (a) the number and duration of hospitalizations during each patient’s terminal disease phase with the location of death between the two groups and (b) the number and length of hospitalizations during each patient’s terminal phase with the number of patients dying in the hospital during the terminal period, and the reason for each hospital admission. In addition, accessible hospice or home-care charts were reviewed for data for a subset of patients whose clinic or inpatient charts were lacking needed information.

RESULTS
Sample description
A total of 166 pediatric patients with a brain tumor were treated for EOL care during the study period.

Table 2. Tumor Types in Study Sample

<table>
<thead>
<tr>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>Diffuse intrinsic pontine glioma</td>
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<tr>
<td>Glioblastoma multiforme</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
</tr>
<tr>
<td>Primitive neuro-ectodermal tumor</td>
</tr>
<tr>
<td>Medulloblastoma</td>
</tr>
<tr>
<td>Peripheral nerve sheath tumor</td>
</tr>
<tr>
<td>Ependymoma</td>
</tr>
<tr>
<td>Atypical teratoid rhabdoid tumor</td>
</tr>
<tr>
<td>Choroid plexus carcinoma</td>
</tr>
</tbody>
</table>

Forty-six of these patients were treated prior to initiation of our standard EOL care program, and 120 of the patients were treated after initiation of the program. Eligible subjects were diagnosed with various types of brain tumors (see Table 2) and were ages 1 to 19 years (at the time of diagnosis). Among the screened charts, 136 patients were eligible: 22 patients in Group 1, 92 patients in Group 2, and 22 patients were excluded because of missing data related to the child’s transfer of care, cause of death other than the brain tumor, incomplete records, or because the child was currently receiving cancer treatment. No patients in Group 1 received continuous infusion midazolam.

Admission prevalence
In Group 1, 12 (54%) of the 22 patients were admitted to the hospital at some point during the terminal phase of their illness. Among these 12 patients, a total of 20 hospital admissions occurred and included a total duration of 81 days, or an average of 4.05 days per hospital admission. Of these 12 patients in Group 1, 4 (18%) of the patients were only admitted to the hospital at the time of death. Therefore, 8 of the 12 patients were admitted to the hospital 16 times during EOL care. In Group 2, 27 (29%) of the 92 patients had a total of 38 hospital admissions (during EOL care or at death). These hospital admissions totaled 115 days, or an average of 3.03 days per hospitalization. Of these children, 21 (23%) of the patients died in the hospital; each having only the single hospital admission. Therefore, the remaining 6 patients accounted for 17 hospital admissions that occurred during EOL care.

Statistical analysis was performed to compare collected data for Groups 1 and 2 using chi-square statistics. The results of the reviewed EOL program did not show a significant decrease in the number of patients dying in the hospital ($\chi^2 = .642, df = 1$).
However, the patients in Group 2 (who had received symptom management by the EOL program) were significantly less likely to be admitted to the hospital during the EOL period than those in Group 1 ($\chi^2 = 5.001$, $df = 1$, $p < .05$). Descriptive analysis of the chart data revealed that the number of hospital admissions for patients in Group 2 was approximately 46% fewer in number than the hospital admissions among patients in Group 1. Also, patients in Group 2 had a shorter average hospital length of stay (3.03 days) than did patients in Group 1 (4.05 days), approximately a 25% decrease. Across the total sample, the average number of hospital admission days decreased by 66% (i.e., 3.68 days per patient in Group 1 to 1.25 days per patient in Group 2; see Table 3).

**DISCUSSION**

This retrospective chart-review study provided data related to specific patient outcomes, designed to act as proxies for evidence of improvement of quality of EOL care for the target sample of pediatric patients with brain tumors. One key finding is that the Group 2 patients (who received care from our standardized EOL program) experienced significantly fewer hospital admissions and also had a decrease in the average length of days in the hospital while receiving EOL care. We suggest that these patients may have had a decreased number of hospital admission days because the EOL program included comprehensive EOL care discussions, continuous infusion midazolam, an assigned clinic nurse or PNP liaison for communication with the family and the hospice nurse, and healthcare provider home visits.

**Hospitalizations for EOL**

In our study, the pediatric patients in Group 1 experienced a higher number of hospital admissions compared to patients in Group 2. Perhaps patients in Group 1 experienced poor symptom control because they did not receive effective symptom management in the absence of a standardized EOL program. In contrast, team members of our standardized EOL program consistently initiated early EOL care discussions with parents for all pediatric patients who were scheduled to receive experimental cancer treatments. No similar studies were found in the literature to allow for any comparisons with the collected data from our study. However, when comparing the data from both groups in our study, the findings revealed that the Group 2 patients who received our comprehensive EOL care program (e.g., utilization of midazolam and home visits) had a lower number of complications in comparison to the Group 1 patients. Therefore, the Group 1 patients may have experienced more hospital admissions because they did not receive effective symptom control.

Another finding identified is the decrease in the rate of hospital admissions among the Group 1 patients who received our EOL program. This finding suggests that the symptoms of affected children were more effectively managed at home. However, the findings did not provide evidence of a decrease in the number of patients who were admitted to the hospital at the time of death. This raises the question of why some patients were admitted at death and what if anything can or should be done to eliminate or change those reasons.

The establishment of a symptom care team in the United Kingdom dramatically increased the number of children with cancer dying at home from 19 to 75% (Goldman, Beardsmore, & Hunt, 1990). Relating these numbers to our study, if all of the patients were combined into one group, a total of only 25 (21.9%) of 114 children were hospitalized at the time of death. When considering the general percentages of children with cancer dying in the hospital versus at home, it is apparent that the EOL care provided at our center, even before the implementation of the program, resulted in a relatively high number of patients that were able to die at home (Klopfenstein et al., 2001; Shah et al., 2011). Perhaps a certain percentage of parents will always want their children to die in the hospital as both ours and the UK study suggest between 20–25% of children died in the hospital. Conflicting evidence exists regarding the benefits of dying at home; however, it has been shown that the majority of parents of chil-

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**Table 3. Hospitalizations and Place of Death**

<table>
<thead>
<tr>
<th></th>
<th>Number of patients</th>
<th>Patients admitted during EOL care (%)</th>
<th>Total number of admissions</th>
<th>Total days spent as inpatients</th>
<th>Average length of stay (in days)</th>
<th>Patients dying in hospital (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>22</td>
<td>12 (54)</td>
<td>20</td>
<td>81</td>
<td>4.05</td>
<td>4 (18)</td>
</tr>
<tr>
<td>Group 2</td>
<td>92</td>
<td>27 (29)</td>
<td>38</td>
<td>115</td>
<td>3.03</td>
<td>21 (23)</td>
</tr>
</tbody>
</table>

*Note: EOL, end of life.*
children dying of cancer prefer to take care of their children at home (Harris, 2004; Hendricks-Ferguson, 2008; Higginson et al., 2003; Sirksia et al., 1998; Wolfe et al., 2002, 2008).

Although our EOL program has shown a decrease in hospital admissions and improved symptom management for pediatric patients, our EOL program did not lessen the probability of a patient dying in the hospital. This finding raises the question of why patients were admitted at the time of death. Thus, the preferences of pediatric patients and their parents and not simply symptom prevalence may have influenced where the child’s death occurred. One possible explanation is that family situations may not be conducive to caring for a dying child at home, such as single parents or the presence of younger siblings. Also, some families may have a shortage of financial resources that preclude them from being constantly available for their ill children (Postovsky & Ben Arush, 2004). In addition, variations in the way parents from different cultures may view the appropriate location to care for the dying child cannot be explained with these data.

Finally, a component of our EOL program that may also have decreased hospital admissions was the important role of the liaison (due to our geographic location). Data were collected from a single pediatric cancer center that provides care to a large geographic area that includes the surrounding eight states. Because our EOL program at this cancer center is provided only by local services, some of the parents may need to travel from 8 to 10 hr to seek health care for their children at this center. Therefore, the nurse or PNP liaison providing weekly phone follow-up and support seemed critical for local hospice nurses who may be inexperienced in providing PC and EOL care to pediatric oncology patients.

**EOL medications**

The study did not specifically gather data regarding management of symptoms; however, continuous infusion medications were an integral part of the EOL program. The literature is sparse on intravenous medications at home for EOL in children. Because most of the children with progressive CNS disease in this chart review also had central venous access in place to receive medications, continuous infusion drug dosing in the home setting could be monitored and regulated. Intravenous medications are easily given at home via continuous infusion with small pumps contained in a backpack. Selected narcotics and benzodiazepines are compatible and can be administered safely together. Patients can remain mobile with two small pumps in one backpack with good symptom control during the final weeks of life. Parents and caregivers can be taught how to escalate the doses as needed to provide symptom management.

Most of the information in the literature regarding the use of midazolam during EOL care is for the treatment of terminal agitation or sedation in adults being cared for in ICUs (Cohen et al., 2002). Research has provided preliminary evidence about the benefits of using palliative sedation with midazolam to render children unconscious when experiencing physical pain during the dying process (Postovsky, Moaed, Krivoy, Ofir, & Ben Arush, 2007). However, we suggest that midazolam can be an effective medication for symptom control at home without oversedation. Midazolam has been shown to be the drug of choice for sedation in the presence of increased intracranial pressure due to its short half-life, making it a good medication for use in children with brain tumors. Continuous intravenous infusion of midazolam is ideal when the dying patient is unwilling or unable to take the medication orally or rectally. Anecdotally, the majority of patients in this study who received continuous infusion midazolam showed signs of being very drowsy, but they later returned to their baseline level of consciousness. Also, the parents of the children reported satisfaction with resolution of symptoms, such as intractable vomiting, seizures, and agitation. Parents also stated that they became more comfortable with their children receiving midazolam. The parents were easily taught to increase the medication doses on the child’s IV pump as needed to help control the child’s symptoms. Under the guidance of the EOL team, the parents conveyed an appreciation for being able to participate in symptom control measures for their children. Ongoing parental education was necessary as the pediatric patients became comatose to clarify that the coma state was induced by the child’s brain tumor rather than the administered medications.

**Study limitations**

This study has several limitations. One limitation is that Group 1 included a smaller sample size than did Group 2. A second limitation is that the Group 1 patients were treated during the 5 years prior to the EOL program and were used as a comparison group due to the difficulty in obtaining records prior to that time.
time. This was a smaller length of time than the 10 years used for Group 2, which added to the difference between the two groups. A third limitation was inclusion of only a heterogeneous sample of children with all types of brain tumors, different treatment protocols, and patients who had varied clinical courses. We also did not collect data on race and gender in this heterogeneous sample. A fourth limitation was that we did not conduct analysis to control for contributing factors, such as personality differences, previous experiences with death, age, gender, race, and socioeconomic status. We did not extract the data of the developmental stage of pediatric patients within the two groups, which also could have affected the parents’ preferences for the children’s EOL care locations. A fifth limitation was use of a convenience sample from one geographic region. Also, the authors were not able to calculate the median or range of length of stay and mean age of the participants. The very fact that in the early years, no documentation may have occurred related to EOL care discussions with parents suggested that parents may have been given little or no EOL information before their children died. Therefore, admissions to the hospital and death location were used as an indirect method of evaluating symptom occurrence because they were readily available in sparse medical records.

Other variables could have affected the changes demonstrated between the two groups. Also, the treatment of brain tumors may have improved during the 15-year time frame of the current study, resulting in children receiving treatments for a longer period of time with potentially no or fewer side effects and less frequent hospitalizations. Perhaps the use of our comprehensive EOL-care program to deliver planned discussions led to increased acceptance by family members of the ultimate risk of death of their children. Also, parents receiving our program may have contributed to children not receiving unnecessary medications that could cause toxic side effects related to the futile hope of achieving a cure that was not possible. These ideas are supported by the recent study on parent decision-making for children with cancer during EOL that revealed a desire for improved patient–provider communication and better understanding of prognosis (Heinze & Nolan, 2012). Lastly, changes in medical policy and insurance practices might have affected the hospitalizations of the population in the study. Hospice and home-care organizations have become increasingly comfortable with caring for dying children in the home environment, possibly leading to a decrease in need for admission when problems arise. Therefore, our findings must be interpreted with caution.

In spite of the improvement of survival of children with brain tumors due to better therapy, a substantial number of these children still die. Improving the quality of care for children dying of brain tumors remains an important priority for all nurses and P.NPs. The nurse is often viewed by the child’s family as the most familiar and trusted healthcare team member due to frequent contact with the family. Therefore, family members may be more receptive to discussions about an EOL program when these discussions include the nurse in whom the family trusts.

**Future directions**

The use of hospitalizations as a means of measuring symptom control is somewhat indirect. Future research should include evaluation of symptoms in children using an instrument that has established psychometric properties, such as the pediatric format of the Memorial Symptom Assessment Scale (MSAS). The original MSAS was designed to measure symptoms among adults with cancer. The MSAS includes two pediatric formats: the MSAS (7–12) that is designed for children ages 7–12 years and the MSAS (10–18) that is designed for children ages 10–18 years (Collins et al., 2000, 2002).

Another symptom checklist for children with cancer is the Therapy-Related Symptom Checklist-Children (TRSC-C). It has recently also shown good measurement properties (Williams et al., 2012). Therefore, future studies, using the MSAS or TRSC-C, could provide more specific information regarding actual symptom prevalence as well as the subsequent response after treatment with midazolam is started. In addition, use of a pediatric brain tumor quality of life tool (e.g., PedsQL Brain Tumor Module) with established psychometric properties could provide stronger data to measure QOL indicators and symptoms experienced among pediatric patients with brain tumors (Palmer, Meeske, Katz, Burwinkle, & Varni, 2007).

Future investigation is warranted in the use of a standardized EOL care program for a large, multicenter replication study. Also, a prospective study design is needed related to the use of midazolam infusions at multiple clinical sites to evaluate the effectiveness of symptom management and medication dose ranges, as well as patient and parental perception of the benefits and drawbacks of using specific symptom management medications.
How might this information affect nursing practice?

The results from the retrospective evaluation of our EOL program, which includes comprehensive discussions with parents, midazolam infusions, a nurse liaison, and home visits by healthcare providers and nurses from the primary brain tumor team, contribute to the body of knowledge of EOL care for patients with pediatric brain tumors. Our comprehensive, standardized EOL program should be examined in future research studies to evaluate its impact on the QOL of children with other life-threatening illnesses. The current study provides preliminary evidence that a comprehensive EOL program that encompasses an active role by staff nurses and PNP’s may reduce the need for increased admission to the hospital setting for a child in need of EOL care.

References


APPENDIX 1

DATE_______________

**NEURO-ONCOLOGY**

**TUMOR RECURRENCE AND END-of-LIFE CARE PREPARATION**

**Patient’s Name_____________________________**

**Brain tumor Diagnosis:________________________**

1. **Topics Reviewed with Parents:**
   - Magnetic Resonance Imaging (MRI) Scans: ____________________________
   - Brain Tumor Treatment Options: ____________________________
   - Alternate Treatment Options (e.g., PC/EOL, Hospice) ____________________________
   - Quality of Life Focus/Issues: ____________________________

2. **Health Care Needs/Equipment (Check those used):**
   - CVL Access____ Mediport needles____ Wheelchair ____ Bed____ Oxygen/suction _____ Other____

3. **Description of Physical Function Losses & Neurologic Deterioration:**

4. **Description of Seizure Activity:**

5. **List of Medications and Observed Side Effects:**
   - Anti-Seizure Medications: ____________________________
   - Pain Medications (Oral and Intravenous medications): ____________________________
   - Steroid Medications: ____________________________

6. **Home Care Support- Name of Health Care Provider and Contact Number:**
   - Primary HCP: ____________________________ Phone Number: ____________________________
   - Secondary HCP: ____________________________ Phone Number: ____________________________
   - Weekend HCP: ____________________________ Phone Number: ____________________________

7. **Hospice Care Support: Name of HCP and Contact Number:**
   - Primary HCP: ____________________________ Phone Number: ____________________________
   - Secondary HCP: ____________________________ Phone Number: ____________________________
   - Weekend HCP: ____________________________ Phone Number: ____________________________

8. **DNR Education Provided:**
   - Yes ____ No; Parent(s) signed DNR Form: ____ Yes ____ No

9. **Description of Post-Mortem Care:**

10. **Assessment of Family Support and Needs:**
   - Physical Care and Respite needs: ____________________________
   - Patient & Sibling Needs: ____________________________
   - Make-a-Wish Program: ____________________________
   - Health Care Coverage & Needs: ____________________________

11. **Follow-up care with staff:**
    - Relationship change with staff: ____________________________
    - Re-involvement with PCP for family: ____________________________

**Definition of Listed Abbreviations in Appendix 1:**

PC/EOL = Palliative Care/End of Life Care

CVL = Central Venous Line


APPENDIX 2

Narcotic/Midazolam
Home Care Orders for End of Life Care

HOME HEALTH AGENCY: ***
Physician Name: ***
Physician Phone Number: *** (NP during working hours, MD after hours)

Weight ________kg

Please initiate hospice nursing and medications.
Please provide oxygen, suction, bedside commode, shower chair, hospital bed as needed.
Please provide in-home emergency hospice medications as needed.

Medications:
Diphenhydramine or Hydroxyzine 1 mg/kg IV/PO as needed for itching with narcotics.

**Narcotic (morphine or hydromorphone): Please dispense 10,000 mg**
1. Start _________*** micrograms/hr as continuous IV infusion.
2. Provide ability to bolus at 50% of continuous infusion with 5 min lock-out.
3. If pain not controlled, double the dose to *** micrograms/hr IV.
4. If pain still not controlled, double the dose to *** micrograms/hr IV.
5. If pain still not controlled, increase to *** micrograms/hr IV.
6. If pain still not controlled, increase in 20% increments X 5.
7. If still not controlled, notify NeuroOncology via phone number above.
   For intractable vomiting, seizures, anxiety.

**Midazolam: Please dispense 10,000 mg**
1. Start Midazolam *** micrograms/hr as continuous IV infusion.
2. Provide ability to bolus at 50% of continuous infusion with 5 min lock-out.
3. If symptoms uncontrolled, double the dose to *** micrograms/hr IV.
4. If symptoms uncontrolled, double the dose to *** micrograms/hr IV.
5. Subsequently, increase in 20% increments X 5.
6. If still not controlled, notify NeuroOncology via phone number above.
   *In the event of a seizure, double the dose of midazolam.
   DO NOT DECREASE MIDAZOLAM FOR OVER-SEDATION.

**Please teach family to be independent in changing pump rates in order to increase medication.
Drug Allergies: Review of patient’s allergies indicates no known allergies.
Dying of brain tumours: specific aspects of care

Tobias Steigleder\textsuperscript{a,b}, Stephanie Stiel\textsuperscript{a}, and Christoph Ostgathe\textsuperscript{a}

\textbf{Purpose of review}
Patients with brain tumours show a high symptom burden, and symptoms are difficult to treat and prone to be overlooked. This review of publications dealing with advanced stages of brain tumours tries to assess the knowledge gained in the past 2 years and to develop an outlook for further investigations.

\textbf{Recent findings}
We searched for publications on advanced brain tumours in a palliative medicine setting. Of 138 publications retrieved by search in PubMed, 22 publications met our criteria for inclusion. We predefined categories of interest: epidemiology and treatment of symptoms; quality of life; and impact on next of kin, caregivers, medico-social system and decision-making.

\textbf{Summary}
Data suggest that patients with primary or metastatic brain tumours often have a high symptom burden and unmet needs for palliative care, and symptoms are hard to diagnose; patients suffer often and early from cognitive impairment but are rarely appropriately prepared concerning end-of-life wishes. This reflects on their caregivers’ burden as well. For symptomatic treatment of common symptoms such as fatigue, depression and cognitive impairment, methylphenidate has established an important role. For assessment of these symptoms, a shortened questionnaire Quality of Life Questionnaire-15-Palliative shows potential. Cancer-directed therapy in advanced stages of brain tumours has to be weighed critically. To assess adequate strategies to help patients and caregivers with the challenges of brain tumour-specific symptoms, randomized intervention studies are necessary. The same accounts for cancer-directed treatment in relation to quality of life in advanced stages of brain tumours.

\textbf{Keywords}
brain tumour, end of life, palliative care, quality of life

\textbf{INTRODUCTION}
Advanced cancer, especially in patients with brain tumours, leads to severe burden on patients and their caregivers \cite{1,2}.

Both primary and metastatic tumours of the brain share common features in clinical presentation, symptoms and the challenges to patients and caregivers, and differences are mostly to be found regarding the rapidity of progression \cite{3–7}.

Decreased mobility and increased dependency on caregivers are frequently found \cite{8–11,12**}, as along with headaches, seizures and dysphagia \cite{9,11,12**}. Personality changes are challenges for patients and caregivers \cite{10,11,12**}. These symptoms are attributable to the tumour and to the secondary effects from treatment \cite{12**}, raising ethical concerns about the goals of treatment in various stages of the disease; meanwhile, cancer-directed options are often overestimated \cite{13,14}.

The rapidity of deterioration and cognitive impairment exact decisions from caregivers, whereas only 6\% of patients established advance care directives beforehand \cite{3,12**,15**}.

The present review will give an overview on patients with advanced brain tumours regarding symptom burden, quality of life (QoL), and social and medico-social impact.

\textbf{MATERIALS AND METHODS}
For this purpose a selective literature search in the PubMed database was conducted on 19 April 2013

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for the time frame between January 2011 and February 2013 (Table 1 and Fig. 1).

We predefined three topics: epidemiology and treatment of symptoms; QoL; and impact on next of kin, caregivers, medico-social system and decision-making.

Of all publications found \(n = 1665\), articles were discarded that dated further back January 2011. The remaining articles \(n = 138\) were scanned by title, if the topic was primary or metastatic cancer of the nervous system. The remaining articles \(n = 65\) were scrutinized for their relevance to the topics and articles had to be excluded not concerning the topics \(n = 43\), being not available \(n = 4\) or other languages than English, French or German \(n = 2\).

The remaining articles \(n = 22\) were retained for the review.

**RESULTS**

Data show that brain tumour patients suffer from high symptom burden that often goes partially undetected; caregiver burden due to patients’ cognitive and functional impairment leads to estrangement and overtaxing in caregiving and responsibility. QoL as a primary treatment goal needs standardized treatment tools and to find its place in developing treatment goals for the patient and deciding upon the means to achieve them.

**KEY POINTS**

- Symptom burden in patients with brain tumours is above average for advanced cancer patients.
- Main symptoms are different from other advanced cancer patients residing more in the cognitive and functional sector and appropriate detection is both difficult and crucial for treatment.
- Caregivers suffer from patients’ symptom burden and are pressured by a perception of high responsibility.
- Discussions concerning end-of-life decisions and advanced directives should be undertaken at an early point of time and in a standardized form to achieve the best possible coverage.
- Patient-centred interventions although improving, patients’ well-being will not automatically do the same for caregivers, so specific interventions are necessary.
- End-of-life decisions and comprehensive discussions about therapeutic goals and the appropriate means need to be considered early, and implementation has to be standardized to improve both the treatment of the patients and well-being of the caregivers.

**Table 1. Feature of the literature search in PubMed**

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<th>Feature of the literature search</th>
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**Epidemiology of palliative care needs and symptoms**

The need for palliative care in a hospital’s population was assessed by Becker et al. [16]. All patients who were discharged within 17 months were included. The treating physician had to answer whether the patient has palliative care needs (PCNs) in ‘yes/no’ format. Data from 39,849 patients (response rate: 96%) were gathered. Approximately 6.9% of all patients and 9.1% of patients older than 65 years were considered to have PCN. Sixty-seven per cent of the patients with PCN were cancer patients. Two entities showed the highest frequency of PCN: cancer of the head and neck (PCN in 28.3%) and primary brain tumours (18.2%). Even more accentuated is the proportion of patients with PCNs in the group of patients with metastases to the brain (38.9%).

In brain tumour patients 20–40% have PCN, but only one of 10 receives palliative care consultation [12].
Gofton et al. [12**] investigated adult patients with primary or metastatic brain tumours (n = 168); primary brain tumours: 65.5%; primary lymphomas of the central nervous system (CNS): 20.2%; metastatic brain tumours: 14.3%. Most common symptoms were gait impairment (65.5%), cognitive/personality change (61.9%), motor deficits (58.3%) and seizures (57.1%). To a lesser extent aphasia (27%), delirium (27%), headache (26%), visual changes (22%) and falls (20%) occurred. Twenty-seven per cent of the patients had PCN [12***].

Another study found falls to occur in 50% of mobile patients with advanced cancer (n = 185) within a period of 6 months. In the subgroup of brain tumours (n = 24) the hazard ratio for falls was 2.5 (P = 0.002) demonstrating the clear-cut increased risk in this group [17*].

Yamanaka et al. [18] investigated symptoms in lung cancer patients with brain metastases (n = 55). They found consciousness disturbance (33.3%), headache (25.9%), fatigue (46.2%) and pain (64.8%), cranial nerve palsy (18.5%, 45.4% in meningeal carcinomatosis) and delirium (18.5%).

Fatigue, anxiety and depression
Depression occurs in up to 50% of patients with brain tumours [15**,19,20]. Previous psychiatric illness, female sex, lower level of education and lower tumour grade were negative prognostic factors. Tumour location did not correlate with depression [15**].

After preliminary findings the first double-blinded randomized study by Kerr et al. [21] shed a light on the effectiveness of methylphenidate in advanced cancer. Both fatigue and depression scores showed improvement between baseline and after treatment and between methylphenidate and placebo. Also appetite is increased and treatment success is rapid [22**].

Cognitive changes, communication and aphasia
Cognitive changes occur in 62% of the patients with either primary or metastatic central tumours [12**]. Furthermore, delirium and aphasia were each found in 27% of the patients [12**].

The causes of cognitive changes are directly tumour-associated: 79% of patients with primary CNS tumours showed cognitive decline before treatment and was independent of treatment modality and degree of surgical resection [15**,23,24].

Tumour therapy also causes changes in cognition as cognitive impairment follows surgery, radiotherapy and chemotherapy [15**,23–25].

Higher age and high-grade tumours are risk factors for cognitive changes [15**,26,27].

Cognitive impairment may also be a predictor of tumour recurrence [15**,28], rapid tumour progression and shortened time to death [15**,29].

Methylphenidate slows cognitive decline [22**,30,31] and improves opioid-induced cognitive dysfunction [22**,32–35].

Headache and pain
Gofton et al. [12**] found headaches in 26% of the patients (n = 168) and pain (unspecified) in 19%. The report of the International Meeting of the French Society of Neurology 2011 (IMFSN 2011) states: diffuse pain occurs in approximately 25% of the patients [27] and headaches affect about one-third of patients with brain tumours [8–11,27]. They are usually controlled by steroid prescription in combination with analgesics [27]. In cases of headache with skull base involvement or meningosis carcinomatosa additional tricyclic antidepressants or anticonvulsants are recommended, if necessary [27].

Seizures
Seizures are common among patients with cerebral tumours [36]. Gofton et al. [12**] found seizures in...
57% of patients (n = 168) [12**]. They will appear foremost in slowly progressive lesions, tumour associated haemorrhages, multiple lesions [27,37] and cortical localization, especially insular or central [27,38]. Generally seizures are attributable to the tumour, but eliminating predisposing causes and specific aetiologies is highly important, such as infections, metabolic factors or cancer-directed treatment [27,39]. The IMFSN 2011 underlines that there are no data to support a prophylactic antiepileptic therapy [27,40], but discusses the fact that emerging antiepileptic drugs with little side-effects and no known enzyme-inducing properties may change this in the future [27].

After surgery the incidence of seizures rises, and a short-term preventive treatment is discussed by Taillandier et al. [27,41]. If decided to treat prophylactically, the authors’ choice would be antiepileptic drugs or benzodiazepines [27,41].

The effect of enzyme-inducing antiepileptic drugs on medication and chemotherapy are unpredictable and drugs proposed are levetiracetam, pregabalin, lamotrigine, topiramate and lacosamide [27,42,43].

Quality of life
Quality of life in patients with brain tumours is challenging to assess appropriately. But proper assessment may provide information on efficacy of treatment and prognosis.

Measuring quality of life
Patients suffering from brain tumours will encounter limitations in answering QoL evaluations. Caisse et al. [44*] assessed a shortened form of the EORTC QLQ-C30 questionnaire, the QLQ-C15-PAL, in patients with metastatic brain tumours (n = 150). The QLQ-C30 has been developed to monitor treatment response, to investigate the relationship between QoL and other factors and as a baseline tool to assess QoL [45–49]. The shortened form reliably detects important aspects of QoL after reducing the number of questions substantially [44*,50].

Quality of life and cancer-directed treatment
Cancer-directed therapy has to pursue QoL and in advanced stages even more so [51,52].

The effect of palliative radiotherapy for brain metastasis on QoL was assessed by applying EORTC QLQ-15-PAL. The authors investigated impact on QoL domains and which might provide prognostic information [53**]. One hundred and fifty-one patients were recruited at 14 centres. Eighty-two per cent of the patients received whole brain radiation therapy. QoL was assessed before radiotherapy and after 3 months. Sixty-two per cent of the patients had survived of whom 70% completed the second questionnaire. QoL scores were significantly worse in: global QoL, physical function, fatigue, nausea, pain, appetite loss, hair loss, drowsiness, motor dysfunction, communication deficit and weakness of legs. Headaches remained unaltered [53**].

Prognostic value of quality of life
One study tried to assess predictors for survival. QoL in the patients who survived a 3-month interval was better at recruitment. Also lower Karnofsky performance status, higher age and higher pain ratings were prognostic of reduced 3-month survival [53**].

Impact on next of kin, caregivers, medico-social system and decision-making
Brain tumours may lead to cognitive changes reducing the patient’s ability to participate in decision-making, thereby increasing the caregiver burden.

Caregiver burden and seizures
Seizures place an immense burden on patients as well as on caregivers. A systematic review on supportive care needs of patients with brain tumours states that seizures pose a definite limitation to caregivers and their perceived options of home care [15**].

Caregiver burden and patients’ symptoms
Caregivers suffer from emotional, physical and economical strain. In a study by O’Hara et al. [54] the question was perused: did ENABLE II (Educate Nurture Advise Before Life Ends), a patient-focused palliative care intervention that increased the patients’ QoL affect caregiver burden? Caregivers completed a caregivers’ burden scale and patients answered queries about QoL, symptom intensity and depressed mood measures. Data were collected at regular intervals. An after-death interview was performed regarding the quality of care of the descendent.

The patients improved in the parent study in all regards. There were no significant differences between intervention and usual care conditions for caregivers.

Caregiver ratings of patients’ mood
Insecurity about the patients’ psychological status increases caregivers’ burden. Concerning the patients’ mood: moderate-to-severe cognitive
impairment will reduce the caregivers capability to assess the patients' mood correctly [15**,55].

**Decision-making at the end-of-life and caregivers' burden**

Gofton et al. [12**] assessed that in their study population of primary and metastatic brain tumour patients, every fifth person dies without hospice discussion and an appointed healthcare proxy. Near to every third patient who dies of primary brain tumours or with metastatic cancer to the brain has no do-not-resuscitate (DNR) order and every fourth patient received cancer-directed therapy in the last month of life [53**]. The median time from hospice discussion and from DNR order to death was 37.5 and 48.8 days, respectively. Placing the time of the decision-making in a time frame when 61% of the patients with primary brain tumours suffered from cognitive impairment, 32% had aphasia and 12% dysarthria, thus reducing their ability to participate in the discussion [53**].

These findings are well reflected in a study on a Dutch population of 101 patients, deceased from high-grade gliomas. Thirty-eight per cent of the patients had no documentation of any end-of-life decisions (ELDs) [1]. The physicians of 61 patients (60%) were aware of their patients' preferences regarding ELD. Of 50 patients, data from both their physicians and their relatives were available. According to their relatives 21 patients (42%) had an advanced directive. In 12 of the cases (57%) physicians were aware of the advanced directive. Five other physicians discussed wishes with the patients, but were unaware of the advanced directive. In 30 patients (30%) the physicians indicated that the patients' ELD probably shortened their patients' lives by hours (10%), days (12%) or weeks (8%) [1].

**DISCUSSION**

The need to characterize the main topics in patient care in advanced stages of brain tumours has been recognized. The specific impairment by tumours of the CNS leads to an increased burden on patients and their relations as the studies by Gofton et al. [12**] and Becker et al. [16] showed. As they are based on a hospital population, they share a common limitation: immobility and cognitive changes often cause inpatient care in nursing homes or hospices and thus possibly preventing admissions in cases of clinical deterioration or new symptoms. It stands to reason that the PCNs may even be more severe than the data suggest. Even more so as cognitive/ personality changes, depression, fatigue and delirium are symptoms, which decisively reduce QoL, but which are less prone to be diagnosed by a physician not specially trained in this field.

Gofton et al. [12**] also showed an overview of symptoms we have to expect mostly in patients with brain tumours: gait impairment, cognitive changes, motor deficits and seizures. The study included a high proportion of primary lymphomas to the brain but data remain valid as there is no relevant difference in symptom occurrence between the lymphoma patients and the other groups of high-grade tumours to the brain and it is well reflected in data collected by Yamanaka et al. [18]. These symptoms share a common feature: they limit the patient's QoL as well as the caregivers'.

To counter this shortcoming, standardized assessment tools may help to better identify the patients in need. The QLQ-15-PAL proofs to be an adequate and feasible tool to this end [44*,53**] and further investigations are necessary to establish whether it is applicable in a routine setting.

Assessing the cognitive status may also help to determine the prognosis as cognitive decline is a predictor for both shorter survival time and tumour progression [15**,56]. To determine the prognostic value of cognitive changes further studies are necessary.

For medical treatment of cognitive impairment, fatigue and depression, methylphenidate seems to be a promising option. Fatigue and depression are independently improved and cognitive function, impaired by both tumour and drugs, is enhanced by methylphenidate. No other medical solution presents itself readily, placing more importance on strategies outside medical treatment to help patients and caregivers to deal with personality and cognitive changes, depression and fatigue.

These symptoms put a heavy strain not only on the patients, but on the caregivers as well [57,58]. Cognitive impairment leads to an increased caregiver burden by both a feeling of increased responsibility [1,15**] and an insecurity about patient's needs, mood and wishes [1,15**].

Falls and seizures pose an immense risk for acute complications, increase caregiver burden and necessitate admissions to a hospital in the last weeks of life. To avoid these complications adequate concepts of care need to be developed [12**,15**,59].

The readiness to accept a multidrug antiepileptic treatment may be higher as patients have lesser capability to recover from seizures; and also because long-term side-effects of medication may not come through in this population. [27]. Primary prophylaxes in cases of increased risk and with well tolerated antiepileptic drugs needs further to be investigated.
Caregivers provide extraordinary care and their efforts remain widely uncompensated [15**,60]. Higher levels of stress and depression, lower subjective well-being and worse physical health are the toll and caregiver burden is an independent risk factor for higher caregivers’ mortality [61]. Concise amelioration of patients’ symptoms improves the patients’ well-being, but not necessarily so the well-being of the caregivers and special focus is needed to improve support there [54,62,63].

The importance of mental competence cannot be stressed enough in this context. Both patients and their caregivers name mental competence as a key factor for QoL [64]. Often cognitive deterioration cannot be prevented and an early and comprehensive dialogue can prepare the patient and his or her caregiver, help to document the end-of-life wishes and give reassurance in times of compromised mental abilities.

The patients state, that QoL is more important for them than prolongation of life [64]. Under this light cancer-directed therapy in advanced stages of brain tumours has to be critically evaluated in every individual patient.

Treatment of primary brain tumours may ameliorate or increase symptom burden depending on the patient’s status, this accounts not only for symptoms of acute radiation exposure but also for symptoms that are primarily targeted by the treatment such as immobility or cognitive impairment [51,65,66].

We know that physicians often estimate patients’ chances of prolonged survival more positively than realistic [13,14]. Especially in the light of this fact both a critical approach to cancer-directed therapies and more information on effectiveness impact on QoL, and prognostic factors are very much in need.

Although observational studies, which are more likely to be done in this specific population, may be worthwhile to better understand the outline of the crucial topics the necessity of randomized intervention trials regarding symptom amelioration, QoL and caregiver burden is growing.

Although all publications in this review dealt with patients in advanced stages of brain tumours, no publication focused especially on the final phase. Further investigations into this topic, there are essentials to meet the challenges appropriately.

**CONCLUSION**

Symptom burden in brain tumour patients is severe and on an average more severe than in other cancer entities. Above that cognitive and functional impairment may hamper detection and proper symptom management.

To address this, problem-specific standardized assessment tools and their routine application are necessary. Caregivers suffer from immense strains partly because of symptoms specific to brain tumours and partly because of difficulties in planning ELDs and advanced directives in a late stage of the disease. Interventions centring on caregivers’ well-being are needed as well as strategies to implement adequate planning ahead of cognitive impairment and deterioration.

**Acknowledgements**

None.

**Conflicts of interest**

The authors declare no conflict of interest.

**REFERENCES AND RECOMMENDED READING**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


A systematic review on psychosocial and supportive needs of patients and caregivers with primary malignant brain tumors. In patients depression and anxiety occurred in up to 48%, in caregivers up to 40%. The study also focused on interventions for patients and caregivers.


In 185 patients with advanced cancer falls occurred in 50.3%. Brain tumors were independently associated with a shortened time to falls with a hazard ratio of 2.5.


Current use of methylphenidate and data on applications and evidence for efficacy. The publication shows beneficial effects of methylphenidate on depression or fatigue in cancer patients, cognitive impairment induced by opioids or brain tumors.


41. A prospective multicentre study to investigate which QoL domains improve or worsen after palliative radiotherapy and which might provide prognostic information. Three months after start of radiotherapy moderate deterioration was observed in several QoL domains. The study calls for addressing critically individual benefit or burden by palliative radiotherapy.


56. Detrimental effects of tumor burden by palliative radiotherapy. Three months after start of radiotherapy moderate deterioration was observed in several QoL domains. The study calls for addressing critically individual benefit or burden by palliative radiotherapy.


Genetics and Genomics of Brain Tumors
The Somatic Genomic Landscape of Glioblastoma

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Abstract

We describe the landscape of somatic genomic alterations based on multi-dimensional and comprehensive characterization of more than 500 glioblastoma tumors (GBMs). We identify several novel mutated genes as well as complex rearrangements of signature receptors including EGFR and PDGFRA. TERT promoter mutations are shown to correlate with elevated mRNA expression, supporting a role in telomerase reactivation. Correlative analyses confirm that the survival advantage of the proneural subtype is conferred by the G-CIMP phenotype, and MGMT DNA methylation may be a predictive biomarker for treatment response only in classical subtype GBM. Integrative analysis of genomic and proteomic profiles challenges the notion of therapeutic inhibition of a pathway as an alternative to inhibition of the target itself. These data will facilitate the discovery of therapeutic and diagnostic target candidates, the validation of research and clinical observations and the generation of unanticipated hypotheses that can advance our molecular understanding of this lethal cancer.

INTRODUCTION

Glioblastoma (GBM) was the first cancer type to be systematically studied by The Cancer Genome Atlas Research Network (TCGA). The initial publication (TCGA, 2008) presented the results of genomic and transcriptomic analysis of 206 GBMs, including mutation sequencing of 600 genes in 91 of the samples. The observations provided a proof-of-concept demonstration that systematic genomic analyses in a statistically powered cohort can define core biological pathways, substantiate anecdotal observations and generate unanticipated insights.

The initial publication reported biologically relevant alterations in three core pathways, namely p53, Rb, and receptor tyrosine kinase (RTK)/Ras/phosphoinositide 3-kinase (PI3K) signaling (TCGA, 2008). Efforts to link the alterations found in these pathways to the distinct molecular and epigenetic subtypes of glioblastoma revealed that coordinated combinations were enriched in different molecular subtypes, which may affect clinical outcome and the sensitivity of individual tumors to therapy (Noushmehr et al., 2010; Verhaak et al., 2010).

Above and beyond these observations, it has become evident that GBM growth is driven by a signaling network with functional redundancy that permits adaptation in response to targeted molecular treatments. Thus, a comprehensive catalogue of molecular alterations in GBM, based on multidimensional high-resolution data sets, will be a critical resource for...
future investigative efforts to understand its pathogenesis mechanisms, inform tumor biology and ultimately develop effective therapies against this deadly cancer.

Toward those ends, TCGA has expanded the scope and depth of molecular data on GBM, including adoption of next-generation sequencing technology (TCGA, 2011, 2012a). Here, we report the efforts of the TCGA GBM Analysis Working Group (AWG) to further our understanding of GBM pathobiology by constructing a detailed somatic landscape of GBM through a series of comprehensive genomic, epigenomic, transcriptomic and proteomic analysis.

RESULTS

Samples and Clinical Data

As summarized in Table 1, the dataset contains molecular and clinical data for a total of 543 patients. Note that different subsets of patients were assayed on each technology platform. The most significant additions to the GBM dataset include sequencing of GBM whole genomes, coding exomes and transcriptomes, expanded DNA methylomes as well as profiling of a targeted proteome. In particular, 291 pairs of germline-tumor native DNAs (e.g. without whole-genome amplification) were characterized by hybrid-capture whole-exome sequencing (WES) and of these, 42 pairs underwent deep coverage whole-genome sequencing (WGS). The transcriptomes of 164 RNA samples were profiled by RNA-sequencing (RNA-seq). Protein expression profiles were generated from 214 patient samples using reverse phase protein arrays (RPPA). The data package associated with this report was frozen on 7/15/2013 and is available at the Data Portal: https://tcga-data.nci.nih.gov/docs/publications/gbm_2013/.

TCGA sample collection spanned 17 contributing sites (SI Table S1). Tier 1 clinical data elements (including age, pathology and survival) are available on 539 of 543 patients (99.6%) and Tier 2 data including treatment information on 525 patients (96.7%) (Figure S1, see Data Portal). Clinical characteristics of this patient cohort are similar to our previous report in 2008 (TCGA, 2008) with a median age of 59.6 years and a male to female ratio of 1.6 (333:209). Median overall survival was 13.9 months with 2-year survival of 22.5% and 5-year survival of 5.3%. Due to TCGA selection of primary GBM, \textit{IDH1} mutation is infrequent in the TCGA cohort compared to other published series. Of the 423 patients with adequate sequencing coverage (by either whole exome next-generation sequencing or previously reported Sanger-based sequencing), 28 (6%) had the \textit{IDH1}-R132H mutation, while one individual had an R132G and one had an R132C mutation. No \textit{IDH2} mutations were found. The associated G-CIMP methylation pattern was present in all cases of \textit{IDH1} mutation (R132H/G/C) while seven G-CIMP cases lacked \textit{IDH1} mutations. Overall, G-CIMP pattern was present in 42 out of 532 cases (7.9%). Clinically-relevant \textit{MGMT} DNA methylation status was estimated from CpG islands as previously described (Bady et al., 2012). Conventional positive prognostic factors were confirmed by univariate analysis: age $< 50$ (OS 21.9 vs. 12.3 months, $p=2.4e-11$), \textit{MGMT} DNA methylation (16.9 vs. 12.7, $p=0.0018$), \textit{IDH1} mutation (35.4 vs. 13.3, $p=1.55e-5$) and G-CIMP DNA methylation (38.3 vs. 12.7, $p=8.3e-9$). Age, MGMT and \textit{IDH1}/G-CIMP status were independently significant in multivariate analysis (SI Table S1).

Patients in this TCGA cohort were diagnosed between 1989 and 2011, with 414 patients (76%) receiving their diagnosis in or after 2002 when the use of concurrent temozolomide (TMZ) with adjuvant radiation became widely adopted. Combined TMZ chemotherapy and radiation treatment is documented for 40% of all patients (217/543), and for 50.2% of the 414 patients diagnosed in or after 2002. Summaries of treatment classification classes are provided in SI.
Whole-exome sequencing identifies significantly mutated genes in glioblastomas

Solution-phase hybrid capture and whole-exome sequencing were performed on paired tumor and normal native genomic DNA obtained from 291 patients. Overall, 138-fold mean target coverage was achieved, with 92% of bases covered at least 14-fold in the tumor and 8-fold in the normal – the threshold which offers 80% power to detect mutations with an allelic fraction of 0.3 (Carter et al., 2012) (see Extended Experimental Procedures). Overall, of the 291 tumor exomes sequenced, 21,540 somatic mutations were identified, with a median rate of 2.2 coding mutations per megabase (lower-upper quartile range: 1.8 – 2.3). Among the somatic mutations were 20,448 single nucleotide variants (SNVs), 39 dinucleotide mutations and 1,153 small insertions and deletions (indels). The SNVs mutations included 5,379 silent, 3,901 missense, 831 nonsense, 360 splice-site and 760 mutations resulting in a frame shift.

Mutations were evaluated across samples to distinguish genes which appear targeted by driver rather than passenger mutations using both MutSig (TCGA, 2008, 2011, 2012a) and InVEx algorithms (Hodis et al., 2012). MutSig assesses mutation significance as a function of gene size, trinucleotide context, gene structure and background mutation rates. InVEx compares the ratio of non-silent exonic mutations to synonymous and intronic/UTR nucleotide variants, an algorithm that is particularly effective for genomes with elevated mutation rates such as melanoma and lung adenocarcinoma. When both InVEx and MutSig algorithms were run on the same dataset, a total of 71 genes were identified as significantly mutated genes (SMG). To validate mutation calls, all 757 SNVs and indels detected by exome sequencing in these 71 SMGs were subject to orthogonal validation by targeted re-sequencing in 259 tumor/normal pairs. At sites with adequate coverage to detect the mutant alleles, 98% of SNVs, 84% of insertions, and 82% of deletions were validated (see Extended Experimental Procedures).

As summarized in Figure 1A, both InVEx and MutSig algorithms identified previously reported genes as significantly mutated in GBM, namely PTEN, TP53, EGFR, PIK3CA, PIK3R1, NF1, RB1, IDH1 and PDGFRα (Figure 1A). In addition, both algorithms identified the leucine-zipper-like transcriptional regulator 1 (LZTR1), mutated in ten samples, as a novel significantly mutated gene in GBM (SI Table S2, SI Figure S2). LZTR1, a putative transcriptional regulator associated with the DiGeorge congenital developmental syndrome (Kurahashi et al., 1995), has not previously been implicated in cancer. It is located at chromosome 22q, and in five of six samples with available copy number data it was simultaneously targeted by hemizygous deletion.

MutSig additionally identified 61 additional genes (71 overall) with mutation frequency above background with a q-value of < 0.1 (SI Table S2). These included spectrin alpha 1 (SPTA1, mutated in 9%), which encodes a cell motility protein that interacts with the ABL oncogene and is related to various hereditary red blood cell disorders; ATRX (6%), a member of the SWI/SNF family of chromatin remodelers recently implicated in pediatric and adult high-grade gliomas (Kannan et al., 2012; Liu et al., 2012; Schwartzentruber et al., 2012); GABRA6 (4%), an inhibitory neurotransmitter in the mammalian brain; and KEL (5%) which codes for a transmembrane polymorphic antigen glycoprotein (SI Figure S2). Albeit at low frequency, several hotspot mutations were found to be significant in this cohort of GBM, most notably the IDH1 R132H mutation. The BRAF V600E sequence variant, which confers sensitivity to vemurafenib in melanoma (Chapman et al., 2011), was detected in five of 291 GBMs (1.7%). Mutation of H3.3 histones, reported in pediatric gliomas (Schwartzentruber et al., 2012), were not observed in this cohort of primary GBM.

To facilitate exploration of mutation data by non-computational biologists, we developed a patient-centric table (PCT) that categorizes each gene in each sample by the type of
mutation (silent, missense, InDel, etc.) observed, and describes the confidence of each call based on the coverage in normal and tumor samples (see Data Portal, Extended Experimental Procedures). To illustrate one potential use of this table, we interrogated the mutation pattern of 161 genes functionally linked to chromatin organization (hereafter referred to as CMG or “chromatin modification genes”, see Extended Experimental Procedures) using this PCT. In total, 135 samples or 46% of the sample cohort harbored at least one non-synonymous mutation in this CMG gene set (Figure 1B). Importantly, CMG mutations were found to be mutually exclusive overall by MEMo analysis (p=0.0008) (Ciriello et al., 2012), suggesting potential biological relevance of chromatin modification in GBM.

Genomic gains and losses in GBM

We expanded our previously reported DNA copy number analysis from 206 GBMs (TCGA, 2008) to 543 samples. The larger data set, coupled with improvement of the analytical algorithm GISTIC (Mermel et al., 2011), resulted in a significant refinement of previously-defined amplification and deletion peaks, thus allowing improved nomination of candidate gene targets for several recurrent somatic copy number aberrations (SCNA) (Figure 1C). The most common amplification events on chromosome 7 (EGFR/MET/CDK6), chromosome 12 (CDK4 and MDM2) and chromosome 4 (PDGFRα) were found at higher frequencies than previously reported (SI Table S3), and often contained only a single gene in the common overlapping region. Additionally, frequent gains of genes such as SOX2, MYCN, CCND1 and CCNE2 were precisely established. Except for the highly recurrent homozygous deletions in CDKN2A/B, all statistically significant DNA losses were hemizygous. Losses were more frequent than amplifications, as has been reported as a general pattern in cancer (Beroukhim et al., 2010). We were able to pinpoint single genes as deletion targets in some cases, most notably in recurrent deletion of 6q26. While the 6q26 deletion has previously been associated with other candidates such as PARK2, our analysis unequivocally defined QKI as the sole gene within the minimal common region and the target of homozygous deletion in 9 cases. The QKI gene was also mutated in 5 cases without evidence of deletion (two frame-shift, two missense and one splice-site mutation). This is consistent with a recent publication demonstrating that QKI functions as a tumor suppressor in GBM by acting as a p53-responsive regulator of mature miR-20a stability to regulate TGFβR2 expression and TGFβ network signaling (Chen et al., 2012). Other single gene deletion targets include LRP1B, Npas3, Lsamp and Smyd3. Similar to the mutation data, we have also algorithmically generated a Patient-Centric Table summarizing DNA copy number aberration and DNA methylation status for each gene and miRNA for each of the cases in the cohort (see Data Portal).

Recurrent structural rearrangements defined by genomic and transcriptomic sequencing

To explore genomic and transcriptomical structural rearrangements, we performed whole-genome paired-end sequencing with deep coverage on 42 pairs of tumor and matched germline DNA samples as well as RNA sequencing (RNA-seq) of 164 GBM transcriptomes (SI Table S4). We detected genomic rearrangements using BreakDancer and BamBam (Sanborn et al., 2013) (see Extended Experimental Procedures), in addition to expressed RNA fusions using PRADA (http://sourceforge.net/projects/prada/). In total, we identified 238 high confidence candidate somatic rearrangements, including 49 interchromosomal, 125 intrachromosomal and 64 intragenic structural variants (Figure 2A and B; SI Table S4). The number of events per sample ranged from 0 to 32 (median: 2), with one sample containing a distinctively high number of rearrangements in the context of local chromothripsis involving a 7.5 Mb region on chromosome 1. No rearrangements were detected in eight samples. Overall, the number of rearrangements generally appeared lower than what has been previously reported for prostate cancer (Sanborn et al., 2013), lung adenocarcinoma...
Imielinski et al., 2012) and melanoma (Berger et al., 2012). Recurrent intragenic events were detected in seven genes: EGFR (n=12), CPM (n=3), PRIM2 (n=3), FAM65B (n=2), PPM1H (n=2), RBM25 (n=2), and HOMER2 (n=2). Since unbalanced structural rearrangements in DNA can be detected as breakpoints in DNA copy number profiles, we investigated whether CNA breakpoints could indicate potential sites of recurrent structural rearrangement using all 492 samples with aCGH data (n=492). Of note, 41 of 129 high-confidence rearrangement events from whole-genome sequencing (WGS) involved genes identified as significant targets of recurrent intragenic copy number breakpoints (iCNA) in the larger cohort of GBM based on DNA copy number profiles (SI Table S4, Data Portal).

RNA seq analysis identified 48 interchromosomal and 180 intrachromosomal mRNA fusion transcripts in 106 of 164 samples (Figure 2C; SI Table S4). Approximately 37% of these were in-frame transcripts, 35% were out-of-frame and the remaining 29% were involved a 3′ or 5′ untranslated region (SI Figure S3A). A substantial portion (44%) of the intrachromosomal events resulted from recombination of genomic loci located less than 1Mb apart. A notable example is the recently reported oncogenic FGFR3-TACC3 inversion (Singh et al., 2012), which was detected in two cases. Interestingly, the FGFR3/TACC3 locus was focally amplified in both samples, suggesting that CNA could serve as a marker of FGFR3-TACC3 rearrangement. Overall, focal amplifications involving FGFR3 or TACC3 were detected in 14 of 537 GBM copy number profiles (2.6%).

Ten of the 42 GBMs with WGS analyses demonstrated rearrangements between EGFR and adjacent genes such as BRIP (n=2) and VOPP1 (n=2), or structural variants of genes surrounding the EGFR locus, such as LANCL2 and PLEXHA (n=2) (SI Table S4). Both types of 7p11 rearrangements were detected in six samples. This pattern was confirmed in the RNA-seq data where eighteen samples of 164 samples showed evidence of transcribed fusion transcripts, such as EGFR-SEPT14 (n=6), SEC61G-EGFR (n=4), LANCL2-SEPT14 (n=1) and COBL-SEPT14 (n=1) (SI Table S4). These fusions tended to be part of a focal gain, suggesting a complex rearrangement (SI Figure S3B).

Genomic rearrangements pertaining to chromosome arm 12q were identified in 11 of 42 whole genomes and 12q-associated fusion transcripts were found expressed in 25 of 164 transcriptomes. A variety of different genomic and transcriptomic variants were found on 12q though none were recurrent (SI Table S4). The majority of 12q lesions occurred in tandem, i.e. as adjacent events in the same GBM. As an illustration, a single sample showed a pattern in which 15 non-adjacent segments (14 from chromosome 12 and one fragment from chromosome 7) were highly amplified (>40 copies) with eight 12q rearrangement events, including the MDM2, CDK4 and EGFR oncogenes (SI Figure S3C). WGS analysis reconstructed two independent circular paths that accounted for all of the amplified segments (SI Figure S3C). Each circle contained at least one oncogene, with one circle (0152-DM-A) containing one copy of CDK4 and two copies of MDM2 and the other circle (0152-DM-B) containing one copy of EGFR. These reconstructed circles are most consistent with extrachromosomal double minute chromosomes (Kuttler and Mai, 2007). Recently, the same data set was used to identify enrichment of genomic breakpoints relating to chromosome 12q14–15, a locus harboring the MDM2 and CDK4 oncogenes, which pertained to less favorable outcome (Zheng et al., 2013), and the reconstruction of double minutes confirmed using orthogonal methods (Sanborn et al., 2013).

**EGFR is frequently targeted by multiple alterations of DNA and RNA**

As anticipated, EGFR was among the most frequently mutated genes and RNA-seq detected a diversity of altered transcripts (Figure 3A). EGFR mutations were accompanied by regional DNA amplification in the majority of cases, leading to a wide range of mutation allelic frequencies. Comparing the allelic frequencies of point mutations in DNA- and RNA-
seq data revealed a high degree of concordance between the type and prevalence of mutations at the DNA level and the composition of expressed mRNA transcripts (SI Figure S4A).

RNA-seq also provided a complete picture of aberrant exon junctions and a semi-quantitative assessment of their expression levels. Transcript allelic fraction (TAF) was calculated as the ratio of each aberrant exon junction to the sum of aberrant and wild-type junctions at the 3′ junction end, corrected for read depth (80% confidence, binomial confidence interval). TAFs for recurrent point mutations and junctions are summarized in SI Table S5. In 11% of tumors, the aberrant exon 1–8 junction characteristic of EGFRvIII was highly expressed (≥10% TAF), while 19% showed at least a low level expression (≥1%). The results were concordant with an independent assessment of EGFRvIII by digital mRNA assay using barcoded probes (nCounter, Nanostring Technologies and by real-time PCR (see Data Portal). While the biological or clinical relevance of low-level EGFRvIII expression remains to be demonstrated, EGFRvIII expression in a minor population of GBM cells has been shown to confer a more aggressive tumor phenotype through paracrine mechanisms (Inda et al., 2010).

A variety of other recurrent non-canonical EGFR transcript forms were detected in the RNA-seq data (Figure 3A, SI Figure S4B). Three different C-terminal rearrangements targeting the cytoplasmic domain of the EGFR were detected at ≥10% TAF in 3.7% of cases and at ≥1% TAF in another 9%. Comparison with WGS data confirmed the presence of C-terminal deletions in 9 cases where sequence data was available. C-terminal deletion variants have previously been associated with gliomagenesis in experimental rodent systems in vivo (Cho et al., 2012). The prevalence of EGFR C-terminal deletion reported here is likely an underestimate since complete loss of the C-terminus may yield aberrant terminal junctions not mappable by transcriptome sequencing. Relative under-expression of C-terminus exons 27–29 (< 3 standard deviations) was readily apparent in another 7.3% of cases without detectable aberrant junctions (Figure 3B).

We identified two relatively uncharacterized recurrent EGFR variants, namely deletions of exons 12–13 (Δ12–13) in 28.7% and exons 14–15 (Δ14–15) in 3%. EGFR Δ12–13 has been previously identified by RT-PCR analysis of glioma (Callaghan et al., 1993). Both Δ12–13 and Δ14–15 appear to be expressed in minor allelic fractions (<10%), raising the question of whether they result from splicing aberration or genomic deletion. Among tumors expressing Δ12–13mRNA, analysis of aberrant junctions in WGS data (BamBam) failed to identify concordant DNA deletion in 14/15 cases where data was available. One case showed a concordant breakpoint as a minor component of a highly rearranged locus. By comparison, EGFRvIII-expressing tumors had concordant deletion spanning exons 2–7 in all 7 cases where WGS data was available (SI Table S5).

In total, 38.4% of cases harbored an EGFR genomic rearrangement or a point mutation expressed in at least 10% of transcripts (Figure 3B; SI Table S5). Overall, 57% of GBM showed evidence of mutation, rearrangement, altered splicing and/or focal amplification of EGFR. While PDGFRA showed no recurrent gene fusions, intragenic deletion of exons 8 and 9 (PDGFRA Δ8,9) was highly expressed (≥10% TAF) in 1 of the 164 samples with RNA sequencing data. Low-level expression of PDGFRA Δ8,9 was far more prevalent in the RNA-seq data (n=29 of 163) and could represent a splice variant. This result is concordant with previously reported estimates of Δ8,9 expression (Ozawa et al., 2010). A novel PDGFRA variant with deletion of exons 2–7 was found highly expressed in a single case (TCGA-28-5216).
The landscape of somatic alterations in glioblastoma

The addition of whole exome and transcriptomical sequencing data has extended the palette of somatic alterations affecting major cancer pathways in GBM. Figure 4 presents a landscape view of the canonical signal transduction and tumor suppressor pathways in GBM based on whole exome sequencing data of 291 patients. Unsupervised analysis of 251 GBMs with both copy number and WES mutation data identified genes sets (modules) in which somatic alterations were significantly mutually exclusive (MEMo, (Ciriello et al., 2012)). This analysis confirmed mutual exclusivity among alterations affecting the p53 pathway (MDM2, MDM4 and TP53), the Rb pathway (CDK4, CDK6, CCND2, CDKN2A/B and RB1), and various components influencing the PI3K pathway (PIK3CA, PIK3R1, PTEN, EGFR, PDGFRA, NF1) (SI Table S6).

As shown, at least one RTK was found altered in 67.3% of GBM overall: EGFR (57.4%), PDGFRA (13.1%), MET (1.6%) and FGFR2/3 (3.2%). Half of the tumors with focal amplification and/or mutation of PDGFRA harbored concurrent EGFR alterations (42.4%, 14/33), as did the majority of MET-altered tumors (3/4), reflecting a pattern of intratumoral heterogeneity that has been previously documented by in situ hybridization (Snuderl et al., 2011; Szerlip et al., 2012).

PI3-kinase mutations were found in 25.1% of GBM (63/251), with 18.3% affecting p110alpha and/or p85alpha subunits and 6.8% in other PI3K family genes. PI3K mutations were mutually exclusive of PTEN mutations/deletions (p=0.0047, Fisher’s Exact), with 59.4% of GBM showing one or the other (149/251). Considering the RTK genes, PI3-kinase genes and PTEN, 89.6% of GBM had at least one alteration in the PI3K pathway and 39% had two or more. The NF1 gene was deleted or mutated in 10% of cases, and never co-occurred with BRAF mutations (2%).

Concordant with the previous TCGA GBM report, the p53 pathway was found to be dysregulated in 85.3% of tumors (214/251), through mutation/deletion of TP53 (27.9%), amplification of MDM1/2/4 (15.1%) and/or deletion of CDKN2A (57.8%). As expected, TP53 alterations were mutually exclusive with amplification of MDM family genes (p=0.0003) and CDKN2A (p=1.99e–7). Concurrently, 78.9% of tumors had one or more alteration affecting Rb function: 7.6% by direct RB1 mutation/deletion, 15.5% by amplification of CDK4/6, and the remainder via CDKN2A deletion.

As reported for lower grade gliomas (Ichimura et al., 2009), 12 of the 13 GBMs with IDH1 hotspot mutations harbored concurrent TP53 mutations. Consistent with recent reports, mutations in SWI/SNF complex gene ATRX often co-occurred in these cases (Figure 4B). Mutations in IDH1 and ATRX appear to be more prevalent in GBM samples without RTK alteration (p=7.2e-5 and 7.3e-4, respectively), tumors genotypically more consistent with secondary GBM (Ohgaki and Kleihues, 2007).

Telomerase reverse transcriptase (TERT) promoter mutations were recently reported in glioma, mapping to positions 124 (C228T) and 146bp (C250T) upstream of the TERT ATG start site (Killela et al., 2013). Of the 42 cases with deep coverage WGS data, 25 samples had adequate coverage (read count >10) of the TERT promoter for mutational analysis. We detected the C228T mutation in 15 of the 25 cases, while the C250T variant was found in another 6 cases (Figure 4C). TERT promoter mutations at these two hot spots were correlated with up-regulated TERT expression at the RNA level (Figure 4C). Interestingly, the four GBMs with non-mutated TERT promoters all harbored ATRX mutations and these were concurrent with IDH1 and TP53 mutations as recently described (Liu et al., 2012). Finally, in line with the role of ATRX in alternative lengthening of telomeres (ALT) (Lovejoy et al., 2012), ATRX-mutant GBM tumors do not exhibit elevated TERT RNA
expression compared to tumors with TERT promoter mutations (Figure 4C). Taken together, these data suggest that maintenance of the telomere either through reactivation of telomerase by TERT promoter mutation-induced increased TERT expression or ALT as a result of ATRX mutation is a requisite step in GBM pathogenesis.

While reported median survival for patients with GBM ranges from 12–18 months, a subset of individuals will survive for more than three years (Dolecek et al., 2012; Dunn et al., 2012). We cross-referenced our data set to identify any factor(s) associated with long-term survival (n=39 or 7.7% of the cohort). Although no specific genomic alteration was significantly over-represented in this subset, amplifications of CDK4 and EGFR and deletion of CDKN2A were observed at decreased frequencies in these long survivors (see Data Portal). Age at diagnosis was found to be a major determinant, with 79% of long-term survivors being diagnosed at younger than 50 years of age. Despite their relatively favorable prognosis, only one third of patients with G-CIMP+ GBM survived beyond three years, suggesting that other factors yet to be identified are contributing to overall long-term survival of GBM patients.

**Molecular subclasses defined by global mRNA expression and DNA Methylation**

Widespread differences in gene expression have previously been reported in GBM, grouping TCGA tumors into proneural, neural, classical and mesenchymal transcriptomic subtypes (Phillips et al., 2006; Verhaak et al., 2010). Samples not included in previously published analysis (n=342) were classified into one of classes using single sample gene set enrichment analysis (Figure 5A, SI Table S7) Similarly, we sought to assign each case in the TCGA cohort to one of the DNA methylation subclasses. The promoter DNA methylation array platforms used by TCGA have evolved with increasing resolution from the Illumina GoldenGate (n=238), Infinium HumanMethylation27 (HM27, n=283) and Infinium HumanMethylation450 (HM450, n=76) platforms (SI Figure S5A). We re-analyzed a total of 396 GBM samples, comprised of 305 new GBM samples profiled on the HM27 (n=192) and HM450 (n=113) platforms in addition to 91 cases profiled on HM27 that were included previously (Noushmehr et al., 2010). Hierarchical consensus clustering of the DNA methylation profiles stratified these 396 GBM cases into six classes, including G-CIMP (Figure 5B, SI Figures S5B and S5C, and SI Table S7). Cluster M1 (35/58, 60%) is enriched for mesenchymal GBMs while cluster M3 (18/31, 58%) is enriched for classical subtype (Figure 5B, red and blue, respectively). As expected, the G-CIMP cluster is enriched for proneural subtype tumors.

To be able to perform more robust exploration of the relationship of G-CIMP phenotype to other genomic alterations, we incorporated the previously reported G-CIMP status (Noushmehr et al., 2010) on 175 additional GBM cases profiled on the GoldenGate platform. A total of 534 GBM cases, were used in the following integrative analyses. The age of GBM diagnosis was statistically different (41yrs vs. 56yrs; p-value = 0.008) between proneural G-CIMP (n=28) and proneural non-G-CIMP (n=22) subtypes, reinforcing the notion that the epigenomics of these transcriptomically similar patients mark distinct etiologies and/or disease characteristics. We observed seven G-CIMP(+) cases lacking IDH1 mutation. These were similar to G-CIMP cases harboring IDH1 mutations with respect to their median age at diagnosis (40yrs vs. 37yrs, p-value = 0.58) and overall survival (mean 913 days vs. 1248 days, p-value = 0.45). IDH2 mutation was not detected in these seven G-CIMP+/IDH1 wildtype GBM, suggesting that alternative pathway(s) responsible for the hypermethylator phenotype.

Next, to identify genomic alterations enriched in each of the transcriptomic or epigenomic subtypes, we referenced the Patient-Centric Tables to count DNA mutation and copy number aberration events per subtype. This analysis confirmed previous reports,
demonstrating significant associations between PDGFRA amplification and the non-G-CIMP+ proneural subgroup, as well as NF1 inactivation and the mesenchymal subtype (Figure 5A). Additionally, the enhanced power of the larger data set identified an enrichment of ATRX mutations and MYC amplifications in the G-CIMP+ subtype, CDK4 and SOX2 amplifications in proneural subtype, and broad amplifications of chromosomes 19 and 20 in the classical subtype (Figure 5A). In contrast to G-CIMP, cluster M6 was relatively hypomethylated, with a predominance of non-mutated IDH1 cases belonging to the proneural subtype (22/37, 59%) with concurrent PDGFRA amplification (Figure 5B).

To explore a plausible connection between chromatin deregulation and DNA methylation, we counted mutations in the 161 CMGs (Figure 1B) per each methylation subclass. In addition to the association of IDH1 and ATRX mutations and G-CIMP, mutations of other CMGs were enriched across the M2, M4 and M6 subclasses (38% of cases in these three subclasses harbor at least one CMG mutation vs. 18% among the other classes, p=0.0015). Furthermore, cases with missense mutation or deletion of MLL genes (n=18) or HDAC family genes (n=4) clustered in the M2 DNA methylation subtype (10/21). These patterns of co-occurrence suggest a functional relationship between chromatin modification and DNA methylation that remains to be elucidated. Recently, Sturm et al. reported that adult and pediatric GBM with alterations of IDH1, H3F3A and receptor tyrosine kinases (RTK) were associated with epigenetic subtypes (Sturm et al., 2012). We compared the Sturm et al methylation-based classification with ours using the 74 TCGA cases that were also classified by those authors. We found that four tumors classified as “IDH” subtype in Sturm et al. were assigned to G-CIMP subtype in our classification scheme (SI Figure S5D). The “Mesenchymal” tumors were assigned to M1 and M2 (21/25), “RTK II ‘classic’” tumors were assigned to M3 and M4 (30/34) and the “RTK I ‘PDGFRA’” tumors were assigned to M6. No TCGA samples were clustered in the Sturm et al’s “G34” or “K27” classes and we found the corresponding histone mutations to be absent across the TCGA sample set.

Lastly, we explored the relationship of molecular subtypes with clinical parameters such as treatment response or survival. In the current larger TCGA cohort, the survival advantage of proneural subtype GBM (Phillips et al., 2006) was definitively shown to be conferred by G-CIMP status, with non-G-CIMP proneural GBMs and not mesenchymal GBM tending to show less favorable outcomes in the first twelve months following initial diagnosis compared to other subtypes (p-value 0.07; SI Figure S6A). While most of the samples clustered in the M6 group were classified as proneural, this methylation subclass was not associated with adverse survival overall (SI Figure S6B) (Noushmehr et al., 2010). This observation reinforces the notion that target genes affected by the G-CIMP phenotype likely contribute to the improved prognosis for this subset of proneural GBM.

DNA methylation of the MGMT gene promoter is a known marker for treatment response (Hegi et al., 2005). We found that the MGMT locus was methylated in 48.5% of patients in our cohort (174 of 359 assessed), and that G-CIMP cases showed an increased likelihood of having MGMT DNA methylation (79% of G-CIMP vs. 46% for non-G-CIMP; SI Figure S6C). When correlated with outcome, MGMT status distinguished responders from non-responders amongst samples classified as classical (n = 96; p = 0.01) but not among samples classified as proneural (n = 66; p = 0.57), mesenchymal (n = 104; p = 0.62) and neural (n = 55; p = 0.12) (SI Figures S6D and E). In summary, our data provides evidence for MGMT DNA methylation as a predictive biomarker in the GBM Classical subtype of GBM, but not other subtypes.

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Regulatory networks of miRNA and mRNA in gliomagenesis

MicroRNAs (miRs) have been found to promote or suppress oncogenesis through modulation of gene expression via mRNA degradation or inhibition of translation (Bartel, 2004; Krol et al., 2010). Recent studies have proposed additional mechanisms of miR-mRNA regulation, including modulation of competing endogenous RNA (ceRNA), which are mRNA with competitive miR binding sites (Sumazin et al., 2011; Tay et al., 2011). Leveraging the existence of matched mRNA and miR profiling data on a large number of samples, we sought to define the salient interactions between specific pairs of miRs and mRNAs through both of these mechanisms.

We employed a relevance network based approach to infer miR:mRNA associations in GBMs with matched miR and mRNA profiles (n=482). Putative regulatory targets of individual miRs were defined as those genes having strong negative correlation with the miR (< −0.3) and prediction support in three commonly used databases (Miranda, Pictar, TargetScan). 133 miR:mRNA associations defined the final putative miR regulatory network (see Data Portal). The most prevalent associations related to molecular subtypes. For instance, hsa-mir-29a (part of the miR29 family, thought to play a role in the TP53 pathway (Park et al., 2009) was predicted to regulate 23 genes. 17 of these 23 genes were expressed at distinctively high levels in the non-G-CIMP+ proneural tumors only, and not in the G-CIMP+ tumors. Interestingly, three (BCL11A, PCFG3, SS18L1) of the 23 genes in this subnetwork are predicted to act as PDGFRA ceRNAs (see below).

Competitive endogenous mRNAs (ceRNAs) are mRNAs co-regulated in trans by a common miR (Sumazin et al., 2011; Tay et al., 2011). Here, we used a correlation- and NLS-based approach, integrating miRNA and mRNA expression and copy number profiles to predict ceRNAs for four GBM signature genes: PDGFRA, EGFR, NF1, and PTEN. Interestingly, predicted PDGFRA ceRNAs significantly overlapped with proneural GBM signature genes (p-value <1e-15), while EGFR ceRNAs significantly overlapped with classical GBM signature genes (p-value=1.2e-14) (see Data Portal). Predicted ceRNAs of NF1 overlapped with proneural signatures (P<1e-15) and PTEN-associated ceRNAs were correlated with the mesenchymal signature. This provocative finding raises the possibility that ceRNA regulation by miR may contribute to the transcriptomic signature that defines the molecular subtypes in GBM, although this hypothesis remains to be tested.

Signaling pathway activation in different molecular subtypes of GBM

To assess whether enrichment of genomic alterations in molecular subtypes translates into downstream pathway activation, we performed targeted proteomic profiling by reverse-phase protein arrays (RPPA). 214 sample lysates were probed with 171 antibodies targeting phospho- and/or total-protein levels among signaling pathways as previously described (TCGA, 2012c). After normalization, co-clusters of correlated signaling molecules within specific signaling pathways were observed (see Extended Experimental Procedures, Data portal) and were utilized as readout of pathway activity status for correlative analyses.

Unsupervised clustering of RPPA data failed to produce a consistent partitioning of the sample cohort into clearly-defined subtypes. However, 127 out of the 171 antibodies were found to correlate significantly with transcriptomal subtype (Kruskal-Wallis, p<0.05; Extended Experimental Procedures). As anticipated, EGFR amplification/mutation was associated with significant elevations in total EGFR expression (p=3.74E-15) and phosphorylation (p=1.44E-12, SI Figure S7A), both prominent in classical subtype tumors (SI Figure S7B). Classical GBMs also showed relative downregulation of pro-apoptotic proteins (including cleaved caspase 7, cleaved caspase 9, Bid and Bak) as well as MAP kinase signaling, including its downstream target p90RSK. Notch1 and Notch3 expression...
were moderately increased in classical tumors, consistent with previous reports linking EGFR and Notch activation in GBM (Brennan et al., 2009).

Mesenchymal subtype tumors exhibited elevated levels of endothelial markers, such as CD31 and VEGF-R2, consistent with previous findings (Phillips et al., 2006), as well as markers of inflammation (e.g., Fibronectin and its downstream target COX-2). Mesenchymal tumors showed moderately increased activation of the MAPK pathway, as evidenced by higher levels of phospho-Raf, phospho-MEK and phospho-ERK (Figure 6). These tumors also exhibited decreased levels of the mTOR regulatory protein, tuberin (TSC2 gene product), which is inhibited by ERK phosphorylation.

In contrast to the mesenchymal subtype, proneural GBMs showed relatively elevated expression and activation of the PI3K pathway including the Akt-regulated mTorC1 activation site (Figure 6). Proneural tumors showed greater inhibition of the 4EBP1 translation repressor, whereas mesenchymal tumors display elevated S6 kinase activation (indicative of mTOR effector pathway activation). Therefore, both subtypes achieve mTOR pathway activation although the specific patterns of steady-state protein activation differ.

G-CIMP+ tumors shared characteristics with their proneural superfamily, but also showed decreased expression of several proteins, including Cox-2, IGFBP2 and Annexin 1. Among the 171 antibodies tested in the TCGA dataset, these three proteins were the most negatively prognostic (Cox proportional hazard test, p<0.0004–0.0013). IGFBP2 and Cox-2 have been independently reported as poor prognostic markers in diffuse gliomas (Holmes et al., 2012; Shono et al., 2001), and low IGFBP2 expression has been associated with global DNA hypermethylation in glioma (Zheng et al., 2011). Members of the annexin family have been associated with glioma growth and migration, and annexin-1 is known to be under-expressed in secondary but not primary GBM (Schittenhelm et al., 2009). Together, the correlations of these proteins with G-CIMP status suggest that their prognostic significance is not independent. Analysis of DNA methylation for IGFBP2, COX2 and ANXA1 found no evidence of hyper-methylation in G-CIMP tumors.

Interestingly, samples with RTK amplification had lower levels of canonical RTK-target pathway activities as measured by phospho-AKT, phospho-S6 kinase and phospho-MAPK co-cluster levels (SI Figure S7C). While PTEN loss and deletion were each associated with incremental increases in AKT pathway activity, PI3K-mutant samples had lower AKT activity than samples lacking PI3K mutations, concordant with findings in breast cancer (TCGA, 2012c). Samples harboring NF1 mutation/deletion showed elevated MAP kinase activity (p-ERK and p-MEK, p-value<0.001), and trended towards decreased PKC pathway activity. These examples of non-linear relationship between protein signaling and underlying genetic mutations speak to complex and likely dynamic signaling in cancers.

DISCUSSION

In this study, we provided a comprehensive catalogue of somatic alterations associated with glioblastoma, constructed through whole genome, exome and RNA sequencing as well as copy number, transcriptomic, epigenomic and targeted proteomic profiling. With the availability of detailed clinical information including treatment and survival outcome for nearly the entire cohort, this rich data set offers new opportunity to discover genomics-based biomarkers, validate disease-related mechanisms and generate novel hypotheses.

In addition to alterations in signature oncogenes of GBM, such as EGFR and PI3K, we found that over 40% of tumors harbor at least one non-synonymous mutation among the chromatin-modifier genes. A role for chromatin organization in GBM pathology, which has
been described for cancer types such as ovarian carcinoma (Wiegand et al., 2010) and renal carcinoma (Varela et al., 2011), is suggested. We also detected mutations in genes for which targeted therapies have been developed, such as \textit{BRAF} (Chapman et al., 2011), and \textit{FGFR1/FGFR2/FGFR3} (Singh et al., 2012), demonstrating the potential clinical impact of this TCGA dataset.

Structural rearrangements that contributed to the overall complexity of the genome and transcriptome were detected in the majority of GBM. A high frequency of structural variants on the q arm of chromosome 12, involving the \textit{MDM2} and \textit{CDK4} genes, was observed and associated with the presence of double minute, extrachromosomal DNA fragments, which may be functionally relevant (Zheng et al., 2013). The identification of complex \textit{EGFR} fusion and deletion variants in nearly half of GBM confirm relevance of this category of somatic alterations to the disease. While the development of a therapeutic strategy targeting mutated \textit{EGFR} could have a major impact on survival and continues to be a topic of great interest (Vivanco et al., 2012), strategies will need to address the possibility that different \textit{EGFR} alterations might exist concurrently in a tumor and yield differential biological activities and/or responses to any given targeted inhibitor.

Another level of biological complexity is revealed by targeted proteomic profile, which showed that the impact of specific genomic alterations on downstream pathway signaling is not linear. The discordance between genomic features and proteomic activation status speak to a complex, and likely dynamic, relationship between signaling and molecular alterations. This observation has provocative clinical implication as it directly challenges the notion that therapeutic inhibition of downstream signaling components along a pathway would yield similar efficacy of targeting the mutated gene itself. Additionally, this observation highlights the limitation of TCGA data, namely its inherent static nature given a single time point analysis, and its inability to map specific genetic or protein changes to the individual cells or cell population given its approach to whole-tumor tissue analysis.

In summary, this report reaffirms the power and value of TCGA’s comprehensive multidimensional and clinically annotated GBM reference dataset in enabling hypothesis generation based on unanticipated observations and relationships emerged from unbiased data-driven analyses. We believe that this public resource will serve to facilitate discovery of new insights that can advance our molecular understanding of this disease.

**EXPERIMENTAL PROCEDURES**

**Patient and Sample Characteristics**

Specimens were obtained from patients, with appropriate consent from institutional review boards. Details of sample preparation are described in the Extended Experimental Procedures.

**Data generation**

In total, 599 patients were assayed on at least one molecular profiling platform, which platforms included: (1) exome sequencing, (2) DNA copy number and single nucleotide polymorphism arrays, (3) whole genome sequencing (4) gene expression arrays, (5) RNA sequencing, (6) DNA methylation arrays, (7) reverse phase protein arrays and (8) miRNA arrays. Details of data generation are described in the Extended Experimental Procedures.

**Whole Genome and Exome Sequencing Data Analysis**

Massively Parallel Sequencing Exome capture was performed by using Agilent SureSelect Human All Exon 50 Mb according the manufacturer’s instructions. All exome and whole
genome sequencing was performed on the Illumina GA2000 and HiSeq platforms. Basic alignment and sequence quality control were done by using the Picard and Firehose pipelines at the Broad Institute. Mapped genomes were processed by the Broad Firehose pipeline to perform additional quality control, variant calling, and mutational significance analysis.

**RNA Sequencing Data Analysis**

Libraries were generated from total RNA and constructed using the manufacturers protocols. Sequencing was done on the Illumina HiSeq platform. Read mapping and downstream data analysis (expression profiles, fusion transcripts, structural transcript variants) were performed using the PRADA pipeline.

**Array Data Preprocessing and Analysis**

To ensure across-platform comparability, features from all array platforms were compared to a reference genome as previously described (TCGA, 2008). Both single platform analyses and integrated cross-platform analyses were performed, as described in detail in the Extended Experimental Procedures.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Exome DNA sequencing in 291 glioblastomas, 42 with whole genome sequencing
- RNA sequencing of 164 glioblastomas identifies recurrent gene rearrangements
- Copy number, DNA methylation, protein, mRNA and miRNA expression profiles of 543 GBMs
- Integrated analysis of somatic alterations, molecular subtypes and affected pathways
Figure 1. Somatic genomic alterations in glioblastoma

(A) Summary of significantly mutated genes from 291 exomes. Specific mutations for LZTR1, SPTA1, KEL, and TCHH are shown in SI Figure S2.

Upper histogram: Number of mutations per sample (substitutions and indels). Left histogram, rate of mutations per gene and percentage of samples affected.

Central heat map: Distribution of significant mutations across sequenced samples, color coded by mutation type.

Left histograms: Overall count and significance level of mutations as determined by log(10) transformation of the MutSig q-value. Red line indicates a q-value of 0.05.

Right histogram: Summary of focal amplifications (red) and deletion (blue) determined from DNA copy number platforms (asterisk denotes inclusion in statistically significant recurrent CNA by GISTIC).

Lower chart: Average fraction of tumor reads versus total number of reads per sample.

Bottom chart: top, rates of non-silent mutations within categories indicated by legend; bottom, mutation spectrum of somatic substitutions of samples in each column.

(B) Mutations in 38 genes related to specific epigenetic function categories (out of 161 genes linked to chromatin modification) across 98 GBMs (out of 292 GBM). IDH1 mutation status is included to illustrate its co-occurrence with ATRX mutation. An additional 37 GBMs harbored mutations in one of the remaining 129 CMGs.

(C) Recurrent sites of DNA copy number aberration determined from 543 samples by the GISTIC algorithm. Statistically significant, focally amplified (red) and deleted (blue) regions are plotted along the genome. Significant regions (FDR<0.25) are annotated with the number of genes spanned by the peak in parentheses. For peaks that contain a putative oncogene or tumor suppressor, the gene is noted.
Figure 2. Structural rearrangements and transcript variants in GBM

(A) Circos plots of structural DNA and RNA rearrangements in six GBMs, selected from 28 cases with available whole genome and RNA sequencing based on their rearrangement frequency. Outer ring indicates chromosomes. Copy number levels are displayed along the chromosome map in red (copy number gain) and blue (copy number loss). Each line in the center maps a single structural variant to the site of origin for both genes (see SI Figure S3 for additional analysis of fusion transcripts derived from RNA sequencing).

(B) The chromosome arm of origin of both ends of each rearrangement detected in whole genome sequencing data from 42 GBM were counted and compared to chromosome arm length.
(C) The chromosome arm of both partners in fusion transcripts detected from RNA sequencing data from 164 GBM were counted and compared to chromosome arm length.
Figure 3. Somatic alterations of the EGFR locus

(A) EGFR protein domain structure with somatic mutations summarized from 291 GBMs with exome sequencing and transcript alterations identified across 164 GBMs with RNA sequencing.

(B) EGFR alterations are summarized by transcript prevalence in 164 GBMs with RNA sequencing. Red, top: focal amplification or regional gain inferred from DNA copy number. Blue: Prevalence of sequencing reads with EGFR point mutation. Green: prevalence of reads with aberrant exon-exon junctions (e.g., 1E–8S is a junction spanning from the end of exon 1 to the start of exon 8, consistent with EGFRvIII mutation). Black: EGFR fusion transcript detected (see rearrangements). See related SI Figure S4 for comparison of EGFR mutations in DNA and RNA and for a summary of EGFR rearrangements.
Figure 4. Landscape of Pathway Alterations in GBM
Alterations affecting canonical signal transduction and tumor suppressor pathways are summarized for 251 GBM with both exome sequencing and DNA copy number data. Rearrangements are underestimated in this summary since RNA-seq data were available for only a subset of cases with exome sequencing data (153/291, 61%).

(A) Overall alteration rate is summarized for canonical PI3K/MAPK, p53 and Rb regulatory pathways.

(B) Per-sample expansion of alterations summarized in 5A. Mutations (blue), focal amplifications (red) and homozygous deletions are selected from the patient-centric tables and organized by function. All missense, nonsense and frame-shift mutations are included.
EGFRvIII is inferred from RNA data and included as a mutation if >=10% transcribed allelic frequency. Deletions are defined by log2 ratios < −1 or <=−0.5 and focally targeting the gene (see Extended Experimental Procedures). Amplifications are defined by log2 ratio>2 or >1 and focal.

(C) **Left:** For a cohort of 25 GBMs for which whole genome sequencing allowed genotyping, TERT promoter C228T and C250T mutations occurred in a mutually exclusive fashion. All four TERT promoter wildtype GBM harbored ATRX mutation, and were enriched in G-CIMP group.

**Right:** TERT promoter mutations are associated with elevated expression.
Figure 5. Molecular subclasses of GBM and their genomic molecular correlates

(A) Genomic alterations and survival associated with five molecular subtypes of GBM. Expression and DNA methylation profiles were used to classify 332 GBMs with available (native DNA and whole genome amplified DNA) exome sequencing and DNA copy number levels. The most significant genomic associations were identified through Chi-square tests, with p-values corrected for multiple testing using the Benjamini-Hochberg method.

(B) Genomic alterations and sample features associated with six GBM methylation clusters. Epigenomic consensus clustering was performed on 396 GBM samples profiled across two different platforms (Infinium HM27 and Infinium HM450). Six DNA methylation clusters were identified (see related SI Figure S5), represented as M1 to M6, where M5 is G-CIMP. These DNA methylation signatures are correlated with 27 selected features composed of clinical, somatic and copy number alterations; DM cluster, G-CIMP status, four TCGA
GBM gene expression subclasses, two clinical features (Age at diagnosis/overall survival in months), somatic mutations (IDH1, TP53, ATRX) and 18 selected copy number alterations.
Figure 6.
Canonical PI3K and MAPK pathway activation determined by reverse phase protein arrays and compared between GBM subclasses: Proneural (P, purple, n=55) and Mesenchymal (M, red, n=45). Activation/expression levels are plotted for principal signaling nodes of the MAPK (phospho-MEK and phospho-p90RSK), PI3 kinase (pS473-Akt) and mTOR (TSC1/2, phospho-mTOR, p235/236 S6, phospho-4EBP1 and EIF4E) pathways (p-values, two-tailed T-test). Mesenchymal tumors showed increased activation of the MAPK pathway (evidenced by higher levels of phospho-MEK and downstream phospho-p90RSK) and decreased levels of phospho-ERK inhibitory target TSC2. In contrast, proneural tumors showed relatively elevated expression and activation of members of the PI(3) kinase pathway including Akt PDK1 target site threonine 308 (p=0.01, not shown) and Akt mTORC2 target site (serine 473). Phospho-ERK levels were not significantly different between these two subtypes.
Table 1
Characterization platforms and data availability

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Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations

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Meningiomas are the most common primary nervous system tumor. The tumor suppressor NF2 is disrupted in approximately half of all meningiomas1, but the complete spectrum of genetic changes remains undefined. We performed whole-genome or whole-exome sequencing on 17 meningiomas and focused sequencing on an additional 48 tumors to identify and validate somatic genetic alterations. Most meningiomas had simple genomes, with fewer mutations, rearrangements and copy-number alterations than reported in other tumors in adults. However, several meningiomas harbored more complex patterns of copy-number changes and rearrangements, including one tumor with chromothripsis. We confirmed focal NF2 inactivation in 43% of tumors and found alterations in epigenetic modifiers in an additional 8% of tumors. A subset of meningiomas lacking NF2 alterations harbored recurrent oncogenic mutations in AKT1 (p.Glu17Lys) and SMO (p.Trp535Leu) and exhibited immunohistochemical evidence of activation of these pathways. These mutations were present in therapeutically challenging tumors of the skull base and higher grade. These results begin to define the spectrum of genetic alterations in meningiomas and identify potential therapeutic targets.

Meningiomas, tumors that arise from the arachnoidal cap cells of the leptomeninges, constitute approximately one-third of primary central nervous system (CNS) tumors1. Most meningiomas (80%) are World Health Organization (WHO) grade I and are treated by surgical resection. However, resection of some meningiomas, particularly at the skull base, is associated with high morbidity. Moreover, 18% of these tumors recur within 5 years, and patients with grade I tumors have significantly reduced long-term survival that is related to both tumor recurrence and stroke risk2. Recurrence rates for grade II and III meningiomas can be as high as 40% and 80%, with 5-year overall survival of approximately 76% and 32%, respectively1,3,4. Although there are recent reports of a stepwise progression of a subset of grade I meningiomas to higher grades5,6, these secondary grade II and III meningiomas may differ fundamentally from spontaneously arising grade II and III tumors. Radiation is frequently used as an adjunct to surgery; however, there are no effective chemotherapeutic options when surgery and radiation fail to offer durable long-term disease control7.

We sequenced DNA from a discovery set of grade I meningiomas using whole-genome (n = 11) and whole-exome (n = 6) techniques to identify somatic copy-number alterations (SCNAs), rearrangements, mutations and insertions and/or deletions (indels) throughout the genome. We then sequenced 645 known cancer-associated genes, including genes altered in the discovery set, in a validation set of 30 additional grade I tumors. We also sequenced these genes in an extrapolation set of 18 grade II or III meningiomas to evaluate whether findings in grade I tumors extended to tumors of higher grade (Online Methods and Supplementary Tables 1 and 2).

Meningiomas in the discovery set had a small number of somatic genetic events compared to other tumor types8–14. We sequenced to high depth (median coverage of 57× for the genome, 181× for the exome and 154× for the validation and extrapolation) and used large-insert libraries in whole-genome samples (500 bp and 800 bp) to optimize our detection of rearrangements and other events. Nevertheless, we found that the median meningioma had SCNAs affecting only 3.3% of the genome, one rearrangement and eight nonsynonymous mutations. Among mutations, cytosine-to-thymidine transitions (deamination (Supplementary Fig. 1a))15. The rates of alteration we observed in meningiomas were significantly lower (often by a factor of ten or more) than previously determined rates for other tumors that have been sequenced to lower depth and with smaller insert sizes (Fig. 1a–c). These findings may reflect differences in mitotic index,

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Nonsynonymous somatic rearrangements disrupt tumor suppressors in several meningiomas. Circos plots show the SCNAs (inner ring heat map) and intra- and interchromosomal rearrangements (green and purple arcs, respectively) in three meningiomas for which the whole genomes were sequenced. Left, chromothripsis of chromosome 1 (enlarged in inset) in an NF2-mutated sample (MEN00017) disrupts the putative tumor suppressor NEGR1. Middle, a second NF2-mutated sample (MEN0018) harbors 29 rearrangements, including disruption of NEGR1. Right, an inversion on chromosome 22 disrupts NF2, and other rearrangements affect NF1 and CDK14 (MEN0009). Data from the remaining tumors are shown in Supplementary Figure 4.
in samples that were not subjected to whole-genome analysis, including a truncating translocation in CHEK2 (Supplementary Fig. 4i) and Supplementary Table 3. The loss of CHEK2 may lead to impaired DNA repair, as has been described in meningioma cell lines\textsuperscript{22}.

The most frequently mutated gene was \(NF2\), which exhibited a total of nine nonsense mutations, nine splice-site mutations and nine frameshift indels in addition to the above-mentioned translocation (Supplementary Fig. 5). The majority of these events overlapped with previously reported events, and our rates of \(NF2\) inactivation were consistent with published rates\textsuperscript{3}. The nonsynonymous mutation rates of tumors with and without focal \(NF2\) alterations did not differ significantly, nor did the spectra of their mutation subtypes (Supplementary Fig. 1b–d).

We also identified nonsynonymous mutations in 190 other genes (Supplementary Tables 4 and 5). We determined the significance of mutation rates in individual genes relative to genome-wide background rates of mutation. Six genes reached statistical significance (Fig. 3, Supplementary Fig. 5 and Supplementary Table 4). Four of these (\(NF2\), \(KDM5C\), \(SMO\) and \(AKT1\)) have previously been implicated in cancer, and the other two (\(RGPD3\) and \(CD300C\)) have not. Isolated or rare mutations that did not reach significance across the entire cohort were observed in 23 genes with known involvement in cancer, including \(TP53\), \(APC\), \(CBL\), \(STK11\) and \(NOTCH2\).

Among genes mutated in our cohort and previously associated with cancer, three (\(KDM5C\), \(KDM6A\) and \(SMARCBI\)) are epigenetic modifiers. Mutations involving these genes affected 8\% of the cohort. Both \(KDM5C\) (also known as \(JARID1C\)) and \(KDM6A\) are histone demethylases\textsuperscript{32}; \(SMARCBI\) (also known as \(SNF5\) and \(INI1\)) is a member of the SWI/SNF chromatin-remodeling complex. \(SMARCBI\) is located 6 Mb away from \(NF2\), and a four-hit model of biallelic inactivation of both genes has been described in familial schwannomas\textsuperscript{33}. The mutation we identified (encoding a p.Arg374Gln alteration) is near a mutational hotspot (p.Arg377His) described in meningiomas\textsuperscript{25}, and germline mutations in \(SMARCBI\), including ones encoding a p.Arg374Gln alteration, have been reported in the congenital disorder Coffin-Siris syndrome\textsuperscript{26}.

We observed mutations of \(SMO\), a member of the Hedgehog signaling pathway, in three tumors (5\%). None of these tumors had focal alteration of \(NF2\). Two samples harbored a mutation in \(SMO\) encoding a p.Trp535Leu alteration, a known oncogenic mutation in basal-cell carcinomas\textsuperscript{27}, and a third harbored a mutation encoding a p.Leu412Phe alteration, previously reported in desmoplastic medulloblastoma\textsuperscript{28}. The allelic fractions of these mutations (45\%, 55\% and 47\%, respectively) were among the highest in these tumors, suggesting clonality. Moreover, dysregulation of the Hedgehog pathway has previously been described in meningiomas\textsuperscript{29,30}. Gorlin syndrome (nevus basal-cell carcinoma syndrome) is caused by a germline \(PTCH1\) mutation, which exhibits a spectrum of somatic mutations in meningiomas. These include frameshift indels in addition to the above-mentioned translocation.

**Figure 3** Significant and selected cancer-related somatic mutations, indels and translocations in meningiomas. Mutation subtypes are denoted by color. If multiple mutations were found in a gene in a single sample, only one is shown. Discovery set tumors are shown in the same order as in Figure 1d. The significance of mutations in each gene is shown to the right by false discovery rate (FDR) \(q\) value. The full list of mutated genes is given in Supplementary Table 4.

**Figure 4** Associations between mutations in Hedgehog and AKT-mTOR pathways and histological findings. (a) Samples with mutations in \(SMO\) and \(AKT1\) are predominantly of the meningothelial subtype \((P = 0.005\) and 0.009, respectively). \(NF2\)-mutated samples are predominantly fibroblastic and transitional \((P = 0.013\). Samples underwent hematoxylin and eosin staining (left). The distribution of samples within different histological subtypes is shown by pie chart (right). (b) Immunohistochemistry indicates activation of the Hedgehog (\(GAB1\)) and AKT-mTOR (\(STMN1\)) pathways in tumors harboring \(SMO\) and \(AKT1\) mutations, respectively \((P = 0.0008\) and 3 \(\times\) 10\(^{-6}\)). Scale bars, 50 \(\mu\)m. WT, wild type.
mutation (upstream of SMO) and is characterized by predisposition to multiple cancers, including desmoplastic medulloblastomas and meningiomas\(^1,2\). Although we did not observe somatic mutations in *PTCH1*, two samples harbored germline alterations that were also present in the tumors (encoding p.Asp346Asn and p.Glu44Gly+ [Pro1282Leu] alterations). However, these individuals did not exhibit manifestations of Gorlin syndrome, such that the functional relevance of these specific events is unclear.

Six samples had mutations of the phosphatidylinositol 3-kinase (PI3K)–AKT-mTOR pathway. None of these had mutations of NF2 or SMO (*P* = 0.03). Five samples harbored identical AKTI mutations (p.Glu17Lys) known to be oncogenic in breast, colorectal and lung cancers\(^33\). The glutamic acid–to-lysine substitution results in constitutive AKT1 activation, which stimulates downstream mTOR signaling. The sixth sample had a new *MTOR* mutation (encoding a p.Asp1279Val alteration).

The mutations in the Hedgehog and PI3K pathways were also associated with particular histopathological subtypes (Fig. 4). Meningiomas with mutated SMO and AKTI –mTOR were predominantly of the meningothelial subtype (*P* = 0.009 and 0.005, respectively, Fisher's exact test). This is in contrast to the grade I NF2-altered tumors, which were predominantly fibroblastic and/or transitional (*P* = 0.013), consistent with previous reports\(^34\). We did not find statistically significant correlations with other clinically relevant variables, including age, tumor location or previous resection. However, one AKTI- and two SMO-mutated tumors were resected from the skull base, a region that offers particular challenges to resection and higher rates of recurrence\(^35\) (Supplementary Table 5).

To validate these findings, we also genotyped an additional 46 grade I and 49 grade II or III meningiomas for AKTI (p.Glu17Lys) and SMO (p.Trp535Leu and p.Leu412Phe) mutations using a mass spectrometry–based method (Sequenom hME). We found one *SMO* mutation (p.Leu412Phe) and three additional AKTI mutations (p.Glu17Lys) (Supplementary Table 6). One of the latter was in a grade III tumor, and the remaining three were in skull base tumors, of which two were of meningothelial histology.

Mutations in SMO, AKTI and *MTOR* also correlated with the presence of markers of pathway activation. GAB1 immunoreactivity is used in medulloblastomas to characterize tumors with Hedgehog pathway activation\(^36\). Of the 65 meningiomas, 7 exhibited strong immunoreactivity for GAB1, including the 3 tumors harboring SMO mutations (*P* = 0.0008). The other four GAB1-positive tumors were grade II meningiomas. Likewise, STMN1 expression is a marker of PI3K-AKT pathway activation\(^37\). Ten meningiomas exhibited strong STMN1 expression, including all six AKTI- and *MTOR*-mutated meningiomas (*P* = 3 \(\times\) 10\(^{-6}\)). Notably, all 3 SMO-mutated meningiomas also exhibited strong (*n* = 2) or moderate (*n* = 1) STMN1 staining. This is consistent with studies that have suggested that the Hedgehog and PI3K-AKT-mTOR pathways may interact\(^38\).

We observed recurrent mutations in signaling pathways and epigenetic modifiers, but our data also highlight the heterogeneity of mutations in these tumors. Among the 17 tumors we comprehensively characterized, 3 (18%) did not have mutations in any of the significantly mutated genes. Genomic characterization of additional tumors, particularly those of higher grade, is likely to reveal additional oncogenic mechanisms.

These observations also have the potential to guide new therapeutic strategies. We observed SMO and AKTI mutations in tumors that pose special therapeutic challenges, including one high-grade and six skull-base tumors. Inhibitors of SMO have generated high response rates in patients with basal-cell carcinoma, many of which are driven by Hedgehog pathway mutations\(^39\). Likewise, inhibitors of the PI3K-AKT-mTOR pathway have shown promise in preclinical and clinical trials in multiple cancer types\(^40\). The paucity of additional genetic events in meningiomas harboring mutations of the PI3K and Hedgehog signaling pathways suggests that patients with these meningiomas may benefit from such targeted therapies already in development or use.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Accession codes.** Data, including sequence data and analyses, are available for download from the database of Genotypes and Phenotypes (dbGaP) under accession phs000552.v1.p1.

**Note:** Supplementary information is available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

P.K.B., P.M.H., I.F.D., R.B. and W.C.H. conceived the study, designed the experiments, analyzed the data and wrote the manuscript. P.M.H. performed the bioinformatics analyses, and A.M., G.G., R.B. and M.D.D. provided analytical advice. S.S., O.A.S.-R. and D.N.L. provided technical support and performed sequencing. W.C.H., I.F.D. and R.B. supervised the study. All authors discussed the results and implications and commented on the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare competing financial interests: details are available in the online version of the paper.


Recurrent somatic alterations of FGFR1 and NTRK2 in pilocytic astrocytoma

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Pilocytic astrocytoma, the most common childhood brain tumor1, is typically associated with mitogen-activated protein kinase (MAPK) pathway alterations2. Surgically inaccessible midline tumors are therapeutically challenging, showing sustained tendency for progression3 and often becoming a chronic disease with substantial morbidities4. Here we describe whole-genome sequencing of 96 pilocytic astrocytomas, with matched RNA sequencing (n = 73), conducted by the International Cancer Genome Consortium (ICGC) PedBrain Tumor Project. We identified recurrent activating mutations in FGFR1 and PTPN11 and new NTRK2 fusion genes in non-cerebellar tumors. New BRAF-activating changes were also observed. MAPK pathway alterations affected all tumors analyzed, with no other significant mutations identified, indicating that pilocytic astrocytoma is predominantly a single-pathway disease. Notably, we identified the same FGFR1 mutations in a subset of H3F3A-mutated pediatric glioblastoma with additional alterations in the NF1 gene5. Our findings thus identify new potential therapeutic targets in distinct subsets of pilocytic astrocytoma and childhood glioblastoma.

Pilocytic astrocytoma is the most common central nervous system (CNS) neoplasm in childhood, accounting for ~20% of all pediatric brain tumors. Tumor locations in our cohort reflect the fact that pilocytic astrocytomas occur throughout the CNS, with about half arising outside the cerebellum (Supplementary Fig. 1). Extracerebellar tumors are often surgically inaccessible, leading to chronic disease with multiple recurrences, visual and neurological impairment and/or side-effects of therapy1,4. Genetic alterations within the MAPK signaling pathway are a hallmark of this tumor, with KIAA1549-BRAF fusion being the most frequent event6–8. A smaller number of tumors harbor BRAF or KRAS point mutations, alternative BRAF-RAF1 fusions or germline NF1 mutations2. Pilocytic astrocytoma has therefore been hypothesized to represent a single-pathway disease2. Previously, however, no MAPK pathway changes were identifiable in 15–20% of tumors (mostly non-cerebellar)2. To investigate the full range of genetic alterations in pilocytic astrocytoma, we performed whole-genome sequencing of tumor and blood DNA from 96 affected individuals (Supplementary Table 1). Corresponding RNA sequencing data and data from mate-pair sequencing with larger inserts (for enhanced detection of structural rearrangements) were generated for 73 and 68 samples, respectively. The average somatic mutation rate was extremely low (<0.1 mutation per megabase), with a mean of 1.6 nonsynonymous single-nucleotide variants (SNVs) per tumor (range of 0–9; Supplementary Table 1), similar to the rate described in NF1-associated pilocytic astrocytomas9.

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medulloblastoma\textsuperscript{10-12} and for several other pediatric solid tumors\textsuperscript{13}. The average number of small insertion-deletion alterations (indels) affecting coding sequences was \(<1\) per case. All coding somatic SNVs and indels are listed in Supplementary Table 2.

In line with other tumor types\textsuperscript{10,14,15}, pilocytic astrocytomas had genome-wide mutation rates that positively correlated with the age of the affected individual (\(r = 0.42; P = 2.3 \times 10^{-5}\)), Pearson's product-moment correlation; Supplementary Fig. 2a). The observed mutations were predominantly cytosine-to-thymine transitions at CpG islands (likely arising from deamination of methylated cytosines), suggesting that the age-dependent increase in mutation frequency may largely be due to background processes occurring in progenitor cells before tumorigenesis, as recently reported in leukemia\textsuperscript{15} (Supplementary Fig. 2b).

Most of the known events activating the MAPK pathway were also found in our series, including KIAA1549-BRAF fusion variants (70 cases), a FAM131B-BRAF fusion\textsuperscript{3,16}, 4 BRAF\textsuperscript{V600E} mutations and 1 BRAF\textsuperscript{P597S} alteration (Supplementary Table 1). Three tumors were associated with neurofibromatosis type 1. This prevalence is lower than would be expected for prospective cohorts (5–10%), as material for biological studies from these typically optically-activated tumors is limited. NF1 has been reported to follow a classical tumor suppressor model in pilocytic astrocytoma, with a somatic second hit in addition to a germline alteration\textsuperscript{3}. This model also held true in our series (Supplementary Table 1).

Analysis of copy number and structural alterations using DNA and RNA sequencing identified four new BRAF fusions (Fig. 1 and Supplementary Fig. 3). As expected, all variants resulted in loss of the N-terminal regulatory region of BRAF. An RNF130-BRAF fusion derived from a reciprocal t(5;7)(q35;q34) translocation was seen in two cases (Fig. 1a), with single examples identified of CLCN6-BRAF, MKRN1-BRAF and GNA11-BRAF fusions (Supplementary Fig. 3a-c). Thus, non-KIAA1549-BRAF fusions comprise a notable minority of activating events, with BRAF seeming to be a promiscuous fusion partner.

Another new BRAF alteration was identified in ICGC_PA65, resulting in a three-amino-acid insertion (p.Arg506_insValLeuArg, insVLR) in the interdomain cleft of BRAF—a structural region linked to BRAF activity\textsuperscript{17} and homodimerization\textsuperscript{18}. Protein modeling predicted that the insertion of these residues stabilizes a dimeric form of BRAF (known to be active independent of RAS stimulation\textsuperscript{19}) (Fig. 1b). Homodimerization was confirmed by immunoprecipitation, and the BRAF\textsuperscript{insVLR} mutant increased extracellular signal-regulated kinase (ERK) phosphorylation as effectively as the BRAF\textsuperscript{V600E} mutant (Fig. 1c,d).

New alterations in KRAS were also observed. ICGC_PA117 and ICGC_PA142 both showed two distinct mutations (encoding p.Glu63Lys\textsuperscript{+}Arg73Met and p.Leu19Pro\textsuperscript{+}Gln22Lys, respectively). DNA and RNA sequencing data confirmed that both alterations affected the same allele (Supplementary Fig. 4). Although there are reports of double KRAS mutations in entities such as colon cancer\textsuperscript{20}, these typically involve at least one hotspot residue (codon 12, 13 or 61) and often represent heterogeneous tumor subclones rather than two hits in one allele (although this has also been described; for example, see ref. 21). The alterations identified in our tumors did not encompass classical mutational hotspots, suggesting that further characterization of downstream effects is warranted.

All but one of the cerebellar tumors in our series harbored a BRAF fusion, with this one exception having a KRAS alteration. Nine of 48 (19\%) of the non-cerebellar tumors, however, lacked the above alterations. Further assessment of structural rearrangements identified two new gene fusions in a total of three samples, involving the region encoding the kinase domain of NTRK2 (also known as TrkB)—an oncogene implicated in the tumorigenesis of neuroblastoma, among other cancers\textsuperscript{22,23}. The related NTRK1 and NTRK3 genes have previously been shown to be activated by fusion events (for example, TPM3-NTRK1 in papillary thyroid cancer\textsuperscript{24} and ETV6-NTRK3 in multiple tumors\textsuperscript{25}). The QKI-NTRK2 and NACC2-NTRK2 fusions identified here were verified by PCR (Fig. 2 and Supplementary Fig. 3d). Both S’ partners contained regions encoding dimerization domains and are therefore predicted to induce ligand-independent dimerization. Notably, N-terminal TrkB truncation has recently been shown to induce transformation of neural crest cells\textsuperscript{26}.

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*Figure 1* New BRAF alterations in pilocytic astrocytoma. (a) Schematic of the RNF130-BRAF fusion gene in ICGC_PA112 resulting from a translocation between chromosomes 5 and 7. A similar fusion was observed in ICGC_PA96. The cDNA sequence at the fusion breakpoint (dashed line) and resulting exon and protein structures are shown. A reciprocal fusion between RUFY1 (encoding RUN and FYVE domain–containing 1) and TMEM178B (encoding transmembrane protein 178B) on the derivative chromosome 5 in ICGC_PA112 was also found to be expressed in RNA sequencing analysis (data not shown). RPM, reads per million; KD, kinase domain. (b) Computational modeling of two BRAF monomers (light and dark gray) with a ValLeuArg insertion (blue and magenta) between Arg506 and Lys507 (green), as identified in ICGC_PA65 (p.Arg506_insValLeuArg, insVLR). Protein Data Bank (PDB) structure 4E26 was used as a template. Val600, a mutational hotspot, is shown in yellow. A new dimer interface is formed between the protomers, with hydrogen bonds formed between the new arginine side chains (dashed lines) and a hydrophobic interaction between the leucine side chains (magenta). (c) Protein blot analysis of NIH3T3 cells transfected with empty vector (EV) or with vector expressing wild-type (WT) BRAF, BRAF\textsuperscript{V600E} or BRAF\textsuperscript{insVLR}. The newly identified BRAF\textsuperscript{insVLR} mutant results in greater phosphorylation of ERK1 and ERK2 (pERK1/2), with phosphorylation at a similar level to that seen with the known oncogenic BRAF\textsuperscript{V600E} form. (d) Pulldown assay with immunoprecipitation (IP) of HA-tagged BRAF\textsuperscript{insVLR}, showing that this new mutant forms homodimers with coexpressed AU1-tagged BRAF\textsuperscript{insVLR} mutant but does not seem to form a strong heterodimer with wild-type BRAF.
The downstream effects of TrkB activation are mediated, at least in part, via MAPK pathway activation.27

A second new recurrent alteration, namely, mutation of two hotspots (codons for Asn546 and Lys656) within the kinase domain of FGFR1, was seen in five tumors (Fig. 3a and Supplementary Table 3). FGFR1 is more commonly activated through amplification in tumors such as breast28 and lung29,30 cancer. Occasional FGFR1 mutations have been observed in adult glioblastoma (GBM)31,32, a highly malignant astrocytoma, as have FGFR1-TACC1 or FGFR3-TACC3 fusion genes.33 Mutations in homologous codons in FGFR2 and FGFR3 are commonly found in other tumor types, particularly bladder, skin and endometrial cancers (see the Catalogue of Somatic Mutations in Cancer (COSMIC) database41). Both mutations result in midbrain hyperproliferation in developing chick embryos.35 The p.Asn546Lys variant alters FGFR1 autophosphorylation, resulting in higher kinase activity and transforming potential16, whereas the p.Lys656Glu variant is also transforming in vitro37. Notably, the latter study suggested that fibroblast growth factor 2 (FGF2, also known as bFGF) ligand was necessary in addition to FGFR1 mutation to maintain neurosphere formation in vitro. Gene expression array data of 118 pilocytic astrocytomas, including 66 from the present series, showed significantly increased FGF2 expression in pilocytic astrocytomas compared with 158 other astrocytic tumors38,39 or normal tissues40. This increase was not restricted to only FGFR1-mutant or wild-type tumors, suggesting that ligand-mediated pathway activation may have a general role in tumorigenesis (Fig. 3b). Immunohistochemical detection of phosphorylated FGFR1 showed strong, diffuse positivity in all seven pilocytic astrocytomas harboring an FGFR1 mutation for which material was available. No positivity was observed in 11 tumors with wild-type FGFR1. All samples showed strong staining for phosphorylated ERK (Supplementary Fig. 5). Notably, ICGC_PA89 harbored an alternative alteration in FGFR1 consisting of a 4.5-kb internal tandem duplication (ITD) of the portion of the gene encoding the kinase domain, reminiscent of the activating internal tandem duplications of the FLT3 kinase observed in acute myeloid leukemia.41 (Fig. 3c).

Further recurrent mutations were found in the phosphatase gene PTPN11 (also called SHP-2) encoding a RAS-MAPK–related adaptor protein (Fig. 3d). Both encoded alterations (p.Glu69Lys and p.Glu76Ala) were previously reported in juvenile myelomonocytic leukemia, which is frequently associated with SHP-2 activation42,43. Notably, both alterations were found in FGFR1-mutant tumors (ICGC_PA84 and ICGC_PA166), suggesting a cooperative role of these factors in tumorigenesis (Supplementary Table 3). Overexpression of mutant SHP-2 alone did not elevate the levels of phosphorylated ERK in vitro, whereas the two FGFR1 mutants, either alone or in combination with mutant SHP-2, upregulated the levels of phosphorylated ERK (Supplementary Fig. 6). This finding supports the hypothesis that PTPN11 mutation alone is insufficient for pilocytic astrocytoma development but may have a modifying role in FGFR1-mutant tumors. Of note, PTPN11 expression was higher in pilocytic astrocytomas compared with other astrocytomas or normal tissues (Fig. 3e), suggesting that this phosphatase has a broader role in the biology of this entity. An additional cohort of 45 non–cerebellar pilocytic astrocytomas, negative for KIAA1549-BRAF fusion, was screened for FGFR1 (exons 12 and 14) and PTPN11 (exon 3) mutations. Nine cases harbored FGFR1 mutations encoding a p.Asn546 or p.Lys656 alteration, and one additionally carried a PTPN11 mutation encoding a p.Glu69Lys change (Supplementary Table 3), confirming our whole-genome sequencing findings. Germline PTPN11 mutations are one of the causes of the hereditary developmental disorders Noonan syndrome44 and multiple lentigines syndrome (also known as LEOPARD syndrome)45. A few case reports have described pilocytic astrocytomas occurring in individuals with these syndromes46–49. Thus, together with NF1, there are three known ‘RASopathies’, characterized by germline MAPK pathway mutations50, linked with pilocytic astrocytoma tumorigenesis. In our germline sequencing data, however, NF1 was the only RASopathy-related gene disrupted at a higher frequency than in the 1000 Genomes Project (see URLs).

Notably, all of the pilocytic astrocytomas in our cohort harbored a MAPK pathway alteration. BRAF, FGFR1, KRAS and NF1 were the only genes found to be significantly mutated using the Genome MuSiC algorithm (see URLs; Supplementary Table 4). With the exception of FGFR1 and PTPN11, each case typically harbored only one pathway alteration (p < 0.0001, permutation test; Fig. 4). Together with the finding that BRAF kinase activation alone is sufficient to induce pilocytic astrocytomas in mice51,52, these data strongly support the concept of pilocytic astrocytoma as a prototypic single-pathway disease driven by a limited number of oncogenic hits (possibly only one in some cases; Supplementary Fig. 7).

One of the FGFR1-mutant tumors (ICGC_PA69) also had an H3F3A mutation encoding a p.Lys27Met alteration and somatic mutations of NF1—both of which are more commonly encountered in pediatric GBM.5 Three experienced neuropathologists agreed on pilocytic astrocytoma histology for this case, although a diagnosis of GBM cannot be conclusively excluded, owing to limited material. By examining previous exome sequencing data for pediatric GBM, we identified 3 of 48 samples (6%) with an FGFR1 mutation. Notably, all three harbored the same constellation of an H3F3A p.Lys27Met alteration, a somatic NF1 alteration and FGFR1 activation (Supplementary Table 3). They were also wild type for TP53, which is mutated in most GBMs or diffuse intrinsic pontine gliomas53 with the H3F3A p.Lys27Met alteration. One tumor reported in a targeted sequencing cohort of medulloblastoma10 had a similar triple alteration,
with an H3F3A p.Lys27Met alteration, an NF1 alteration and an FGFR2 p.Lys659Glu alteration (homologous to FGFR1 p.Lys656Glu), making a total of five cases with this combination. Gene expression analysis indicated that this tumor was likely a GBM previously misclassified as medulloblastoma. It is not currently clear why these alterations occur in concert, and additional work will be required to assess their roles. One possibility is that NF1 mutation may mimic elevated PTPN11 expression, as activation of SHP-2 inhibits the recruitment of Ras GTPase–activating proteins (RasGAPs, including NF1) to the plasma membrane.

All FGFR1-mutant tumors were extracerebellar, mostly in midline locations (Supplementary Table 3), suggesting a link between cell of origin and/or microenvironment with FGFR1-driven tumorigenesis. The H3F3A p.Lys27Met alteration is also associated with midline GBM.\(^{59}\) Notably, FGFR1 has a role in neural stem cell self-renewal\(^{55}\) and is essential for midline glial cell development.\(^{56}\) This spatial clustering may also reflect differential sensitivity of distinct neural precursors to activating stimuli, particularly NF1 loss.\(^{57,58}\) The type and timing of second hits (H3F3A or NF1 mutation) and/or the differentiation stage of the cell of origin may contribute to determining a fate of oncogene-induced senescence and slow growth (pilocytic astrocytoma)\(^{59,60}\) versus aggressive malignancy with poor outcome (GBM).

In summary, this study has provided new insights into the tumorigenesis of pilocytic astrocytoma. Each tumor harbored very few mutations, in keeping with generally benign behavior. Our findings confirm the concept that pilocytic astrocytomas are predominantly

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**Figure 3** FGF pathway signaling molecules are recurrently altered in pilocytic astrocytoma. (a) Schematic of the domain structure of FGFR1, indicating the position and frequency of the hotspot alterations in pilocytic astrocytomas sequenced in the present study (including replication cases). Ig, immunoglobulin-like domain; TM, transmembrane domain; TK, tyrosine kinase domain. (b) Gene expression data for FGFR1 indicating significantly elevated expression in pilocytic astrocytomas (red) compared with other astrocytic tumors (blue), normal cerebellum (black) and other normal tissues (green); \(P < 0.001\), two-sided t test. The pilocytic astrocytomas with expression data that harbor FGFR1 alterations (four mutants plus FGFR1-ITD) are circled. Horizontal gray bars indicate mean expression values per group. PA, pilocytic astrocytoma; DA, World Health Organization (WHO) grade 2 diffuse astrocytoma; AA, anaplastic astrocytoma; K27, G34 and PTP, protein tyrosine phosphatase domain. (c) Schematic of an additional alteration in FGFR1 identified in ICGC_PA89 comprising an internal tandem duplication of part of intron 10, exons 11–17 and part of exon 18 (boundaries highlighted by dashed lines). The duplicated amino acids are residues 478–820 (numbered according to the αA1 isoform), with an additional 40-residue linker sequence encoded by part of intron 10. The whole kinase domain is therefore duplicated in the resulting predicted protein (TK1’ and TK2’). (d) Schematic of the structure of SHP-2 (PTPN11), indicating the position and frequency of alterations in pilocytic astrocytomas sequenced in the present study, SH2, src homology 2 domain; PTP, protein tyrosine phosphatase domain. (e) Gene expression data for PTPN11 indicating significantly elevated expression in pilocytic astrocytomas compared with other groups as defined in b; \(P < 0.001\), two-sided t test. The pilocytic astrocytomas with expression data that harbor FGFR1 alterations (four mutants plus FGFR1-ITD) are circled.
driven by aberrant activation of the MAPK pathway. Most notably, however, we report new recurrent mutations in NTRK2, FGFR1 and PTPN11, which were mutually exclusive with other RAF and RAS changes. Combined with the observation of FGFR2 and PTPN11 over-expression, these results indicate upstream contributors to MAPK pathway activation in this entity. Emerging preclinical data suggest that BRAF inhibitors may trigger paradoxical activation in tumors harboring KIAA1549-BRAF fusions, that is, the majority of pilocytic astrocytomas61. Single-drug or combination therapy with FGFR, NTRK2 and/or MAPK/ERK kinase (MEK) inhibitors, several of which are currently in preclinical and clinical trials62–64, may therefore represent rational treatment options. BRAFV600E-specific agents may also be a logical choice for ~5% of patients. Finally, the identification of recurrent FGFR1 mutations in a subset of pediatric GBMs provides an opportunity for the therapeutic targeting of FGFR signaling in these clinically challenging brain tumors.


METHODS

Methods and any associated references are available in the online version of the paper.

Accession code. Sequencing data have been deposited at the European Genome-phenome Archive, which is hosted by the European Bioinformatics Institute (EBI), under accession EGAS00001000381.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS


COMPETING FINANCIAL INTERESTS

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35. Liu, A. et al. FGFR1b and FGFR1b have different midbrain regulatory properties from FGFR1b, activated FG receptors. Development 130, 6175–6185 (2003).
Advances in understanding pediatric high-grade glioma (pHGG) genetics have revealed key differences between pHGG and adult HGG and have uncovered unique molecular drivers among subgroups within pHGG. The 3 core adult HGG pathways, the receptor tyrosine kinase-Ras-phosphatidylinositol 3-kinase, p53, and retinoblastoma networks, are also disrupted in pHGG, but they exhibit a different spectrum of effectors targeted by mutation. There are also similarities and differences in the genomic landscape of diffuse intrinsic pontine glioma (DIPG) and pediatric nonbrainstem (pNBS)-HGG. In 2012, histone H3 mutations were identified in nearly 80% of DIPGs and ~35% of pNBS-HGG. These were the first reports of histone mutations in human cancer, implicating novel biology in pediatric gliomagenesis. Additionally, DIPG and midline pNBS-HGG vary in the frequency and specific histone H3 amino acid substitution compared with pNBS-HGGs arising in the cerebral hemispheres, demonstrating a molecular difference among pHGG subgroups. The gene expression signatures as well as DNA methylation signatures of these tumors are also distinctive, reflecting a combination of the driving mutations and the developmental context from which they arise. These data collectively highlight unique selective pressures within the developing brainstem and solidify DIPG as a specific molecular and biological entity among pHGGs. Emerging studies continue to identify novel mutations that distinguish subgroups of pHGG. The molecular heterogeneity among pHGGs will undoubtedly have clinical implications moving forward. The discovery of unique oncogenic drivers is a critical first step in providing patients with appropriate, targeted therapies. Despite these insights, our vantage point has been largely limited to an in-depth analysis of protein coding sequences. Given the clear importance of histone mutations in pHGG, it will be interesting to see how aberrant epigenetic regulation contributes to tumorigenesis in the pediatric context. New mechanistic insights may allow for the identification of distinct vulnerabilities in this devastating spectrum of childhood tumors.

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malignant transformation, the process whereby a low-grade lesion progresses to a high-grade tumor, is a common event in adults but infrequent in children. Genetic analyses have illuminated molecular differences driving pediatric and adult high-grade gliogenesis.

**The 3 Core aHGG Pathways Show a Different Spectrum of Alteration in pHGG**

As the genomic landscape of aHGG came into view, it shaped initial work into the pediatric disease. The first pHGG studies focused primarily on investigating the involvement of high-frequency recurrent events found in adult tumors. For example, epidermal growth factor receptor (EGFR) is the most commonly altered receptor tyrosine kinase (RTK) in aHGG; with the corresponding gene locus undergoing amplification or intragenic deletion or both in ~50% of cases. First identified in adult glioblastoma, EGFRvIII is the most common EGFR variant in aHGG and is formed by deletion of exons 2-7 resulting in a constitutively active kinase. Accordingly, EGFR variant in aHGG and is formed by deletion of exons 2-7 resulting in a constitutively active kinase. 

A number of studies found that EGFR alteration was less frequent in pHGG, although gene amplification and EGFRvIII expression were detected in some pHGGs. Through genome-wide studies, PDGFRα, which encodes platelet-derived growth factor receptor alpha (PDGFRα), was identified as the most commonly targeted RTK in both DIPG and pediatric nonbrainstem (pNBS)-HGG. Alterations in the gene itself include amplification, mutation, or both. Experimentally, overexpression of wild-type (WT) or mutant PDGFRα confer a growth advantage to astrocytes, an effect that is diminished by the introduction of the adenosine triphosphate–competitive inhibitors crenolanib or dasatinib. PDGFRα mutants drive glioma formation in vivo, with murine-derived HGGs recapitulating critical features of the human disease such as histopathologic characteristics and expression profiles. In an effort to target PDGFR therapeutically, pediatric trials using dasatinib, crenolanib, or imatinib have been launched. Unfortunately, the benefit derived from selective RTK inhibitors may be marginal at best. pHGGs show evidence of intratumoral heterogeneity, with some cells coamplifying multiple RTK genes or discrete cell populations within the same tumor amplifying different genes, suggesting that resistant populations are likely to be present even before treatment with targeted agents.

Both PDGFRα and EGFR are part of the RTK-Ras-phosphatidylinositol 3-kinase (PI3K) signaling cascade, which is altered in nearly 90% of aHGGs. Additionally, ~80%-90% of adult tumors show evidence of retinoblastoma (RB) and p53 pathway dysregulation. For this reason, many of the first genetic pHGG studies focused on these same networks (Fig. 1).

In adults, the most commonly targeted components of the RTK-Ras-PI3K axis downstream of RTKs include activation of PI3K itself, or loss of function of phosphatase and tensin homolog (PTEN), the main negative regulator of PI3K signaling, or NF1, a negative regulator of Ras-mediated signaling. Activation of PI3K signaling caused by mutations of PIK3CA, encoding the catalytic p110α subunit of PI3K, or PIK3R1, encoding the regulatory subunit of PI3K, is usually present in mutually exclusive patterns, occurring in approximately 20% of aHGGs and in a similar frequency of pHGG, including DIPG. The PTEN tumor suppressor is located on chromosome 10q. It remains unclear whether all tumors with loss of chromosome 10q are targeting PTEN loss of function when a WT PTEN allele is still retained. However, there are examples in experimental systems where PTEN haploinsufficiency contributes to tumorigenesis. Loss of heterozygosity (LOH) of chromosome 10q, with or without concurrent PTEN mutation, is very frequent in adult glioblastoma, with 10q LOH in approximately 80% and PTEN mutation in 25%-40%, whereas the frequency is significantly lower in pHGGs, with 10q LOH in approximately 30% and PTEN mutation in less than 5%-15%.

RB pathway dysregulation is common in both pNBS-HGGs and DIPG. The CDKN2A locus codes for 2 tumor suppressors, p16INK4a and ARF. Notably, homozygous deletion of CDKN2A/B appears to be almost exclusive to pNBS tumors and largely absent in DIPGs. In contrast, amplification of CDK4/6 or CCND1/2/3 is found in approximately 30% of DIPG. CDK4/6 codes for cyclin D–dependent kinases that phosphorylate the RB protein, facilitating G1-S cell cycle progression. To become active, these kinases must bind to cyclin D family members (encoded by CCND1/2/3), which themselves confer substrate specificity. Therapeutic inhibition of this cyclin-CDK complex, using PD-0332991, a highly-selective non–adenosine triphosphate–competitive CDK4/6 inhibitor, significantly increased survival in a murine model of DIPG, both as a single agent or following irradiation.

TP53 mutations occur in up to 35% of pNBS-HGGs (range: 18%-35%) and appear to be more common in DIPGs (40%-50% of cases).

From the aforementioned data, we can conclude that although the 3 main signaling pathways affected in aHGG are also affected in pHGG, pediatric and adult tumors differ regarding the most frequently mutated effectors.

**Copy Number Imbalances and Gene Expression Profiling**

Despite some common copy number imbalances such as 13q and 14q loss in approximately one-third of patients with HGG regardless of age or location, aHGG and pHGG also exhibit a unique constellation of gains and losses that distinguish one from the other, and the same can be said for DIPGs and pNBS-HGGs. This suggests that unique combinations of genetic drivers underlie adult and pediatric...
tumorigenesis, and among childhood HGG, DIPG and pNBS-HGG tumorigenesis.

Transcriptional analysis of tumors supports similar conclusions. Clustering of gene expression signatures from aHGGs identifies 3-4 major gene expression subgroups, with the most robust distinction between proneural and mesenchymal subgroups. Unlike the WNT and sonic hedgehog subgroups of medulloblastoma, the mutations associated with particular HGG subgroups are much less consistent. These same subgroups were identified in pHGGs by unsupervised comparisons, showing a clear relationship in the gene expression signatures of gliomas among different age groups and locations. However, supervised comparisons revealed expression signatures that distinguished adult from pediatric tumors, and within childhood glioma, DIPGs from pNBS-HGGs.

Because biopsy on patients with DIPG is not routinely performed in the United States, most research material is acquired at autopsy from irradiated patients. The vast majority of these samples are designated World Health Organization grade IV. In contrast, clinicians in France regularly perform pretreatment biopsies, and although most samples were HGGs, some were classified as low grade, raising the possibility that there may be a low-grade to high-grade malignant transformation in the genesis of DIPG. Importantly, there is a high degree of similarity in the copy number imbalances and expression signatures from DIPGs collected as biopsy samples before treatment and those collected at

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**Figure 1** The p53, RB, and RTK-Ras-Pi3K pathways are dysregulated in pHGG. (A) The p53 and RB pathways regulate G1 cell cycle checkpoints. Mitogenic signaling activates the cyclin D–dependent kinases CDK4 or CDK6, coupled with cyclin D family members (CCND1/2/3). This complex phosphorylates pRB, releasing E2F and promoting transcription of genes responsible for G1-S cell cycle progression. Gene amplifications of CDK4, CDK6, or any of the 3 Cyclin D family members are found in pHGG, with greater frequency in DIPG. The tumor suppressor locus CDKN2A encodes 2 different proteins through translation of 2 different reading frames, p16INK4A and p19ARF. p16INK4A inhibits the activity of the cyclin D–dependent kinases CDK4 and CDK6. Oncogenic signals, DNA damage, or induction of p19ARF induces p33, leading to cell cycle arrest, apoptosis, or senescence. Homozygous deletions of CDKN2A occur almost exclusively in NBS-HGGs, whereas TP53 mutations are common in both pHGGs and DIPG. (B) Mutations in the RTK-RAS-Pi3K pathway transduce unregulated signals for cell proliferation, growth, and survival. RTK signaling begins when growth factor ligand binding leads to receptor dimerization. In pHGG, PDGFRα is the RTK most frequently targeted by amplification or mutation or both. Upon dimerization, RTKs transphosphorylate one another at tyrosine residues in their cytosolic tails. p85, the regulatory subunit of Pi3K, can then either directly bind to these phosphorylated tyrosine residues or connect to RTKs through adaptor molecules and Ras. Pi3K comprises catalytic (p110) and regulatory (p85) subunits, both of which are targeted by mutation, usually in a mutually exclusive pattern, in pHGG. pRB, RB protein. (Color version of figure is available online.)
autopsy, which were treated by radiation in most cases, with or without chemotherapy.16,24 Furthermore, expression signatures of pediatric brainstem LGGs are much more closely related to nonbrainstem LGG and not to DIPGs, emphasizing a different etiology underlying genesis of LGG and HGG arising in the brainstem.

Histones Make Their Mark

The aforementioned work established the concept that oncogenic events driving pediatric HGG were different from those arising in adults. This appreciation, however, was not fully cemented until early 2012, with the discovery of recurrent histone mutations in pHGG (Fig. 2). As the first reports of histone mutations in human cancer, these mutations implicated novel mechanisms in pHGG tumor biology that are not found to play a significant role in the adult disease.

Whole-genome sequencing of 7 DIPGs and matched germ-line DNA, as part of the St. Jude Children’s Research Hospital-Washington University Pediatric Cancer Genome Project, identified somatic mutations in H3F3A leading to a p.K27M substitution in histone H3.3 in 4 of 7 cases. A fifth case contained an analogous mutation in HIST1H3B.59 Similar frequency of histone H3 mutation was found in an independent cohort; however, the proportion of H3F3A and HIST1H3B mutations varied between the groups, likely owing to patient age, with HIST1H3B mutations arising in younger children.59,60 In pNBS-HGG, H3F3A and HIST1H3B p.K27M substitutions were found in 19% and 3% of cases, respectively. Additionally, 14% of pNBS-HGGs harbored somatic mutations in H3F3A leading to p.G34R
substitution, whereas no such alteration was identified in any DIPG. Most of the DIPG samples evaluated were collected at autopsy. However, of 8 DIPG samples collected from patients who had not received adjuvant therapy, 7 contained p.K27M substitutions. Hence, histone H3 alterations were not necessarily secondary to therapy.59

Collaborating groups in Canada and Germany performed whole-exome sequencing of 48 pNBS-HGGs and identified p.K27M, p.G34R, and p.G34V alterations in 19%, 10%, and 2% of cases, respectively, with all changes affecting H3F3A encoding the histone H3.3 variant. Additional targeted sequencing of the H3F3A locus in more than 700 samples of gliomas of various grades from patients of different ages revealed these mutations to be exclusive to high-grade tumors and significantly enriched in pediatric cases. Of the 11 adult cases harboring H3F3A mutation, all were missense substitutions of G34, and the majority were identified in young adults aged 20-30 years. The specific histone mutation was associated with anatomical location. p.K27M mutations occurred in tumors involving midline structures (such as the brainstem, cerebellum, and thalamus), whereas p.G34R/V mutations occurred in nonmidline supratentorial lesions34,61 (Fig. 3).

Nucleosomes are the basic unit of chromatin, in which DNA is wrapped around a nucleosome core composed of a histone octamer with 2 copies each of histones H2A, H2B, H3, and H4 (Fig. 2). H3F3A and HIST1H3B code for the 2 histone H3 variant isoforms H3.3 and H3.1, respectively. There are 3 isoforms of histone H3. Histone H3.1 and H3.2 are encoded by 10 and 3 separate genes, respectively, and are synthesized during S phase of the cell cycle to package newly replicated DNA. Histone H3.3 is synthesized throughout the cell cycle and selectively incorporated into promoter regions of active genes, and through interactions with ATRX and DAXX, into pericentromeric heterochromatin and subtelomeric regions.62 Notably, recurrent ATRX loss-of-function mutations were found in approximately one-quarter of pHGG, which were also associated with alternative lengthening of telomeres. All tumors with H3F3A p.G34R/V mutation carried concomitant ATRX mutations, suggesting synergy between the 2 mutations.34

All identified histone mutations occur in the N-terminal tails of histones, unstructured regions that undergo extensive posttranslational modification. These modifications in turn facilitate recruitment of effector proteins that regulate transcriptionally active or silent chromatin states.63

Understanding Mutant Histone Gain-of-Function

All histone H3 mutations in pHGG were heterozygous, and in any individual tumor, only 1 of 16 genes encoding histone H3 was mutated. This pattern clearly indicates a dominant gain-of-function effect.

Lysine 27 on histone H3 (H3K27) is a residue that can be acetylated or monomethylated, dimethylated, or trimethylated (H3K27me3). Although mutant histone H3.1/3.3 make up a minority of the total cellular histone H3 pool,64 p.K27M mutations led to loss of total H3K27me2/3 of the entire cellular H3 pool, most of which is WT.64-67 This dominant negative effect appears to be caused by the inhibition of the H3K27 methylase EZH2 owing to interaction with p.K27M mutant histone H3.64,65 Globally, both p.K27M and p.G34R/V tumors exhibit DNA hypomethylation.61,65 In an unsupervised comparison of genome-wide DNA methylation signatures, tumors with p.K27M and tumors with p.G34R/V form independent clusters based on histone mutation status. Many genes with differentially methylated promoters showed differential gene expression that was associated with the anatomical origin of the
tumors, suggesting that both DNA methylation and gene expression may be a result of the origin of the tumor that may be further influenced by histone mutations.\textsuperscript{51,63,66,68}

The dominant mechanism of action is less apparent for mutations affecting G34. Although there is no clear dominant effect on modification of the nearby H3K36, there appears to be an altered genome-wide distribution of H3K36me3 binding.\textsuperscript{35,39-41} Interestingly, one of the most upregulated genes in such tumors is MYCN, a well-known oncogene.\textsuperscript{68}

**Additional Genetic Associations With pHGG Subgroups**

Genome-wide sequencing approaches revealed that 20%-32% of DIPGs harbored somatic missense mutations in ACVR1, also known as \textit{ALK2},\textsuperscript{35,39-41} which encodes a receptor serine-threonine kinase mediating bone morphogenetic protein–induced signal transduction.\textsuperscript{66} These mutations frequently co-occur with histone H3.1 pK27M substitutions. Both alterations tend to occur in younger patients with DIPG and were not found in pHGG arising outside the brainstem.\textsuperscript{35,39-41} Thus, these mutations further clarify molecular subgroups within DIPG. Surprisingly, some of the somatic mutations found in DIPG were the same as previously reported \textit{ACVR1} germline mutations in fibrodysplasia ossificans progressiva, a disease characterized by heterotopic bone formation exacerbated by inflammation that is not associated with cancer predisposition.\textsuperscript{70}

RNA sequencing analysis allowed the identification of fusion genes that were generated by genomic rearrangements. Expression of fusion genes was common in pHGG, including DIPG, although most of the fusion genes were not recurrent. Strikingly, recurrent chimeric genes encoding N-terminal sequences from a number of different genes fused to the kinase domain of the neurotrophic tyrosine receptor kinase (NTRK) family members were found in 40% of infant NBS-HGGs, and at much lower frequency in pHGG overall.\textsuperscript{35} The prognosis for pNBS-HGG in children younger than 3 years is significantly better than for older children.\textsuperscript{71} The NTRK fusion genes may provide a useful new therapeutic target for this patient population. NTRK fusions were also identified in adult glioblastoma as well as pLGG, but they do not appear to be as enriched as in infant NBS-HGG.\textsuperscript{72-74}

We are grounded in knowing that our understanding of the protein coding genome far surpasses that of the remaining 98% of DNA sequence, which potentially hosts a vast network of regulatory elements. Furthermore, we are just beginning to piece together the role of epigenetics in normal and neoplastic contexts. The high frequency of histone mutations in pHGG strongly suggests that epigenetic dysregulation plays a major role in tumors arising within the pediatric setting. We must also recognize that our analyses yield snapshots of an ever-changing process. The genetics and epigenetics of tumors are dynamic, and the portrait of each cancer landscape evolves as a function of any selective pressure introduced, therapy included.\textsuperscript{75}

The aforementioned studies, both large and small scale, genome-wide and targeted, have given today’s researchers and clinicians a better understanding of pHGG than ever before. The biologies of DIPG, pNBS-HGG, and infant NBS-HGG are distinctly different, and these differences in turn require specific clinical consideration in terms of appropriate intervention. With a 2-year survival rate of less than 20%, the prognosis for pHGG remains unacceptably poor.\textsuperscript{76} However, there is a palpable excitement within the community that meaningful change is within reach.

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Biology of Brain Tumors
Harnessing preclinical mouse models to inform human clinical cancer trials

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The urgent need for better cancer treatments has stimulated interest in employing small-animal models to evaluate potential drug therapies. Robust mouse models of many human cancers have been generated using sophisticated technologies for engineering germ-line mutations. As we enter into an age of targeted therapeutics, these strains provide novel platforms for validating new anticancer drugs, assessing therapeutic index, identifying surrogate markers of tumor progression, and defining epigenetic and environmental influences on tumorigenesis.

The availability of strains of genetically engineered mice (GEM) that develop a spectrum of cancers similar to those found in humans offers an unprecedented opportunity to efficiently evaluate the efficacy and therapeutic index of novel anticancer therapies in preclinical models in advance of human trials. While straightforward in principle, executing preclinical studies in mice that allow for meaningful and immediate application to the treatment of human cancer is difficult. Moreover, the potential use of GEM cancer models to accelerate the process of bringing effective new treatments to patients is largely theoretical, as few examples exist in which mouse preclinical data has been successfully translated to clinical practice.

The current development process for anticancer drugs

Taking a drug from discovery to market is an arduous process that frequently takes longer than 15 years and costs more than $800 million. Most agents that are advanced into early-phase human clinical trials fail. Recent advances in the fields of cancer biology and high-throughput screening have identified numerous potential molecular targets for drug discovery; however, most of the proteins and pathways deregulated in cancer cells also have essential roles in normal cells. It is therefore difficult to predict when a drug will prove tumor-selective. Moreover, developing new therapies against specific molecular abnormalities in well-defined subsets of cancers can be prohibitively expensive.

The use of GEM cancer models as an initial “filter” to identify tumors and molecular targets that, when inhibited, will selectively kill tumor cells is one potential strategy for streamlining the overall process of cancer drug development.

Preclinical mouse models of human cancer

Numerous small-animal models of human cancer have been generated. These include inbred strains that spontaneously develop cancer (1–4), rodents in which cancer is caused by intrauterine or postnatal exposure to chemical mutagens (5–9), and mice in which tumors are produced by viral or bacterial infection (10–13). In addition, xenograft models that were generated by directly implanting cancer cell lines established from human tumors into mice have been widely used for drug discovery (14–17). The major limitations of these explant models are the requirement for an immunocompromised host and the inability of these models to fully recapitulate the complex relationship between the tumor and its microenvironment (e.g., angiogenesis). Most importantly, the ability of xenografts to accurately predict drug efficacy in human cancer patients has been disappointing (18).

GEM cancer models are becoming increasingly sophisticated in their ability to accurately mimic the histology and biological behavior of human cancers. Numerous tissue-specific GEM models have been developed that exhibit many biologic hallmarks of human cancer, including angiogenesis and stromal interactions, as well as similar histopathologic and genetic abnormalities (19).

The major advantages of GEM models are that: (a) the initiating genetic event is known; (b) the mice are immunocompetent; and (c) the tumors develop spontaneously in their appropriate tissue compartments. Moreover, GEM cancer models, which allow assessment of therapeutic efficacy on a uniform genetic background, are particularly useful for performing preclinical studies of rare cancers and for assessing synergy between therapeutic agents. They can also potentially provide the tools needed to learn more about the histologic and biochemical effects of specific agents prior to human testing.

While GEM models offer many advantages, the cancers typically arise from genetic events that are expressed simultaneously in many cells throughout an animal or in an entire tissue. By contrast, most human tumors are believed to arise from single cells or from a small population of mutant cells. To overcome this limitation, strategies have been developed that allow mutant alleles to be expressed in small populations of cells in vivo (20, 21).

Opportunities to employ mouse models

The availability of robust GEM models facilitates a detailed analysis of human cancer that cannot be easily accomplished by studying primary human tumors (see Opportunities provided by employing GEM cancer models). First, the ability to more effectively target human cancers requires a detailed understanding of molecular and cellular pathogenesis to identify specific molecular targets. Second, there is also a great need to define those individuals at greatest risk for developing cancer as well as those most likely to respond to any
given therapeutic regimen. These studies require large numbers of individuals and are often not possible for less common cancers. Last, the identification of surrogate markers of tumor formation and early response to therapy, which would have tremendous impact on current treatment strategies, is another unmet need.

**Evaluation of standard human antitumor therapies.** One of the often neglected uses of GEM cancer models is the validation of conventional therapies employed for the treatment of cognate tumors in humans. For example, accurate GEM models of astrocytoma or pancreatic cancer should ideally respond to the same treatments currently used to treat these cancers (i.e., temozolomide and gemcitabine, respectively). In addition, GEM models provide the opportunity to define the mechanism(s) underlying the antitumor effects. Tumors from mice treated with anticancer therapies can be analyzed to determine whether regression results from decreased cell growth, increased cell death, decreased tumor angiogenesis, or necrosis. Failure to observe any effects on GEM tumors may reflect problems with bioavailability (e.g., inability to cross the blood-brain barrier), differences in the metabolic processing of drugs in rodents (e.g., pharmacokinetic and pharmacodynamic [PK/PD] issues), and/or genetic differences between mouse strains that dictate the response to therapy (e.g., modifier loci).

Experience with a mouse model of acute promyelocytic leukemia (APL) suggests that GEM models respond to human cancer treatments and can be used to improve therapy. In APL, blasts are arrested at the promyelocytic stage of differentiation due to chromosomal translocations that fuse the retinoic acid receptor alpha (RARA) gene to a variety of partner genes including promyelocytic leukemia (PML) and promyelocytic leukemia zinc finger (PLZF). All-trans-retinoic acid (ATRA) induces complete remissions in approximately 80% of patients with APL who have a PML-RARA translocation by relieving the differentiation block (22) but does not induce remission in those individuals with PLZF-RARA fusions (23). Similarly, ATRA induces remissions in PML-RARA transgenic mice but is ineffective in a PLZF-RARA strain that also develops APL (24). In addition, mouse models of APL have been harnessed to test new therapeutic approaches such as arsenic trioxide (As$_2$O$_3$) and the potential synergy between ATRA and As$_2$O$_3$ (25, 26).

**The role of specific cancer genes.** GEM strains have been generated that model the inactivation of genes mutated in inherited cancer syndromes (e.g., neurofibromatosis 1 [NF1], NF2, APC), in sporadic cancers (e.g., KRAS, PML-RARA), and in both types of cancer (e.g., TP53) (27–46). GEM models based on these tumor suppressors and oncogenes provide unique opportunities to clearly define the causative role of each of these genetic changes in tumor formation and progression. This information is critical for the design of targeted (biologically based) therapies for individual cancers with these specific tumor-associated mutations.

**Target validation.** GEM cancer models can be used to determine whether the success or failure of a given therapy reflects the ability of the drug to reach the tumor and inhibit its target. An illustrative example of how GEM cancer models can provide insights into mechanisms of drug activity comes from studies that evaluated the efficacy and putative biochemical targets of farnesyltransferase (FTase) inhibitors (FTIs). Ras processing is initiated by cytosolic prenyltransferases, which attach either a farnesyl or geranylgeranyl isoprenoid lipid to the thiol group of the cysteine. Geranylgeranyl transferase 1 (GGTase-1) and FTase catalyze the transfer of isoprenoid groups, which are donated by geranylgeranyl pyrophosphate and farnesyl pyrophosphate, respectively. FTIs were developed as cancer therapeutics based on their potential as Ras inhibitors in xenograft models. However, KRAS and NRAS are also good GGTase-1 substrates and are processed by this enzyme when FTase is inhibited. Preclinical studies of the efficacy of FTIs gave variable results in transgenic mouse models of breast cancer induced by expressing oncogenic HRAS or KRAS from the murine mammary tumor virus promoter (47–49) and in a model of myeloproliferative disease induced by inactivating the Nf1 tumor suppressor (50), which encodes a GTPase-activating protein that negatively regulates Ras signaling. Importantly, careful biochemical investigation of tumor tissues from these mouse models unequivocally showed no inhibition of KRAS or NRAS processing at the maximally tolerated dose (MTD) of FTI. Based on these data, it was concluded that any therapeutic effects of FTIs were due to “off-target” activities that were not related to the original goal of inhibiting hyperactive Ras.

**Defining the discrete steps of tumorigenesis.** GEM cancer models can be used to dissect the cellular and molecular changes associated with each stage of neoplasia, including tumor formation, tumor maintenance, and malignant progression. Studies focused on defining the events associated with tumor formation in multistep cancers are essentially chemoprevention investigations. Direct chemoprevention studies in people at risk for cancer are difficult, owing to the genetic heterogeneity in human populations and the difficulties in accurately measuring exposure, which necessitate large and enormously expensive long-term studies. By contrast, experiments in GEM cancer models can be performed on a uniform genetic background in which environmental exposures are rigorously controlled. GEM cancer models have been employed to establish causal relationships with environmental exposures (e.g., asbestos in mesothelioma, tobacco and lung cancer; diet in colon cancer) (51–55).

The ability of a tumor to continue to survive and proliferate in an otherwise inhospitable environment requires additional molecular and cellular changes. Studies of tumor maintenance are typically focused on defining the key signals required for these processes and form the basis for targeted chemotherapy. Studies in GEM models

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**Opportunities provided by employing GEM cancer models**

Provide initial “filter” to identify molecular targets that, when inhibited, kill cancer cells

Investigate mechanisms underlying responsiveness and resistance to conventional cancer therapies

Define discrete steps of tumorigenesis

Determine the role of the microenvironment in tumor formation and progression

Identify surrogate markers of tumor growth and response to therapy

Define epigenetic and environmental influences on tumorigenesis
and in human patients have implied that molecular changes important for cancer formation are also necessary for maintenance. For example, studies in which tetracycline-regulatable alleles of oncogenic RAS and MYC were “shut off” in established tumors resulted in dramatic tumor regression (56–58). Furthermore, the emergence of imatinib-resistant mutant alleles of BCR-ABL in patients with chronic myeloid leukemia (59, 60) argues strongly that the cancer-initiating mutation remains central to the tumor’s growth advantage. However, other data suggest that cancer cells can escape from dependence on the initiating oncogenic lesion under some circumstances (61, 62). The exact mechanisms underlying “tumor escape” have not been fully elucidated; but they may reflect a change in the histologic phenotype of the tumor, loss of expression of the initiating oncogene, or the acquisition of additional genetic changes (63). The ability of some cancers to free themselves from dependence on the initiating molecular event likely has implications for the design of targeted therapies for recurrent tumors.

Tumors frequently evolve from a benign neoplastic lesion to a more malignant cancer. This progression involves the acquisition of additional genetic changes, which also serve as targets for chemotherapeutic drug design. For example, during the progression to malignant cancer, some low-grade astrocytomas somatically acquire a constitutively active version of the EGFR. This signature genetic event formed the basis for the development of targeted therapies directed against this mutant EGFR in both mice and humans (64, 65). GEM models were important in demonstrating that the EGFR mutation is a causative genetic change that accelerates malignant transformation (66, 67).

Tumor microenvironment. GEM cancer models have been powerful tools for examining the contribution of the tumor microenvironment to tumor formation. Studies of peripheral and central nervous system tumors in a mouse model of the NF1 familial cancer syndrome demonstrated that tumor formation requires that loss of NF1 expression in Schwann cells (neurofibromas) or astrocytes (optic glioma) occur in the context of a heterozygous germ line NF1 mutation (43, 44). These data demonstrate that heterozygous NF1 mutant cells in the microenvironment of preneoplastic lesions participate in tumorigenesis. Nonmalignant stromal cells also contribute to mammary carcinoma, in which loss of TGF-β receptor expression in fibroblasts promotes mammary ductal carcinoma growth and invasion by upregulating specific signaling networks (68, 69). Last, angiogenesis plays a fundamental role in tumor formation and progression and has formed the biological basis for numerous clinical trials using antiangiogenic therapies (70, 71). GEM models have been instructive in defining the molecular basis for new blood vessel formation by tumors and the impact of angiogenesis on tumor progression (72, 73).

Radiologic and serum biomarkers. The ability to define individuals at high risk of developing cancer and the ability to noninvasively monitor disease burden during and after cancer treatment have substantial implications for clinical practice. GEM models have been employed to identify serum biomarkers for cancer using advanced proteomics methods. While these studies are still in their early phases of discovery, one serum biomarker has been identified for murine prostate cancer that correlated well with tumor weight and response to hormone therapy (74). In addition to serum biomarkers, MRI has recently been evaluated for its ability to provide information regarding therapeutic efficacy in brain tumors. MRI of mice bearing brain tumors demonstrated that the tissue diffusion values obtained early after standard chemotherapy correlated with tumor response (75). These results prompted an investigation of human brain tumors, which showed that tissue diffusion values obtained 3 weeks after the initiation of chemotherapy could predict patient response (76). Similar to serum biomarkers, the ability of MRI to define patients with recurrent disease or who do not respond to first-line therapy would allow for early intervention and the administration of alternative therapies.

Modifier genes. Unlike humans, GEM models can be generated on homogeneous genetic backgrounds, which greatly facilitate identifying modifier genes that influence the incidence or clinical behavior of specific cancers. Numerous candidate genetic loci have been found that influence tumor number and size in mouse lung and colon cancer (77–79) as well as tumor type in mice harboring identical genetic mutations. For example, the tumor spectrum in mice harboring mutations in the p53 and Nf1 genes is dictated by the genetic background, which led to the identification of a locus on mouse chromosome 11 that determined susceptibility to astrocytoma (80). Last, genes that function to identify DNA polymerase errors during DNA replication (DNA mismatch repair genes) have been shown to modify colon cancer tumor burden and survival in GEM (81–83).

Performing preclinical studies in mice
Evaluating conventional cancer therapies in human patients. There are well-established paradigms for testing new drugs in human patients. New agents are typically evaluated in 3 phases. As the primary goal of a phase 1 trial is to determine the MTD of a drug, these studies typically involve administering a single agent to patients with a variety of different tumor types who have failed to respond to standard therapies. Phase 2 trials are designed to measure response rates in a group of patients with refractory or recurrent cancers treated at the MTD. Responses are traditionally reported as “complete” (objective regression of all detectable lesions), “partial” (some regression), or “mixed” (regression of some lesions with growth of others). Compounds that show significant promise in phase 2 studies are advanced to randomized phase 3 trials, in which the new drug is compared, either alone or in combination with other agents, to the “standard” treatment for a specific cancer. In contrast to phase 1 and phase 2 trials, phase 3 studies include newly diagnosed patients and are frequently performed in the setting of cooperative multi-institutional networks. Because phase 2 and phase 3 studies are logistically challenging, expensive, and time consuming, pharmaceutical and biotechnology companies are understandably most interested in testing agents that might be approved to treat patients with common cancers. Importantly, phase 1 trials in patients with refractory cancers may not accurately mimic response rates in persons with de novo disease, and it has been difficult to test drug combinations in phase 1 and phase 2 trials. GEM cancer models offer the possibility of overcoming these 2 problems.

Using GEM cancer models to investigate responsiveness and resistance to conventional anticancer agents. Relatively few studies of conventional cytotoxic agents have been performed in GEM models. This is due, in part, to the fact that many investigators who generate GEM cancer models lack expertise in performing preclinical studies. Although much less expensive than human clinical trials, investigating drugs in mice is challenging due to factors that include the need to generate and maintain cohorts of mice that spontaneously develop tumors, difficulties in assessing the responses of tumors that can only be visualized by small-animal
chemotherapeutic agents (84, 85). Later become resistant when patients relapse. Pioneering studies in an Eμ-Myc B cell lymphoma model have shown that some of the genetic lesions that contribute to cancer, such as Tp53 inactivation or deregulated Bcl2 expression, also modulate resistance to chemotherapeutic agents (84, 85).

Evaluating molecularly targeted inhibitors in humans and in GEM models. Some traditional strategies for evaluating new cancer therapies are being reconsidered as more specific agents are developed (86). Target inhibition, rather than overt cellular toxicity (e.g., MTD), may represent a better endpoint for phase I testing of drugs with a well-defined biochemical target. Additionally, there are now many examples that underscore the importance of preselecting patients with specific molecular abnormalities for targeted therapies trials. In this regard, the beneficial effects of ATRA are largely limited to APL; the efficacy of imatinib correlates with biochemical inhibition of specific mutant kinases (BCR-ABL, c-kit, and PDGF); and the presence of activating EGFR mutations predicts clinical responsiveness to gefitinib in lung cancer (25, 87–91). In a recent study, coexpression of a mutant EGFR receptor and an intact PTEN gene correlated with the response of high-grade malignant astrocytoma to EGFR inhibitors (92). Similarly, although RAS and BRAF mutations both encode proteins that deregulate MEK/ERK signaling in melanoma, cancer cell lines with BRAF mutations are highly sensitive to MEK inhibitors, whereas cells with RAS mutations are not (93).

Harnessing GEM cancer models to enhance the development of new therapies. Academic researchers, pharmaceutical companies, government agencies, and patient advocacy groups have all expressed concern about the apparent “disconnect” between our growing understanding of cancer biology and the relatively few instances in which these advances have been successfully translated into better cancer treatments. The authors recently participated in a meeting that examined how mouse models of tumors that develop in persons with NF1 and NF2 could be efficiently employed to inform human clinical trials (“Barriers and Solutions in the Use of Mouse Models to Develop Therapeutic Strategies for Neurofibromatosis-Associated Tumors,” Banbury Center, Cold Spring Harbor Laboratories, November 3–5, 2005). Individuals with NF1 are predisposed to the development of specific benign and malignant tumors, including cutaneous and plexiform neurofibromas, low-grade astrocytoma, juvenile myelomonocytic leukemia (JMML), and malignant peripheral nerve sheath tumor (MPNST), while persons with NF2 develop schwannoma, meningioma, and ependymoma (94). Because NF1- and NF2-associated tumors are relatively uncommon, pharmaceutical and biotechnology companies are not actively engaged in developing drugs for these specific indications. However, the molecular genetics of human NF1 and NF2 are understood in detail, and elegant mouse models of most NF-associated tumors are available. Many companies are developing drugs that interfere with components of the RAS signaling network, which might prove effective in some NF1-associated tumors. Unfortunately, performing clinical trials in NF1 patients is difficult for a variety of reasons, including the slow and predictable growth rates of many of these tumors, the propensity to affect children, and relatively small patient numbers. GEM models of JMML and MPNST are characterized by rapid growth and relative ease of measuring treatment responses (45, 46, 95, 96). These in vivo models, and tumor cells from these mice, could be used to rapidly screen candidate drugs for a beneficial therapeutic index, and promising agents might be investigated further by performing detailed PK/PD studies. By contrast, evaluating therapeutics in the existing neurofibroma and optic glioma GEM models is more difficult due to their relatively slow growth rates and requirement for small-animal imaging (43, 44). These models might be more useful for studies of preventive agents or for “front-line” preclinical studies of compounds that target cells in the tumor microenvironment. The overall goal of this type of strategy (Figure 1) is to optimally employ the available GEM tumor models as “filters” to select agents for human trials that have the greatest likelihood of succeeding in the clinic. In this proposed strategy, new drugs could be rapidly screened for efficacy, target validation, and potential “off-target” effects in GEM models that lend themselves well to rapid throughput (e.g., NF1 MPNST and leukemia GEM models). Drugs active in these paradigms would be further studied to define

**Figure 1**
Use of GEM tumor models as “filters” to select agents for human clinical trials. One strategy has been proposed for use in the Nf1 GEM models community, which involves the evaluation of new therapies in multiple mouse strains. New drugs would be rapidly screened using MPNST and leukemia GEM models for efficacy (therapeutic index), target validation, and potential “off-target” effects. These GEM models would be utilized for initial evaluation, based on the rapid growth of the tumors and the relative ease of measuring tumor growth. Drugs that exhibit activity in these models would be further analyzed in detailed PK/PD studies in other tumor models, such as orthotopic tumor explant models and transgenic mice harboring specific deregulated cancer-associated molecules or pathways. Optic glioma and neurofibroma (plexiform neurofibroma) GEM models may be better suited for chemoprevention studies as well as investigations of drugs that target specific cells in the tumor microenvironment (e.g., microglia and mast cells). Collectively, the combined use of each of the available robust preclinical GEM models would afford researchers the opportunity to comprehensively evaluate drugs prior to considering human clinical trials. Adapted with permission from a summary presentation by Susan Blaney, Cold Spring Harbor Laboratories, Banbury Center conference on “Barriers and Solutions in the Use of Mouse Models to Develop Therapeutic Strategies for Neurofibromatosis-Associated Tumors,” November 3–5, 2005.
their bioavailability and pharmacokinetics in other GEM model systems (e.g., orthotopic transplant or transgenic mouse models). Secondary evaluation of candidate drugs would then entail the use of additional GEM strains (e.g., NFI optic glioma and neurofibromatosis GEM models) in which tumor microenvironment plays an important role in cancer formation. These latter GEM models are uniquely suited for chemoprevention studies as well as for examining drugs directed against specific cell types in the tumor microenviron- 

ment (e.g., immune system cells, endothelial cells). The combined use of multiple complementary preclinical model systems provides an excellent opportunity to comprehensively evaluate lead compounds under conditions that closely approximate the human condition prior to the initiation of human clinical trials.

Given the pressing need to develop new cancer therapies, it is important to establish preclinical testing paradigms that provide the greatest opportunities to optimally translate results obtained in GEM cancer models into the clinic. We recommend that inves-

gtigators take advantage of the multiple complementary GEM can-
cancer models now available to evaluate new agents in order to best inform subsequent human clinical trials.

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The Neurobiology of Neurooncology

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The histological classification of brain tumors currently is based on the morphological appearance and protein expression patterns that reflect specific cell types within the central nervous system. Recent studies have suggested that the cells of origin for brain tumors may persist in the fully formed tumors, and that these “cancer stem cells” might represent the relevant cellular targets for anticancer therapy. In this regard, insights into the developmental neurobiology of brain tumors has significant impact on our understanding of the molecular and cellular pathogenesis of these devastating cancers, as well as the development of new strategies for treating brain tumors.

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Our knowledge of the cause of brain tumors has increased tremendously over the past 20 years and is leading to a deeper understanding of the molecular events essential for tumor formation. Simultaneously, we have gained insights into the developmental processes that cause the diversity of the normal cells that comprise the brain, and we are beginning to recognize the overlapping and common mechanisms regulating tumorigenesis and development in the nervous system. Understanding the interplay between tumorigenesis and development may have important implications for both neuroscience and neurooncology. Among these, one of the most important factors is determining the cell of origin for brain tumors. Identifying the cell from which a given tumor arises would allow us to compare tumor cells with their normal counterparts, so that key differences and vulnerabilities of tumor cells can be discovered. Furthermore, identifying the cell of origin would allow us to create more robust and relevant animal models with which to study brain tumor etiology, pathology, and treatment. Finally, recent studies suggest that the cell of origin, or a cell that resembles it, may persist in mature tumors, and this cell type may be critical for the continued growth and propagation of brain tumors.1 Therefore, identifying the cell of origin for a particular central nervous system tumor may be critical for designing effective approaches to therapy. In this regard, certain subpopulations of cells in other cancers (e.g., melanoma) exhibit distinct sensitivities to chemotherapy as a result of differential expression of P-glycoprotein and adenosine triphosphate–binding cassette proteins.2 Herein, we review what is known about the cell of origin for two major classes of brain tumors, medulloblastoma (MB) and astrocytoma, and discuss new approaches to addressing this important neurobiological issue.

Histological Classification of Brain Tumors

Brain tumors currently are classified according to the World Health Organization (WHO) system, which derives from the pioneering work of Bailey and Cushing.3 This classification system names tumors after the cell type that tumor cells resemble most in the developing embryo or adult. Based on these criteria, neuropathologists distinguish among glioma (astrocytoma), oligodendroglioma, and neuronal tumors.4 Astrocytic tumors comprise a wide range of glial neoplasms, which are subdivided into four malignancy grades (WHO grades I-IV) based on the presence of specific criteria, such as nuclear atypia, mitotic activity, necrosis, and microvascular proliferation. Oligodendrogliomas are subdivided into those tumors composed of pure oligodendroglial tumor cells and those with a mixed oligodendroglial and astrocytic appearance. Neuronal tumors constitute a large proportion of brain tumors seen in children, and they include central neurocytoma, ganglioglioma, supratentorial primitive neuroectodermal tumors, and MB. MB, the most common pediatric brain tumor, is a malignant invasive neoplasm of the cerebellum composed of cells that exhibit primarily neuronal differentiation.

While the current WHO classification scheme is

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used widely, it has some notable limitations. First, many tumors consist of atypical-appearing cells that do not resemble any normal cell type in the brain. Second, central nervous system tumors are often morphologically diverse, and classification may rely on identification of a specific area within the tumor most characteristic of that particular tumor type. This may lead to inaccurate histological assignment based on a small region of tumor within an otherwise heterogeneous cellular mass. Third, tumor classification often depends on the use of immunocytological techniques to identify specific antigens or cell types. Because there are few antibodies that reliably or exclusively identify specific cell lineages, the presence or absence of a particular antigen suggests only a tumor type and does not necessarily allow definitive classification.

Although the WHO classification scheme implies a cell of origin for many brain tumors, the cell of origin has not been unequivocally identified for any of them. It is hypothesized that MBs originate from neuronal precursors, whereas astrocytoma and oligodendroglioma arise from astrocytic or oligodendrogial precursors. In this fashion, brain tumors may form as a result of the acquisition of specific genetic changes in stem cells, progenitor cells, or differentiated cell types (Fig 1). These genetic changes deregulate cell growth and differentiation control pathways important for normal brain development and lead to increased cell growth as an initiating step in tumor formation. Cancer-causing genetic mutations in stem cells and progenitor cells likely result in the increased growth of immature cell types, whereas differentiated cell types (e.g., astrocytes) may acquire stem cell-like or progenitor cell-like properties as a result of specific genetic mutations. In the following sections, we discuss what is known and what remains to be learned about the origins of MB and astrocytoma.

Cellular Origin of Medulloblastoma

Among central nervous system tumors, few have evoked more discussion and speculation regarding their cell of origin than those primarily composed of primitive neuroepithelial cells. The most representative tumor in this group, the cerebellar MB, was distinguished from other brain tumors in 1910 by James Homer Wright, and later more specifically by Percival Bailey and Harvey Cushing. In light of its predominant neuronal morphology, Wright suggested that MBs derived from restricted neuronal precursors, or neuroblasts. In contrast, Bailey and Cushing noted that these tumors often contained glial cells as well, and proposed that their cell of origin was a new embryonic neuroepithelial cell type (“medulloblast”) capable of generating both glial and neuronal cells.

In addition to the debate about the neuronal/glial potential of the cell of origin, there has also been significant disagreement about the location of this progenitor cell within the developing cerebellum. It has long been recognized that the cerebellum contains two distinct germinal zones: the ventricular zone (VZ) that forms the innermost boundary of the cerebellum, and the external germinal layer (EGL) that lines the outside of the cerebellum (Fig 2). In their original description of MB, Bailey and Cushing suggested that the medulloblast was located in the VZ, and that the tumor originated from this region. In contrast, many investigators have proposed that MBs arise from the EGL. Because developmental studies have suggested that the VZ generates both neurons and glia, whereas the EGL contains primarily neuronally restricted granule cell precursors (GCPs), proponents of the EGL as the site of origin tend to favor the notion that these tumors arise from neuroblasts rather than multipotent progenitors.

Over the years, evidence has accumulated in support of both restricted neuronal progenitors and multipotent precursors as the cells of origin for MB. Most of this information has come from immunohistochemical staining and gene expression analyses of tumor tissue. For example, studies of human MB have shown that some tumors express markers associated with EGL-derived GCPs, such as p75NTR, TrkC, Zic1, and Math1. However, many MBs express markers of
VZ-derived progenitors, such as calbindin-D28K, parvalbumin, nestin, vimentin, and glial fibrillary acidic protein (GFAP). Although some of these markers (e.g., nestin) can be found both in the EGL and in the VZ, most MBs express either EGL markers or VZ markers, but not both.

In an attempt to explain these discrepancies, a number of investigators suggested that different classes of MB may originate from distinct progenitors. Although the WHO lists several histological subtypes of MB, the majority of tumors are described as either “desmoplastic” or “classic.” Desmoplastic MBs account for 15 to 20% of all MBs, with a higher incidence in adult patients. These tumors are most often located in the cerebellar hemispheres, display extensive nodularity, and have a relatively favorable prognosis. In contrast, 75 to 80% of tumors are regarded as classic MBs. These are commonly located in the center of the cerebellum (the vermis), grow as relatively uniform sheets of cells with a high nuclear/cytoplasmic ratio, and have a tendency to invade adjacent brain and leptomeninges.

Several studies have suggested that desmoplastic and classic MBs may have different origins. Desmoplastic tumors tend to express markers of the granule cell lineage (such as Math1 and P75NTR), and have therefore been suggested to arise from GCPs in the EGL. Classic MBs more frequently express markers associated with non-granule neurons (e.g., calbindin) and, hence, have been suggested to originate from the VZ. Gene expression profiling also supports the concept of distinct origins for the different subtypes of MB. For example, both classic and desmoplastic MBs have separable genetic profiles, with desmoplastic MBs expressing genes associated with proliferating GCPs in the EGL. In contrast, classic MBs express a distinct set of markers that are more heterogeneous and not clearly associated with any particular cerebellar cell type. These data suggest that desmoplastic MBs may derive from the EGL, whereas classic MBs arise from the VZ.

Although the dichotomy between EGL-derived desmoplastic tumors and VZ-derived classic MBs makes for an appealing model, some studies have cast some doubt on this view. First, some microarray analyses of human MB have failed to find a clear correlation between lineage markers and desmoplastic/classic histology. In addition, a number of recent studies have suggested that both desmoplastic and classic MBs express high levels of stem-cell markers, including the VZ-associated glycoprotein CD133. CD133 stemlike cells isolated from MBs can form proliferating clones characteristic of neural stem cells (“neurospheres”), can undergo self-renewal (ie, form secondary and tertiary neurospheres in culture), and under appropriate conditions, can be induced to differentiate into both neurons and glia. Finally, these CD133+ cells can generate tumors after transplantation into immunocompromised mice. These data suggest that both histological subtypes of MB contain cells that resemble multipotent neural stem cells, but whether the tumors actually arise from stem cells remains unknown.

A number of genes and signaling pathways have been implicated in the genesis of MB, and these also shed some light on the cell of origin. The two most studied examples are the sonic hedgehog (SHH)-patched (PTCH) and the WNT signaling pathways (Fig 3). Patients with germline PTCH mutations (which activate the hedgehog pathway) acquire Gorlin’s syndrome, a disease characterized by recurrent basal cell carcinomas of the skin, craniofacial abnor-
malities, and an increased incidence of MB. In addition, 20 to 30% of sporadic MBs harbor activating mutations in the SHH/PTCH pathway mutations, and mice engineered with patched mutations experience development of MB. Because SHH signaling has been shown to control proliferation of GCPs, it has been suggested that tumors arising from SHH pathway mutations are likely to arise from these cells. However, SHH/PTCH signaling may also influence multipotent neural stem cell growth. Regardless of the cell of origin of SHH pathway tumors, there is hope that these tumors will be sensitive to small-molecule inhibitors of the hedgehog pathway. Such inhibitors have already been shown to dramatically improve the survival of tumor-bearing Ptch mutant mice. Their effectiveness in treating human MB is not yet known, but is likely to be evaluated in the near future.

The WNT signaling pathway has likewise been implicated in a subset of MBs. Turcot’s syndrome, which results from germline mutations in the adenomatous polyposis coli gene, have a high incidence of colon cancer and brain tumors, primarily MBs. Although adenomatous polyposis coli mutations are relatively rare in sporadic MBs, 5 to 15% of these tumors have been reported to contain mutations in β-catenin or Axin, each of which can result in WNT pathway activation. Unlike the SHH pathway, the WNT pathway has not been implicated in growth or survival of GCPs. However, WNT signaling is known to be critical for the specification of the midbrain-hindbrain boundary from which the entire cerebellum develops and may therefore be important for the growth and survival of multipotent progenitors in the embryonic cerebellum. Alternatively, there may be other classes of progenitors in the developing cerebellum that depend on WNT signaling for proliferation or self-renewal. The cellular targets of transformation in WNT pathway–associated MBs remain an important area of investigation.

Cellular Origins of Astrocytoma
As with MB, there has been a considerable amount of controversy and discussion regarding the cellular origin of astrocytoma. In 1846, Virchow described the presence of glial cells in the brain and named them “neuroglia.” He postulated that these cells may be causally related to the development of a number of brain tumors, and coined the term glioma. Ramon y Cajal and Del Rio Hortega further defined this heterogeneous group of neuroglia, subdividing them into astrocytes and oligodendroglia. In 1926, Bailey and Cushing proposed that astrocytic tumors are related to either the maturation of progenitor cells (bipolar spongioblasts) or astrocytes.

Using human tumor tissues, it has not been possible to conclusively demonstrate which cell type causes astrocytoma, and debate continues whether astrocytomas arise from differentiated astrocytes, astroglial progenitor cells, or neural stem cells. Immunohistochemical studies have shown that astrocytoma tumor cells express protein markers typically found in glial progenitor cells, including GFAP, nestin, brain lipid-binding protein, and OLIG-2. However, identifying the cell of origin based on these lineage-specific markers is problematic. In this regard, although GFAP has long been regarded as a marker for differentiated astrocytes, recent studies have shown that GFAP expression begins in midembryogenesis and marks cells in the subventr..
tricular zone with the ability to function as true neuroglial stem cells. With the recognition that GFAP identifies a wide variety of cell types, ranging from stem cells to mature astrocytes, its use as a marker for differentiated astrocytes has been called into question. Efforts to identify additional differentiation markers for the astroglial lineage have been sought by studying glial cell differentiation in vitro; however, it is not clear that the “lineage-specific” markers expressed by differentiating astroglial cells grown in vitro reflect the different phases of astroglial cell maturation that occur in the intact animal in vivo. Finally, similar to MBs, all histological grades of human astrocytoma contain CD133 stem cells, which, when explanted into naive mouse brains, result in the development of astrocytomas histologically identical to the original parental tumor. As discussed earlier, these studies support the hypothesis that stem cells exist in human astrocytomas, and that these cells can regenerate astrocytomas in naive recipient mice, but do not prove that astrocytomas arise from these cells.

A number of specific genetic changes that influence astroglial cell differentiation from stem cells in vivo have been identified that have particular relevance to gliomagenesis (Fig 4). These include growth factors (epidermal growth factor [EGF] and platelet-derived growth factor [PDGF]), proteins of the interleukin family (interleukin 6 [IL-6] and leukemia inhibitory factor), and members of the SHH transcriptional control program (OLIG and GLI transcription factors). In this regard, PDGF is a potent mitogen for astroglial cell precursors and is a critical growth factor that specifies oligodendrocyte development. EGF has been shown to promote astroglial cell differentiation from neural stem cells at the expense of neurons both in vitro and in vivo, and mice lacking the EGF receptor exhibit abnormal astrocyte maturation. Astrocyte differentiation is dependent on IL-6 and leukemia inhibitory factor receptor function, such that leukemia inhibitory factor receptor–deficient mice exhibit a significant reduction in the number of GFAP+ cells in the brain. Lastly, SHH has been implicated in the maintenance of neural progenitor cells and in the differentiation of oligodendroglial and astroglial cells.

It should not be surprising that some of the cancer-associated changes important for astrocytoma formation involve the same genes important for astroglial cell differentiation during development (see Fig 4). In this regard, mutations in these genes would release the normal brakes on cell proliferation and the terminally differentiated state and facilitate the acquisition of a less differentiated and more proliferative cellular phenotype. Activating mutations in the EGF receptor are commonly seen in high-grade astrocytoma, and mice engineered to express a mutationally activated EGF receptor in combination with other genetic changes develop astrocytoma. Similarly, astrocytomas of many grades exhibit increased PDGF and PDGF receptor expression. PDGF has been shown to dedifferentiate cultured astrocytes in vitro and result in oligodendroglioma formation in vivo. Using a glioblastoma transgenic mouse model, IL-6 was found to be required for tumor formation. Lastly, altered expression of members of the SHH pathway have been reported in astrocytomas and some of its downstream transcriptional targets have been implicated in astrocytoma formation.

A number of these regulators of brain and astrocytoma development are also potential targets for therapeutic drug design. Inhibition of EGF and PDGF receptors by specific small-molecule tyrosine kinase inhibitors are currently being investigated in clinical trials. Similarly, monoclonal antibody therapy against EGF receptor is in preclinical evaluation for the treatment of glioma. It is likely that additional tar-
Finding the Cell of Origin
Despite intense scientific investigation, it is fair to say that the origins of human brain tumors remain unresolved. Moreover, several characteristics of human tumors may make it difficult to resolve this issue definitively. First, because human tumors can be studied only once they have already developed, the cell of origin can merely be inferred retrospectively from markers expressed in its progeny. In this regard, that a tumor cell expresses a marker of a particular lineage does not necessarily mean that the tumor arose from cells of that lineage. Second, because tumor cells undergo significant molecular changes as a result of transformation, they may express markers that are not expressed by their normal counterparts during development. Finally, the heterogeneity of human tumors and the discrepancy among the histopathological criteria used to classify tumors further complicates studies of the cell of origin for human brain tumors.

However, these limitations and obstacles do not mean that searching for the cell of origin for brain tumors is futile. A powerful alternative to studying the cell of origin in human tumors involves the use of genetically or virally engineered animal models. Mouse models based on a specific genetic mutation have a number of significant advantages. First, they are less genetically heterogeneous than human tumors, making it easier to draw conclusions about the cell of origin for any particular tumor. Second, using retroviral gene delivery or transgenic technology, genetic alterations can be introduced into specific subpopulations of normal cells, and the resulting animals can be used to prospectively test hypotheses about the cell of origin. Finally, mouse tumors can be studied at both early and late stages, so that progressive molecular and phenotypic changes in the cell of origin can be tracked as they happen, instead of being inferred from the end stages of the disease. A number of such studies have already been performed, and these have important implications for our understanding of the origins of brain tumors.

Studies in genetically engineered mice have provided important insights into the cellular origins of MB. In this regard, conditional knock-out methods (Cre-Lox technology) have been used to assess the role of the retinoblastoma (Rb) and p53 tumor suppressor genes in neoplastic transformation. Mice expressing the Cre recombinase under the control of the GFAP promoter were mated with mice in which the Rb and p53 genes were flanked by LoxP sites, so that Rb or p53 would be deleted in GFAP+ cells. Mice lacking both Rb and p53 expression in GFAP+ cells experienced development of MBs. In this model, Rb and p53 inactivation occurred in astrocytes, but also in a population of neuronal precursors in the EGL. In a complementary approach, the RCAS-TVA viral transduction system has been used to target oncogenes to neural progenitors in the cerebellum. Nestin-TVA transgenic mice, which express the avian retrovirus receptor (TVA) in nestin+ cells, were infected as neonates with avian retroviruses encoding SHH. In this model, MB formation was observed in 9 to 15% of mice, suggesting that SHH-induced MB can arise from nestin+ progenitors in the postnatal cerebellum.

Similar to the approaches described for MB, mouse modeling studies have shown that specific oncogenic changes can also result in astrocytoma formation when introduced into either GFAP+ or nestin+ cells in vivo. In addition, astrocytomas form in mice explanted with either neural stem cells or astrocytes engineered to harbor glioma-associated genetic changes. However, there is a tendency for nestin+ cells to be more sensitive than GFAP+ cells to these glioma-associated genetic changes.

The use of animal models offers a powerful new approach to studying tumor origins, and in the long run, it may lead to definitive conclusions regarding the cell of origin. However, developing appropriate models depends on at least two critical types of information: (1) the identification of specific genes with deregulation that is sufficient to cause tumors in mice, and (2) the identification of cell type–specific promoters that can be used to drive expression of these genes in the appropriate cell types. The list of genes that have been shown to be mutated or misexpressed in human MB and astrocytoma is growing rapidly, but most have not yet been shown to cause tumors in mice. Similarly, a number of transgenes and mutations have been demonstrated to cause MB and astrocytoma in mice, but their contribution to the development of human brain tumors remains unknown. Further study of the key molecular switches that govern astrocyte and neuronal differentiation is likely to yield important insights into the specific genes and growth regulatory pathways deregulated in MB and astrocytoma.

Similarly, we have currently identified only a small number of cell-specific promoters for the in vivo interrogation of the cell of origin of MB and astrocytoma. For astrocytoma and MB, most genetically engineered mouse modeling studies have used the nestin or GFAP promoters, the interpretation of which is complicated by uncertainty regarding the identity of the cells in the...
developing brain that express GFAP or nestin. In this regard, GFAP expression has been reported in both astrocytes and neural stem cells, whereas nestin expression has been observed in GCPs, neural stem cells, and radial glia. Future studies will be required to identify and use more specific genetic markers for astrocytes, neural stem cells, and GCPs that would enable the generation of additional genetically engineered mouse models to evaluate the contribution of these cell types to the origin of brain tumors. Progress in these areas will undoubtedly lead to new animal models for MB and astrocytoma and to a clearer definition of the cell of origin of these tumors.

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We apologize to those investigators whose work we did not cite due to space limitations.

References


Meeting Report

Report from the Fifth National Cancer Institute Mouse Models of Human Cancers Consortium Nervous System Tumors Workshop

David H. Gutmann, Charles D. Stiles, Scott W. Lowe, Gideon E. Bollag, Frank B. Furnari, and Al Charest

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Cancers of the nervous system are clinically challenging tumors that present with varied histopathologies and genetic etiologies. While the prognosis for the most malignant of these tumors is essentially unchanged despite decades of basic and translational science research, the past few years have witnessed the identification of numerous targetable molecular alterations in these cancers. With the advent of advanced genomic sequencing methodologies and the development of accurate small-animal models of these nervous system cancers, we are now ideally positioned to develop personalized therapies that target the unique cellular and molecular changes that define their formation and drive their continued growth. Recently, the National Cancer Institute convened a workshop to advance our understanding of nervous system cancer mouse models and to inform clinical trials by reconsidering these neoplasms as complex biological systems characterized by heterogeneity at all levels.

Keywords: brain tumor, genetically engineered mice, glioma, medulloblastoma, xenograft.

Tumors of the nervous system comprise a heterogeneous group of neoplasms that vary in location, age at onset, histologic features, tendency for progression and migration, and response to therapy.\(^1\) In this regard, these tumors exhibit a wide spectrum of histologic subtypes reflecting their potential cell of origin, causative molecular changes, local microenvironments, and clinical behavior (Table 1). Recent studies have underscored this heterogeneity, even within a histologically defined tumor subtype, demonstrating that histologically similar tumors represent several distinct molecular subtypes,\(^2\)–\(^4\) each with a unique pattern of deregulated growth control pathways.\(^5\) Similarly, other CNS tumors (e.g., medulloblastoma, ependymoma) harbor distinct gene expression patterns that suggest that this molecular heterogeneity may be harnessed to develop more individualized therapies for these deadly cancers.\(^6\)–\(^8\)

To begin to address this issue of heterogeneity, the National Cancer Institute convened the fifth Mouse Models of Human Cancers Consortium (MMHCC) Nervous System Tumors Workshop, held in Montreal, Canada, on November 18, 2010 (Table 2). The workshop was divided into four topics, each moderated by an expert in the field. The meeting opened with presentations on the identification and characterization of the cell of origin of brain tumors in different mouse models, followed by talks that focused on the role of the microenvironment.
in tumor initiation and growth. The meeting concluded with sessions on genomics and systems biology as well as the use of mouse models for therapeutic target discovery and evaluation.

### Table 1. Diversity of Nervous System Cancers

<table>
<thead>
<tr>
<th>Tumor type&lt;sup&gt;a&lt;/sup&gt;</th>
<th>WHO grade&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Age&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Location</th>
<th>Genetic Alterations&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrocytic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pilocytic astrocytoma</td>
<td>I</td>
<td>0–20</td>
<td>Optic nerve, hypothalamus, thalamus, basal ganglia</td>
<td>NF1 loss</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>II</td>
<td>30–40</td>
<td>Frontal and temporal lobes, brain stem, spinal cord</td>
<td>p53 loss, PDGFRA, IDH1/2 mut (R132H)</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>III</td>
<td>30–60</td>
<td>Cerebral hemispheres</td>
<td>p53, Rb, Cdkn2a, PTEN loss, CDK4 amp</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>IV</td>
<td>45–70</td>
<td>Subcortical white matter of cerebral hemispheres</td>
<td>PDGFRA and EGFR mut/overexpression, IDH1/2 mut, Cdkn2a, PTEN, NF1 loss</td>
</tr>
<tr>
<td>Oligodendroglial</td>
<td>II–III</td>
<td>40–60</td>
<td>Cortex and white matter of cerebral hemispheres</td>
<td>LOH chr 1p, 19q, EGRF, PDGFRA +ligands overexpression, loss of CDK2a</td>
</tr>
<tr>
<td>Oligoastrocytic</td>
<td>II–III</td>
<td>35–45</td>
<td>Cerebral hemispheres</td>
<td>LOH chr 1p, 19q, loss of p53</td>
</tr>
<tr>
<td>Ependymal</td>
<td>I–III</td>
<td>0–16 and 30–40</td>
<td>Along the ventricular system and spinal canal</td>
<td>NF2 loss</td>
</tr>
<tr>
<td>Embryonal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>IV</td>
<td>0–20</td>
<td>Cerebellum</td>
<td>c-myc amp, p53, pthc loss</td>
</tr>
<tr>
<td>PNET</td>
<td>IV</td>
<td>0–10</td>
<td>Supratentorial</td>
<td>n-myc amp, p53 loss</td>
</tr>
<tr>
<td>Cranial/peripheral nerves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schwannoma</td>
<td>I</td>
<td>0–60</td>
<td>Peripheral nerves of head and neck region</td>
<td>NF2 loss</td>
</tr>
<tr>
<td>MPNST</td>
<td>II–IV</td>
<td>30–60</td>
<td>Large and medium nerves</td>
<td>NF1 and p53 loss</td>
</tr>
</tbody>
</table>

Abbreviations: PDGFRA, platelet-derived growth factor receptor; PNET, primitive neuroectodermal tumor; CDK4, cyclin-dependent kinase 4; IDH1, isocitrate dehydrogenase 1; LOH, loss of heterozygosity.

Based on World Health Organization (WHO) Classification of Tumours; *Pathology and Genetics of Tumours of the Nervous System*, P. Kleihues and W. Cavenee, eds.

WHO grading system, from benign (grades I–II) to malignant (grade III–IV).

Peak incidence range.

Most common alterations listed.

### Table 2. MMHCC Nervous System Tumors Workshop participants

<table>
<thead>
<tr>
<th>Participant</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suzanne Baker, Ph.D.</td>
<td>St. Jude Children’s Research Hospital, Memphis, TN</td>
</tr>
<tr>
<td>Michael Berens, Ph.D.</td>
<td>The Translational Genomics Research Institute, Phoenix, AZ</td>
</tr>
<tr>
<td>Gideon Bollag, Ph.D.</td>
<td>Plexxikon Inc., Berkeley, CA</td>
</tr>
<tr>
<td>Al Charest, Ph.D.</td>
<td>Tufts University School of Medicine, Boston, MA</td>
</tr>
<tr>
<td>Charles Eberhart, M.D., Ph.D.</td>
<td>Johns Hopkins University, Baltimore, MD</td>
</tr>
<tr>
<td>Frank Furnari, Ph.D.</td>
<td>Ludwig Institute, University of California–San Diego, La Jolla, CA</td>
</tr>
<tr>
<td>Marco Giovannini, M.D., Ph.D.</td>
<td>House Ear Institute, Los Angeles, CA</td>
</tr>
<tr>
<td>David Gutmann, M.D., Ph.D.</td>
<td>Washington University School of Medicine, St. Louis, MS</td>
</tr>
<tr>
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<tr>
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<tr>
<td>David Largaespada, Ph.D.</td>
<td>University of Minnesota, Minneapolis, MN</td>
</tr>
<tr>
<td>Scott Lowe, Ph.D.</td>
<td>Cold Spring Harbor Laboratories, Cold Spring Harbor, NY</td>
</tr>
<tr>
<td>Silvia Marino, M.D.</td>
<td>Barts and The London School of Medicine and Dentistry, London, England</td>
</tr>
<tr>
<td>John Ohifest, Ph.D.</td>
<td>University of Minnesota, Minneapolis, MN</td>
</tr>
<tr>
<td>Karlyne Reilly, Ph.D.</td>
<td>NCI, Frederick, MD</td>
</tr>
<tr>
<td>Joshua Rubin, M.D., Ph.D.</td>
<td>Washington University School of Medicine, St. Louis, MS</td>
</tr>
<tr>
<td>Jann Sarkaria, M.D.</td>
<td>Mayo Clinic, Rochester, MN</td>
</tr>
<tr>
<td>Charles Stiles, Ph.D.</td>
<td>Dana Farber Cancer Institute, Boston, MA</td>
</tr>
<tr>
<td>Rob Wechsler-Reya, Ph.D.</td>
<td>Sanford-Burnham Medical Research Institute</td>
</tr>
<tr>
<td>William Weiss, M.D., Ph.D.</td>
<td>University of California–San Francisco, San Francisco, CA</td>
</tr>
</tbody>
</table>

**Modeling Nervous System Tumors in Mice**

Brain tumor models in mice are being used to study many aspects of tumor biology and in preclinical settings
to evaluate potential treatment modalities. In general, small-animal models can be divided into two basic categories: (1) those that implant tumor cells into recipient mice (xenograft) and (2) those that induce tumors in mice de novo (genetically engineered mouse [GEM] models) (Fig. 1). The first generation of nervous system tumor xenograft models employed tumor cell lines that had been maintained under artificial cell culture conditions for extended periods of time (often decades). Typically, the tumors generated from these cell lines fail to accurately reproduce the classical histopathologic appearances of their human counterparts, display no molecular resemblance to the original human tumor, and, more importantly, are not predictive of drug response in preclinical trials.

Over the past several years, a number of laboratories have developed orthotopic xenograft models using primary nervous system tumor cells from freshly isolated human brain tumors. These tumor models recapitulate certain features of the human tumors, including their invasive behaviors and tissue architecture. More recently, the isolation of brain tumor-initiating cell populations from dissociated patient tumors using cell surface markers has refined our understanding of glioblastoma multiforme (GBM) heterogeneity with respect to renewal and tumor-initiating capacities. Dr David James (University of California, San Francisco) provided a nice overview of the human glioma xenografts generated in his laboratory and outlined their use for preclinical therapeutic studies. Similarly, Dr Jann Sarkaria (Mayo Clinic) demonstrated...
that these human high-grade glioma xenografts retain most of the seminal genetic alterations observed in the original patient tumors, which were remarkably stable over time. While these brain tumor models have the advantage of deriving from actual human tumors, they are grown in mice lacking a functional immune system or a relevant microenvironment.

In contrast, GEM models are designed to induce brain tumors using relevant cancer-causing genetic changes in the context of an intact immune system and nervous system microenvironment. Their use for functionally validating the role of specific genetic changes to tumor formation and progression has been particularly instructive, and they have revealed important roles for local and genomic environments in tumorigenesis and continued tumor growth. In preceding studies in human tumors, accurate small-animal nervous system GEM strains have been used to test the efficacy of novel drugs and compounds in preclinical settings.

**Cell of Origin and Developmental Neurobiology**

The session on the interface between neuro-oncology and developmental neurobiology was moderated by Dr Charles Stiles (Dana Farber Cancer Institute) and focused on the various methodologies employed to identify the cell types that give rise to various brain tumors. A recurrent theme in this session was the concept of the varying degrees of permissiveness of stem and progenitor cells to specific cancer-causing genetic alterations. In this regard, tumorigenesis in the nervous system is dependent on a combination of specific cancer-associated genetic mutations occurring in receptive cell types during permissive periods of nervous system development.

Dr Rob Wechsler-Reya (Sanford-Burnham Medical Research Institute) presented data from mouse medulloblastoma modeling experiments suggesting that more personalized brain tumor treatments may come from a more complete understanding of the interplay between genetic mutations and the specific stem and progenitor cells in which these mutations occur. In these studies, he employed a combination of human xenograft and GEM models to re-create various genetic subtypes of medulloblastoma. He showed that forced expression of c-myc in cerebellar stem cells is mitogenic and results in transient hyperplasia, while simultaneous expression of c-myc and mutant p53 results in aggressive tumors that resemble human large-cell anaplastic medulloblastoma. Further characterization of these tumors indicated that they were molecularly distinct from those driven by 

\[ P_{ch} \]

mutation and exhibited different responses to therapy.

Dr Silvia Marino (Barts and The London School of Medicine and Dentistry) demonstrated that loss of \( p\text{53} \) and \( R\text{b} \) in two different populations of progenitor cells—cerebellar granule cell progenitors and cerebellar stem cells of ventricular zone (VZ) origin—gave rise to medulloblastomas in mouse models. In these studies, conditional inactivation of \( R\text{b} \) and \( p\text{53} \) was obtained in these cells either in vivo, through granule cell progenitors, or in vitro followed by orthotopic transplantation, through VZ-derived stem cells of nongranule cell lineage. Both populations gave rise to medulloblastomas with identical histopathologic appearances; however, tumors originating from VZ progenitors preferentially expressed stem cell markers. This set of markers was shown to identify a subset of human medulloblastomas associated with a poorer clinical outcome.

Dr William Weiss (University of California, San Francisco) presented studies that focused on identifying the cell of origin in two glioma models. First, using advanced labeling techniques, he demonstrated that in an astrocytoma GEM model (GFAP-HaRas) developed by Dr Abhijit Guha, gliomas arise from SVZ-derived stem cells, whereas in the S100-vErb oligodendroglioma model tumors originated from white matter NG2+ glial progenitor cells. He further demonstrated that NG2+, but not CD133+, cells isolated from human oligodendroglioma tumors were capable of forming tumors following implantation into immunocompromised mice. Collectively, these data support a model in which gliomas may develop from stem cells, whereas oligodendrogliomas derive from NG2+ progenitor cells.

Dr Charles Eberhart (Johns Hopkins University) described the differences between various Notch isoforms in inducing glioma formation in the optic nerve and retina. In his studies, he showed that while Notch3 robustly induced optic nerve gliomas, tumors were not generated following expression of activated Notch1 or Notch2. These experiments clearly demonstrate differences in the susceptibility of tissues for oncogenic transformation by the Notch gene family. Using chimeric Notch constructs, the oncogenic portion of the Notch3 gene was found to reside in the carboxyl terminal domain of the protein.

Dr Marco Giovannini (House Ear Institute) described a new mouse model of schwannomatosis. In human schwannomatosis, \( N\text{F2} \) mutations are common; however, mutations in the \( \text{IN11} \) gene are observed in 30% of familial and 7% of sporadic cases. Whereas targeted deletion of \( \text{In11} \) in mice is lethal, Schwann cell precursors with conditional \( \text{In11} \) inactivation resulted in olfactory nerve, third cranial nerve, and trigeminal nerve tumors. Current studies are focused on developing mice with combined \( \text{In11} \) and \( \text{Nf2} \) inactivation in Schwann cell precursors.

**Stromal Influences on Tumorigenesis**

As has been reported for other cancers, it is becoming increasingly clear that the local microenvironment plays a critical role in brain tumor development and growth. This session was moderated by Dr Frank Furnari (Ludwig Institute, University of California, San Diego) and focused on the use of GEM strains to elucidate the complex relationship between neoplastic and nonneoplastic cells in the tumor microenvironment.

In particular, two presentations employed the inherited cancer predisposition syndrome, neurofibromatosis
type 1 (NF1), to demonstrate that specific cell types and signals from the tumor microenvironment are important for gliomagenesis and continued glioma growth. The use of NF1 as a model system to study nervous system tumor-stroma interactions derives from studies first published by Dr Luis Parada (Southwestern University), in which targeted loss of NF1 in Schwann cell precursors is insufficient for tumorigenesis unless coupled with heterozygosity for an inactivating NF1 gene mutation in nonneoplastic cells.[16] These initial observations have been extended to glia[17,18] and used to identify specific growth factors and cytokines that drive tumorigenesis and continued glioma growth.[19,20]

Dr David Gutmann (Washington University School of Medicine) described the critical role that microglia play in NF1 optic glioma growth. Using a combination of approaches, he demonstrated that pharmacologic and genetic microglia silencing inhibits optic glioma growth. Moreover, he described studies in which optic nerve microglia are uniquely sensitive to the effects of NF1 heterozygosity during early glioma formation, leading to studies aimed at disrupting the interactions between microglia and preneoplastic/neoplastic cells during critical phases of gliomagenesis.

Dr Joshua Rubin (Washington University School of Medicine) next reported on his discovery of one key chemokine expressed in the nonneoplastic optic glioma microenvironment. He showed that CXCL12 (stroma-derived factor-1α) normally induces astrocyte apoptosis, whereas in NF1−/− astrocytes, CXCL12 treatment leads to inappropriate astrocyte survival in vitro. This reduced apoptosis reflects decreased intracellular cyclic adenosine monophosphate (cAMP) production, prompting Dr Rubin to explore the possibility that ectopic suppression of cAMP in regions of the brains of NF1 optic glioma might induce glioma formation. Indeed, cAMP reduction resulting from viral expression of phosphodiesterase-4–induced gliomas in the forebrain of these mice. Collectively, these studies highlight the critical interdependent relationship between neoplastic cells and signals from their nonneoplastic neighbors relevant to gliomagenesis and glioma maintenance.

Genomics and Systems Biology

With the recent explosion of comprehensive genomic studies on brain tumors, it is becoming increasingly clear that one has to view individual genetic mutations in the context of a global network. In the session chaired by Dr Scott Lowe (Cold Spring Harbor Laboratories), presentations focused on the various combinations of genetic mutations required for nervous system tumorigenesis.

Dr Suzanne Baker (St Jude Children’s Research Hospital) presented data demonstrating the profound differences of Pten gene inactivation on gliomagenesis. Whereas postnatal, adult Pten ablating in astrocytes had no effect, combined deletion with other tumor suppressors induced astrocytomas with high penetrance. Co-deletion of Pten and Rb failed to induce astrocytomas, but co-deletion of Pten and p53, p53 and Rb, or Pten, p53, and Rb all induced astrocytomas. Secondary mutations within the phosphoinositide-3 kinase and retinoblastoma signaling pathways were found in tumors that were induced by inactivation of tumor suppressors in the same pathways. Tumors formed within and outside of proliferative niches in adult brain.

Dr David Largaespada (University of Minnesota) described work on the use of the sleeping beauty (SB) transposon system for mutagenesis screens in mice conditionally deleted for Pten and p53. Using this approach, he was able to generate cerebellar tumors with different complements of genetic alterations. For example, one of the genes inactivated by SB in this genetic screen was Slit3, which his laboratory demonstrated was also inactivated by mutation or promoter methylation in human medulloblastoma.

Dr Karlyne Reilly (NCI Frederick) presented her work on the identification of genetic modifiers of NF1:p53-Gis–driven malignant peripheral nerve sheath tumor (MPNST). In these studies, she leveraged the differential susceptibility to MPNST in A/J compared with C57Bl/6J mice. One candidate gene was found to be an imprinted gene. Using a targeting strategy, she discovered that this modifier gene acts in a tumor suppressive manner when inherited from the mother. These findings support a model in which the severity of MPNSTs depends on whether maternal or paternal copies of chromosomes are altered during tumorigenesis.

Therapeutic Targets

In the final session of the meeting, approaches to discovering and exploiting therapeutic targets were discussed. This session was moderated by Dr Gideon Bollag (Plexxikon) and emphasized the complexities associated with performing preclinical trials in mice and the adaptability of tumors to therapeutic interventions.

Dr Al Charest (Tufts University School of Medicine) presented work using a GBM model driven by wild-type epidermal growth factor receptor (EGFR). Drawing upon the observation that human GBMs overexpressing wild-type EGFR also express EGFR ligands, he described a model by which somatic expression of EGFR and of transforming growth factor-α (an EGFR ligand) in the context of loss of cdkn2a and/or Pten tumor suppressor gene function yields tumors with molecular and histopathologic features of “classical” GBM tumors. He also described differences in the sensitivity of cdkn2a-null and cdkn2a;Pten-null tumor cells to EGFR inhibitors. His laboratory found that this difference arises from tumor cells switching their dependence for mitogenic signaling from one receptor tyrosine kinase to another. These data illustrate one molecular mechanism for the primary resistance of GBMs to EGFR tyrosine kinase inhibition.
Dr Michael Berens (The Translational Genomics Research Institute) described the Ivy Genomics-based Medicine Project, which is a 9-institute preclinical study relating chemovulnerability to molecular profiling in human primary GBM orthotopic xenografts. Funded by the Ben and Catherine Ivy Foundation, the update reported survival outcomes of 21 GBM models tested with 4 treatment regimens; genomic data (expression profiling, array comparative genomic hybridization, cytosine-phosphate-guanine methylation, and selected DNA sequencing of the models) are being produced. Engagement of pharmaceutical companies to provide a larger spectrum of targeted therapeutic agents remains in motion. Using extensive genomic profiling of 40 GBM xenograft lines, one initial objective was to establish a proband set of xenograft tumors with genomic signatures that represent the spectrum of patients with GBM as portrayed in The Cancer Genome Atlas. A follow-up study proposes to use the molecular profiling data to inform treatment planning (clinical trial) by matching the therapeutic responses of the various xenograft lines to their genetic signatures and aligning these against patient tumor signatures.

Dr Eric Holland (Memorial Sloan Kettering) described a procedure to generate a recurrent model of GBM using the replication-competent ALV splice acceptor (RCAS) virus/Tva model system. Tumor-bearing animals were given fractionated ionizing radiation or temozolomide therapy. Gene expression profiling was performed before and after treatment to identify genetic signatures most predictive of recurrence-free survival. In parallel, Dr Holland also presented data on the
relative sensitivity of the various cell types within the GBM tumor to therapeutic intervention in vivo. Using differential cell purification methods, he was able to identify specific genes within the Olig2+ population of cells that might mediate resistance to radiation.

Dr John Ohlfest (University of Minnesota) described the importance of the multidrug resistance system in the treatment of brain cancer. There are two dominant mechanisms responsible for poor blood-brain barrier (BBB) penetration of certain molecularly targeted drugs: the efflux systems coded for by the Bcrp and Pgp genes. Ohlfest advocated that although the BBB in the tumor core is leaky, allowing for systemic drug delivery, the tumor-infiltrated normal brain (the site of recurrence) has an intact BBB, which prevents drug delivery. Using a combination of knockout mice and specific pharmacologic inhibitors, he demonstrated that BCRP and PGP cooperate by synergistically effluxing gefitinib, sorafenib, and dasatinib from the brain. However, in the case of sorafenib, Bcrp was dominant, while for gefitinib and dasatinib, Pgp was dominant. Using a mouse glioma model based on SB-delivered oncogenes, he discovered that loss of function of both the Bcrp and Pgp genes more than doubled survival after treatment with dasatinib relative to wild-type mice. In addition, western blot data revealed that dasatinib markedly inhibited phosphorylation of Src only in the Bcrp Pgp compound knockout mice. Collectively, these results suggest that optimal penetration of these drugs into tumor-infiltrated normal brain where the BBB is intact is dependent on Bcrp and Pgp function, such that administration of single inhibitors of Pgp or Bcrp would have minimal clinical advantage over chemotherapeutic agent alone. More importantly, these data stress the need to consider penetration of molecularly targeted agents in the tumor-infiltrated normal brain where the BBB is intact.

Summary—Leveraging Heterogeneity

One of the common themes of this meeting was heterogeneity. Heterogeneity affects nervous system tumor formation and treatment in many ways (Fig. 2). First, tumor susceptibility is influenced by genomic heterogeneity, such that both tumor formation and response to therapy are dictated in part by modifier genes in our individual genomes. Subtle polymorphisms in specific genes may change the local microenvironment, expression of specific tumor suppressor genes, or drug metabolizing enzymes. Second, progenitor cells and stem cells in distinct regions of the brain and during different times of development are unique, and may be differentially affected by cancer-causing genetic changes. For example, Nf1 inactivation in astrocytes or neural stem cells from the cortex has little effect on astrocyte proliferation or astrogliogenesis in vitro and in vivo, whereas Nf1 loss in brainstem neural stem cells or astrocytes results in increased proliferation and gliomagenesis. Third, the local microenvironment harbors specialized cells and signals capable of initiating and maintaining tumors in the nervous system. Fourth, the signaling pathways and transcriptional factor networks are highly adaptable and dynamic. In considering future therapies for brain tumors, we will need to employ a systems-based approach that integrates this heterogeneity at all levels to effect a more personalized treatment for these deadly cancers.

This workshop also provided a glimpse into future directions for glioma model research. Several laboratories are focusing on expanding the complexities of their models to better mimic human tumors for preclinical studies. As such, a great deal of resources and efforts are invested in studying therapeutic responses in models that are genetically designed to mirror patients’ tumors. In addition, more sophisticated studies on basic mechanisms of gliomagenesis are arising. Specifically, the concept of permissibility and resistance of glia and neuro stem vs non stem cell to oncogenic assault will no doubt reveal basic themes for CNS cancers. In the coming years, we will witness an unprecedented level of sophistication in these models and their use that will translate into major advances for both clinical and basic research.

Acknowledgments

We thank Dr Cheryl Marks, Janice Embry, Cynthia Graddy, and Jan Esenwein for their assistance in organizing this workshop.

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References


Traditionally, cancer studies have primarily focused on mutations that activate growth or survival pathways in susceptible pre-neoplastic/neoplastic cells. However, recent research has revealed a critical role for non-neoplastic cells within the tumor microenvironment in the process of cancer formation and progression. In addition, the existence of regional and developmental variations in susceptible cell types and supportive microenvironments support a model of tumorigenesis in which the dynamic symbiotic relationship between neoplastic and non-neoplastic cell types dictate where and when cancers form and grow. In this review, we highlight advances in neurofibromatosis type 1 (NF1) genetically engineered mouse brain tumor (glioma) modeling to reveal how cellular and molecular heterogeneity in both the pre-neoplastic/neoplastic and non-neoplastic cellular compartments contribute to gliomagenesis and glioma growth. *Oncogene* (2011) 30, 1135–1146; doi:10.1038/onc.2010.519; published online 15 November 2010

Keywords: glioma; astrocytoma; neurofibromatosis type 1; NF1; stroma; microenvironment

Introduction

Based on seminal studies in colon cancer, tumor development is often thought to result entirely from an accumulation of acquired genetic changes that allow a cell to escape the constraints that normally control cell proliferation, death and migration. These constraints are typically provided by the local microenvironment (stroma) in the form of growth factors, chemokines and extracellular matrix (ECM) molecules. The presence of each type of stromal molecule instructs the cell to divide, differentiate, die or migrate, and thus regulates the orderly behavior of that cell within its natural tissue environment. However, traditional models of tumorigenesis have largely focused on identifying cancer-causing genetic changes present in the neoplastic cells without considering the local microenvironment. In these models, tumor formation is envisioned as a stochastic series of events that allow neoplastic cells, through a process of natural selection, to predominate and culminate in cancer. Although these models have been incredibly instructive, they fail to account for the spatial and temporal patterns of tumor formation, and do not fully explain several unique features of pediatric brain tumors.

In this review, we will explore brain tumor formation as a developmental abnormality involving interactions between neoplastic and non-neoplastic cells, in which molecular alterations in non-neoplastic cells not only affect tumor proliferation and maintenance, but also influence the propensity for tumor initiation and formation through the co-evolution of a permissive microenvironment. Both before and during tumor development, non-neoplastic and neoplastic species change the cellular and molecular composition of their local milieu by recruiting new cell types, activating existing cell types and modifying the regional expression profile of specific molecules (for example, growth factors, cytokines and ECM proteins). The cumulative effect of these changes over time creates a permissive microenvironment that provides the necessary substrate for the expansion of pre-neoplastic and neoplastic cells during the process of oncogenesis and continued tumor growth.

Traditional models of oncogenesis

The multistep model of tumorigenesis was initially proposed by Vogelstein and colleagues for colorectal cancer, based on the identification of a series of genetic mutations arising in colorectal cancers at various stages of malignant progression (Fearon and Vogelstein, 1990). The observation that some genetic changes occurred at the earliest stages of oncogenesis (epithelial hyperplasia) whereas others were only detected in more malignant cancers suggested a model in which the orderly acquisition of cancer-causing genetic changes explained the progression from the pre-neoplastic lesion (polyp) to advanced metastatic colon cancer (Figure 1). However, it is more likely that it is the complement of genetic changes, rather than the chronological order of acquisition, that drives the process of tumorigenesis and malignant progression.

Weinberg and colleagues experimentally defined a series of six hallmark changes necessary for the progression from cancer initiation to tissue invasion and metastasis. These include growth signal self-sufficiency, antigrowth signal insensitivity, apoptosis evasion, limitless replicative potential, angiogenesis, and tissue invasion and metastasis (Hanahan and Weinberg, 2000).
In this regard, that these hallmark properties reflect escape from the susceptible pre-neoplastic cell, it should be recognized (mutational loss of the Deleted in Colorectal Carcinomas intracellular proliferative or survival signal. Similarly, molecule obviates the need for these extracellular kinases. The presence of a constitutively active H-Ras ways that normally would be governed by extracellular activation of pro-proliferative and antiapoptotic pathways, respectively, and resulted in oncogenic transformation (Hahn et al., 1999). Similarly, this collection of genetic alterations was also shown to be sufficient for the transformation of normal astrocytes to malignant astrocytomas (Sonoda et al., 2001).

Although these changes were introduced into a susceptible pre-neoplastic cell, it should be recognized that these hallmark properties reflect escape from the normal constraints provided by the local environment. In this regard, HRAS mutation results in constitutive activation of pro-proliferative and antiapoptotic pathways that normally would be governed by extracellular ligands binding to their cognate receptor tyrosine kinases. The presence of a constitutively active H-Ras molecule obviates the need for these extracellular growth/survival factors by providing an equivalent intracellular proliferative or survival signal. Similarly, mutational loss of the Deleted in Colorectal Carcinomas (DCC) gene during colorectal cancer transformation is thought to reduce normal cell–cell and cell–ECM adhesions (Fearon and Vogelstein, 1990), whereas TP53 mutation results in loss of p53-mediated cell cycle arrest and apoptosis (Baker et al., 1989; Vousden, 2002). These tumor suppressor gene inactivation events provide escape from the growth and survival constraints normally provided by the local microenvironment.

**Incorporating the tumor microenvironment**

The concept that oncogenic changes in a pre-neoplastic cell type are necessary, but not completely sufficient, for tumorigenesis is highlighted by the differential patterns of metastatic colonization. For example, B16 melanoma cells injected into mice only form tumors in pulmonary or ovarian tissue, but not in renal tissue (Hart and Fidler, 1980). Furthermore, human breast cancer cell lines injected into the mammary glands of naïve mice widely disseminate, but metastatic tumors only develop in the lungs and lymph nodes, even though dormant cells could be recovered from organs without metastasis (Suzuki et al., 2006). These findings raise the intriguing possibility that determinants beyond the neoplastic cell are critical for progression.

The microenvironment of a solid tumor is composed of numerous non-neoplastic cell types, such as fibroblasts, infiltrating and resident immune cells, and recruited macrophages and mast cells. These cell types, although not tumorigenic, have the capacity to produce growth/survival factors, chemokines, ECM and angiogenic molecules that change the local milieu in which these pre-neoplastic/neoplastic cells live. The specific composition of this microenvironment further varies depending on the developmental age, tissue type and stage of malignant progression (Egeblad et al., 2005; Coussens and Werb, 2010; Rozario and DeSimone, 2010).

Some of the first studies to demonstrate the influence of the microenvironment on tumorigenesis examined the relationship between fibroblasts and epithelial cells during wound healing (Gabbiani et al., 1972, 1978) and breast cancer progression (Tremblay, 1979). Cancer-associated fibroblasts, or myofibroblasts, exhibit embryonic-like migratory properties (Schor et al., 1988) and stimulate prostate tumor progression (Olumi et al., 1999) as well as support the initiation and progression of breast cancer (Barcellos-Hoff and Ravani, 2000). Matrix metalloproteinases secreted from these cancer-associated fibroblasts change the ECM and alter the signal transduction responses of epithelial cells to growth factors, thus forming a reactive stroma that enhances tumorigenesis (Lee and Streuli, 1999; Sternlicht et al., 1999). One of the key signaling molecules produced by cancer-associated fibroblasts is transforming growth factor-β (TGF-β): TGF-β activates fibroblasts to increase ECM formation (Keski-Oja et al., 1988) and promotes epithelial cell and fibroblast proliferation, depending upon the complement of growth factors present in the local microenvironment (Roberts et al., 1985). Collectively, these studies support a model of tumorigenesis in which TGF-β signaling creates a permissive stromal state for epithelial cancer initiation and progression (Bhowmick et al., 2004; Cheng et al., 2005).

Other stromal components, including immune system cells, also can participate in the process of tumor formation. In the initial stages of tumor formation, recruited immune cells may suppress tumor formation through ‘immunoediting’ (Dighe et al., 1994; Dunn et al., 2002), which represents a process of cancer cell elimination by immune system cells. Following this initial period of elimination, some tumor cells will acquire the ability to escape immune recognition and exist in a state of dynamic equilibrium with the immune system...
system (Shankaran et al., 2001). Eventually, these tumor cells will proliferate and expand.

Similar to fibroblasts, mast cells and macrophages can also be recruited to the tumor by chemotactic factors (Dabbous et al., 1986; Graves et al., 1989). Recruited mast cells activate fibroblast collagen synthesis and induce angiogenesis through the secretion of the mast cell serine proteases monocyte chemoattractant protein-4 and monocyte chemoattractant protein-6 (Coussens et al., 1999). Mast cells can also increase ECM degradation in rodent mammary carcinomas, thereby facilitating tissue invasion and metastasis (Dabbous et al., 1986). Similarly, recruited macrophages can also degrade elastin, glycoproteins and collagen to promote invasion through secreted proteinases (Werb et al., 1980; Coussens et al., 2000) and regulate angiogenesis and metastasis through production of proangiogenic factors (Lin et al., 2006; Pollard, 2008).

The brain as a specialized ecosystem

Similar to tumors in other organs, brain tumors are composed of an evolving ecological community composed of non-neoplastic and pre-neoplastic/neoplastic cells. However, the normal brain is a specialized niche that lacks fibroblasts and is essentially lymphocyte free, both of which are key players in the microenvironment of other solid cancers. The environment of the brain consists of blood vessels, ECM, microglia, pericytes, oligodendrocytes, progenitor cells, astrocytes and neurons. As the normal brain develops and matures, these cell types and the signals they elaborate vary over time and in different brain regions.

The spatial and temporal pattern of brain tumor formation is illustrated by the age of onset and location of glial cell malignancies (Louis et al., 2007). In children, gliomas predominate in infratentorial locations, including the optic pathway, brainstem and cerebellum, whereas in adults, gliomas are more commonly found in supratentorial regions, such as the cerebral hemispheres. Even within the brainstem, gliomas tend to form in the pons (Ueoka et al., 2009). Moreover, the histological grade of gliomas differs in children compared with adults. World Health Organization grade I pilocytic astrocytomas are more frequent in children, whereas higher grade malignancies are found in adults. Interestingly, pilocytic astrocytomas rarely progress to high-grade glial cancers compared with the progressive nature of adult grade II gliomas. These unique patterns of gliomagenesis could reflect differences in susceptible pre-neoplastic cell types, causative oncogenic mutations and/or unique features of the microenvironment in these different brain locations.

Insights into the critical interplay between pre-neoplastic/neoplastic cells and non-neoplastic cell types in the evolving tumor microenvironment have derived from studies of inherited cancer syndromes. Individuals with these cancer pre-disposition syndromes are born with one mutated copy of a tumor suppressor gene in all cells throughout their bodies. For example, in familial retinoblastoma (RB), patients are heterozygous for an inactivating mutation in the retinoblastoma tumor suppressor gene. RB tumors develop following bi-allelic inactivation of the RB gene (Cavenee et al., 1983; Dryja et al., 1984). As children born with a germline RB mutation require only one additional genetic alteration, the loss of the one remaining functional allele, the incidence of RB tumor formation is much higher and occurs at an earlier age than observed in the general population, where both RB alleles must be inactivated in the same cell for tumors to develop (Knudson, 1971).

Neurofibromatosis-1 as a model system

The most common of the inherited tumor pre-disposition syndromes in which affected children develop brain tumors (gliomas) is neurofibromatosis type 1 (NF1). This disorder is characterized by the presence of optic pathway gliomas, neurofibromas (peripheral nerve sheath tumors), as well as pigmentary changes, such as café-au-lait macules, skinfold freckling, and Lisch nodules (Gutmann et al., 1997). There are several features of NF1-associated glioma formation and growth that merit further discussion: First, while the vast majority of patients with NF1 form peripheral nerve sheath tumors (Huson et al., 1988), only 15–20% of children with NF1 develop brain tumors. Second, gliomas predominate in the optic pathway as opposed to the cerebellum, where they more commonly arise in the general population (Listernick et al., 1995). Third, NF1-associated optic gliomas arise at a younger age (mean = 4.5–5.8 years) than in the general population (mean = 5.1–12 years) (Stern et al., 1980; Listernick et al., 1989, 1994; Singhal et al., 2002; Thiagalingam et al., 2004). Fourth, unlike their sporadic counterparts, NF1-associated optic gliomas are less likely to progress and require treatment (Listernick et al., 1997). This unique spatial and temporal pattern of gliomagenesis suggests that additional factors, including susceptible cell types, permissive microenvironments and genomic modifiers, may dictate when and where gliomas form in individuals with NF1.

Recapitulating NF1-associated brain tumors in NF1 genetically engineered mice

Genetically engineered mouse (GEM) models are powerful tools to study the molecular mechanisms and cellular origins of brain tumors. In genetic pre-disposition models, GEM provides unprecedented opportunities to define the developmental changes in critical cell types throughout the natural history of tumor initiation, proliferation and progression. In this regard, mice engineered to lack Nf1 gene expression in glial fibrillary acidic protein-positive glial progenitor cells did not develop brain tumors (astrocytomas or gliomas) in vivo, although there was increased growth in Nf1-deficient
astrocytes (Bajenaru et al., 2002). To more accurately model the NF1 human condition, mice heterozygous for an inactivating Nf1 mutation (Nf1+/−) in every cell in their bodies were designed to also lack Nf1 gene expression (Nf1−/−) in glial progenitors (Bajenaru et al., 2003; Zhu et al., 2005). These mice developed low-grade optic gliomas, recapitulating the predominance of optic pathway gliomas seen in NF1 patients (Bajenaru et al., 2005). The fact that a brain microenvironment composed of Nf1−/− cells is required for gliomagenesis and that the combination of Nf1 loss in glial progenitors and stromal Nf1 heterozygosity results in gliomas restricted to the optic pathway supports the use of Nf1 GEM strains as tractable models to illustrate the complex interplay between pre-neoplastic cells, non-neoplastic cells and genomics in gliomagenesis.

**Susceptible cell type**

As described previously, the preferential spatial pattern of NF1-associated gliomagenesis raises the possibility that glial cell types in different brain regions may be differentially responsive to NF1 gene inactivation. Evidence for this diversity derives from studies that demonstrate unique molecular signatures from glial tumors, as well as normal astrocytes and progenitor cells arising from different brain regions (Taylor et al., 2005; Sharma et al., 2007). One impact of this molecular diversity is variation in the expression levels of specific tumor suppressor genes. For example, Nf1 mRNA and protein expression was significantly reduced in astrocytes from the neocortex compared with astrocytes from the optic nerve, cerebellum, or brainstem, such that Nf1 inactivation in the neocortical astrocytes did not result in increased proliferation (Yeh et al., 2009). In addition, similar brain region-specific effects of Nf1 inactivation have recently been reported for neural stem cells (Lee et al., 2010). Neural stem cells from the brainstem, but not the cortex, exhibit increased proliferation and glial cell differentiation following Nf1 inactivation in vitro and in vivo. Collectively, these findings support the notion that not all neural stem cell or glial populations will proliferate in response to Nf1 inactivation, and that cellular heterogeneity may in part contribute to the spatial pattern of gliomagenesis in this inherited cancer syndrome.

An additional level of heterogeneity is conferred by the differential effects of Nf1 protein, neurofibromin, on downstream growth control pathways (Figure 2). Sequence analysis of the predicted Nf1 protein sequence revealed that it contains a small domain with striking similarity to the catalytic segment of a family of proteins termed GTPase activating proteins. Neurofibromin was subsequently shown to be a Ras-GTPase activating protein, accelerating the conversion of active GTP-bound Ras to inactive GDP-bound Ras (Ballester et al., 1990), such that loss of neurofibromin expression results in increased Ras activity and increased Ras-driven cell proliferation (Basu et al., 1992; DeClue et al., 1992).

However, neurofibromin may not negatively regulate all Ras isoforms in every cell type (Walsh and Bar-Sagi, 2001; Ehrhardt et al., 2004). For instance, only the K-Ras isoform is activated in Nf1-deficient astrocytes despite equal expression of all three Ras isoforms (Dasgupta et al., 2005a). Similar findings have also been reported for other Nf1-deficient cell types (Khalaf et al., 2007; Morgan et al., 2007). This heterogeneity will need to be considered when selecting effective drugs for NF1-associated cancer treatment. This is well illustrated by the poor clinical response of NF1-associated peripheral nerve sheath tumors to farnesyltransferase inhibitors that inhibit Ras (Widemann et al., 2006), as these drugs preferentially inhibit H-Ras, rather than K-Ras (Prendergast and Rane, 2001).

Moreover, the Ras downstream signaling pathways that transduce this proliferative message vary between cell types. Proliferation or survival is mediated through Ras/MAPK signaling in Nf1-deficient or heterozygous mast cells (Khalaf et al., 2007; McDaniel et al., 2008) and vascular smooth muscles (Li et al., 2006; Xu et al., 2007). Multiple pathways contribute to increased osteoclast activity and gain of function (Yang et al., 2007).
2006; Yan et al., 2008; Li et al., 2009) as well as abnormal myeloid cell survival and proliferation (Bollag et al., 1996; Donovan et al., 2002). However, \textit{Nf1}-deficient cell growth regulation is dependent on Ras activation of the mammalian target of rapamycin (mTOR) pathway in astrocytes (Dasgupta et al., 2005b; Sandmark et al., 2007) and Schwann cells (Johannessen et al., 2005; Johansson et al., 2008). In astrocytes, neurofibromin loss leads to mTOR-dependent increases in cell proliferation, which reflect mTOR regulation of Rac1 (Sandmark et al., 2007) and STAT3 (Banerjee et al., 2010). These differences have profound implications with respect to therapeutic drug design and support the use of rapamycin, an immunosuppressant drug, to specifically inhibit the mTOR pathway in \textit{Nf1}-deficient gliomas (Hegedus et al., 2008) and Schwann cell malignancies (Johansson et al., 2008), and MEK inhibitors for other NF1-associated tumor types.

**Tumor microenvironment**

Although the heterogeneity of cell types in different brain regions partly accounts for the patterns of gliomagenesis seen in NF1 patients, additional spatial and temporal signals from the tumor microenvironment are likely required to fully explain the unique features of glioma development and growth in NF1 (Figure 3). Studies using \textit{Nf1} GEM strains have been particularly instructive for identifying specific stromal cell types and molecules that provide the optimal substrate for pre-neoplastic cell proliferation and gliomagenesis.

Non-neoplastic stromal cells function in part to provide a permissive microenvironment for tumorigenesis through the release of factors that expand the pre-neoplastic/neoplastic cell populations (gliomagens) as well as promote the formation of a local milieu rich in other non-neoplastic cell types (stromagens). This is facilitated by both pre-neoplastic/neoplastic cells and non-neoplastic cells that can produce stromagens to attract other stromal cell types, such as chemokine recruitment of microglia or endothelial cells to the region of a developing tumor. These recruited stromal cells elaborate molecules that promote pre-neoplastic/glial cell proliferation, survival and invasion. Thus, during tumor evolution, there is a dynamic relationship established between neoplastic and non-neoplastic cells through the elaboration of stromagens and gliomagens that together facilitate tumor formation and subsequently promote tumor maintenance and progression.

**Stromal determinants of glioma formation and growth**

Microglia are one of the key cell types in the tumor surround that provide a permissive environment for tumorigenesis, as they can produce both gliomagens and stromagens. Tumor-associated microglia constitute the main population of brain immune cells (Graeber et al., 2002) and are important for monitoring their local environment, regulating function and apoptosis, and secreting proinflammatory cytokines (Banati et al., 1993; Elkabes et al., 1996; Marin-Teva et al., 2004; Roumier et al., 2004). These resident brain immune system mononuclear cells can be recruited by glioma tumors cells to the local tumor microenvironment by vascular

![Figure 3 Co-evolution of neoplastic and non-neoplastic cells in gliomas. Loss of heterozygosity (grey to red) of critical tumor suppressor genes, such as the \textit{NF1} gene, in susceptible astroglial cell types is an initial step in gliomagenesis. These \textit{NF1}-deficient astrocytes release factors (stromagens) to recruit or activate microglia as well as other stromal cell types (for example, endothelial cells and non-neoplastic astrocytes) to create a supportive microenvironment. Stromal cells elaborate gliomagens that promote the growth of these pre-neoplastic/neoplastic astroglia. A dynamic relationship forms between the pre-neoplastic/neoplastic and non-neoplastic stromal cell populations through stromagen and gliomagens release, which together facilitate tumorigenesis, tumor maintenance and glioma progression.](image-url)
endothelial growth factor (Forstreuter et al., 2002; Kerber et al., 2008), hepatocyte growth factor (Budie et al., 1999; Kunkel et al., 2001), monocyte chemoattractant protein-1 (Martinet et al., 1992; Leung et al., 1997) and the chemokine CX3CL1 (fractalkine) through activation of the CX3CR1 receptor (Held-Feindt et al., 2010). Monocyte chemoattractant protein-1 can also increase the surface expression of CX3CR1 on microglia (Green et al., 2006), thus providing amplification circuits for further microglia recruitment. Once present in the tumor microenvironment, microglia can secrete inflammatory molecules such as interleukin-10 and interleukin-6 (Wagner et al., 1999; Williams et al., 2000). Elevated levels of these two interleukins are correlated with the degree of malignancy in gliomas (Huetttner et al., 1995; Sasaki et al., 2001) as well as increased proliferation and migration of glial cell lines (Huetttner et al., 1997; Wang et al., 2009). In particular, interleukin-6 ablation prevents tumor formation in a v-src-induced model of gliomagenesis (Weissenberger et al., 2004).

Moreover, it should be appreciated that microglia, like astrocytes, may have different properties depending on their local microenvironment. Cerebral microglial expression of CD40 is lower than in cerebellar microglia, and CXCR3, a chemokine that regulates cell migration, is higher in cerebral microglia than in those from the hippocampus (de Haas et al., 2008). These observations suggest that different brain regions harbor unique populations of microglia that can differentially contribute to the spatial and temporal patterns of glioma formation and growth.

Using an Nfl optic glioma GEM model, increased numbers of microglia were found along the optic nerve before histologically and radiographically obvious glioma formation (Bajenaru et al., 2005). The microglia in these pre-neoplastic optic nerves are Nfl+/− resident brain microglia, with increased proliferation, motility and activation compared with their wild-type counterparts (Gutmann and Daginakatte, 2007; Daginakatte et al., 2008). The importance of microglia to mouse optic glioma proliferation is underscored by two experimental findings: First, microglia inactivation using the minocycline antibiotic results in reduced optic glioma proliferation (Gutmann and Daginakatte, 2007). Second, Nfl+/− microglia have increased activation of the c-Jun kinase signaling pathway, whereas Nfl−/− deficient astroglial cells have no c-Jun kinase hyperactivation (Daginakatte et al., 2008). Inhibition of c-Jun kinase activation results in reduced Nfl+/− microglia proliferation and motility in vitro, such that inhibition of Nfl+/− microglia function with crude c-Jun kinase inhibitors attenuates optic glioma proliferation in vitro.

Determining how Nfl−/− brain microglia promote Nfl−/− deficient glial cell proliferation required the identification of the relevant gliomagens (Gutmann and Daginakatte, 2007). Two complementary approaches have been applied to date. First, using a microarray strategy, Nfl+/− microglia were found to express increased levels of the hyaluronidase-like protein, meningioma-expressed antigen-5 (MGEA5). Cultured media from Nfl+/− microglia increased the proliferation of Nfl−/− astrocytes in vitro, which could be inhibited using crude hyaluronidase inhibitors (Gutmann and Daginakatte, 2007). The ability of MGEA5 to promote Nfl−/− deficient astrocyte proliferation reflected MAPK activation, a pathway not previously found to drive Nfl−/− glial cell growth. Although this pathway may not be a major mitogenic signaling pathway in glial cells, it is possible that MAPK activation cooperates with other stromal signals to increase Nfl−/− astrocyte growth.

A second approach to identifying Nfl−/− microglia gliomagens involves the examination of known molecules that regulate signaling pathways controlled by neurofibromin. Previous studies from our laboratory and others have shown that neurofibromin positively regulates cyclic adenosine monophosphate (cAMP) levels (Tong et al., 2002; Dasgupta et al., 2003) in the brain in addition to negatively regulating Ras activity. The regulation of both Ras and cAMP is one of the properties of G-protein-coupled receptors, which suggests that chemokines present in the tumor microenvironment might increase Nfl−/− astrocyte growth. One of these chemokines, CXCL12, is developmentally and spatially regulated, such that high levels of CXCL12 is found in young mammals along the optic pathway (Warrington et al., 2007). Moreover, Nfl+/− microglia express three times more CXCL12 than their wild-type counterparts.

CXCL12 acts on its receptor, CXCR4, to regulate both cAMP levels and Ras activity (Klein and Rubin, 2004), and CXCL12 treatment of wild-type astrocytes causes increased cell death through apoptosis (Warrington et al., 2007). In striking contrast, CXCL12 treatment of Nfl−/− astrocytes resulted in increased cell survival. The major pro-survival effect of CXCL12 on Nfl−/− deficient astrocytes is mediated by cAMP. In this regard, restoration of wild-type levels of cAMP by several methods attenuates Nfl−/− astrocyte survival. This observation prompted a preclinical study to determine whether elevating cAMP in Nfl optic glioma mice would reduce glioma growth. Although the effects of Rolipram, which blocks phosphodiesterase-4-mediated cAMP degradation, were transient, treatment resulted in attenuated glioma growth (Warrington et al., 2010).

The finding that CXCL12 expression is one of the key stromal determinants directing where gliomas form in NF1 raises the intriguing possibility that the pattern of tumorigenesis could be changed by ectopically expressing CXCL12 in regions of the brain where gliomas do not form in NF1 GEM strains. However, ectopic lentiviral CXCL12 expression in the forebrain of NF1 optic glioma mice did not induce gliomas with high penetrance (Sun et al., 2010), arguing against CXCL12 as the only spatial determinant important for NF1-associated gliomagenesis. In contrast, ectopic expression of phosphodiesterase-4 to lower cAMP levels in the forebrain of NF1 optic glioma mice did lead to glioma formation (Warrington et al., 2010). Interestingly, there are significant variations in the levels of cAMP in different brain regions, with high levels of cAMP in the forebrain compared with the optic nerve (Warrington
Collectively, these findings support the hypothesis that a combination of Nf1+/− stromal cell types, permissive cell types and basal signaling levels contribute to the pattern of gliomagenesis in NF1.

The tumor microenvironment impact on non-neoplastic cells

In addition to the effects of the NFI+/− microenvironment on gliomagenesis, additional studies using Nf1 GEM strains highlight the impact of NFI heterozygosity on the response of non-neoplastic cells to tumor formation. Although most children with NF1-associated optic gliomas will not require treatment for progressive visual loss, nearly 50% of children with NF1 and an optic glioma initially present with visual impairment (Listernick et al., 1995). Using evoked potentials to measure visual function in Nf1 optic glioma mice, reduced potentials were detected early during tumor evolution, suggesting that disrupted relationships between neurons and their associated glial cells may result in abnormal neuronal transmission (Hegedus et al., 2009). To this end, before the development of a radiographically and histologically evident optic glioma, progressive increases in optic nerve axon calibers were found, followed by axonal swelling and apoptosis in the retinal ganglion cell layer. Further examination revealed that Nf1+/− retinal ganglion cells have shorter neurites, growth cones and survival in vitro, such that axon injury or optic glioma formation leads to increased neuronal death (Brown et al., 2010). Interestingly, unlike peripheral nervous system neurons in which Nf1 loss results in inappropriate cell survival as a consequence of Ras–Akt pathway hyperactivation, reduced neurofibromin expression in central nervous system neurons has the opposite effect that instead results from reduced neurofibromin-mediated cAMP generation. Particularly relevant to future neuroprotective strategies, elevating cAMP using phosphodiesterase-4 inhibitors results in attenuated retinal ganglion neuron death in response to glioma formation in vivo (Brown et al., 2010).

Importance of the genomic background

One of the unresolved issues in NF1 gliomagenesis revolves around the incomplete penetrance of this tumor phenotype. In contrast to the peripheral nerve sheath tumors (neurofibromas), only 15–20% of children with NF1 develop optic gliomas. This clinical observation suggests that gliomagenesis requires more than a permissive environment and susceptible progenitor cells. As nearly 100% of Nf1 GEM develop optic gliomas when maintained on a C57BL/6 genetic background, and rare mice lacking optic gliomas are almost invariably born with a white coat color, it is highly likely that tumorigenesis is influenced by genomic determinants.

Support for a role for genomic modifiers in NF1-associated gliomagenesis derives from elegant studies by Reilly and associates using a different Nf1 GEM model. In this system, astrocytomas formed with high frequency in NPCIs (Nf1+/−;Trp53+/−) mice when the genetic background was C57BL/6J, but only rarely in mice with the 129S4/SvJae background (Reilly et al., 2000, 2004). This observation suggests that there may be epigenetic or polymorphic differences between these strains that could confer additional resistance or susceptibility to gliomagenesis (Hawes et al., 2007; Dong et al., 2008).

The existence of modifier loci creates two interesting and clinically relevant opportunities. First, modifier loci could function by changing the expression or function of genes important for glial cell biology or neurofibromin growth regulation. In this way, it is possible that subtle polymorphic changes in genes that encode proteins of the Ras or cAMP regulatory pathways could increase or decrease the effect of neurofibromin loss on glial cell growth. Similarly, polymorphisms that alter chemokine function or microglia activity could likewise attenuate or amplify the impact of NFI heterozygosity on susceptible cell types in the tumor microenvironment. Finally, it is possible that strain-dependent changes lead to differences in overall neurofibromin expression, which alter the effect of NFI heterozygosity on non-neoplastic cell function (Pemov et al., 2010).

Second, the finding of strain differences in glioma susceptibility suggests that genomic polymorphisms might determine which children with NF1 will develop optic gliomas. The identification of single nucleotide polymorphisms predictive of glioma risk would have tremendous impact on the management of children with NF1, and would allow clinicians to stratify children from an early age into clinically relevant subgroups for surveillance and potential treatments.

In addition, there could be other etiologies beyond our current understanding of neurofibromin growth control pathways, microenvironmental determinants, brain region-specific cellular heterogeneity and genomic modifiers that influence how oncogenesis occurs in patients with NF1. Identifying these factors may provide future targets for treatment that are not obvious at this time.

Conclusion

Recent advances in mouse brain tumor modeling support the notion that these cancers represent neurodevelopmental abnormalities. As would be expected, the rules that govern proliferation, apoptosis, differentiation and migration of cells during the process of normal brain formation and maintenance apply to tumors arising in the brain. Cell types and signals that are normally tightly regulated can become de-regulated and co-opted during tumorigenesis, such that these instructive cues become inappropriately active in response to specific genetic mutations.

Although Nf1 inactivation in glial lineage cells is a necessary step in oncogenesis, it must occur in a cell type capable of expanding in response to loss of neurofibromin growth regulation. This capacity is dictated not only by brain location, but also by the
developmental stage. Elegant studies by a number of groups have demonstrated a more limited capacity for accelerated growth following Nf1 inactivation in differentiated astrocytes compared with glial progenitor cells (Zhu et al., 2005; Alcantara Laguno et al., 2009). Together, these observations establish a regional and developmental context in which bi-allelic Nf1 inactivation will lead to glioma formation.

In addition to Nf1 loss in a susceptible cell type, environmental factors are critical determinants of gliomagenesis. These include supportive cell types, such as microglia, reactive astrocytes and endothelial cells. Although the role of microglia in Nf1 glioma formation and growth has gained traction, there are fewer data currently available on the important roles that reactive astrocytes and endothelial cells have. Microglia are known in other pathological conditions to increase endothelial cell migration and proliferation as well as to stimulate reactive gliosis. In this manner, microglia-induced neoangiogenesis might create a supportive niche for cancer stem cells, as has been reported for high-grade gliomas (Ludwig et al., 2000; Ren et al., 2004; Calabrese et al., 2007; Charles et al., 2010), and account for the highly vascular nature of these otherwise relatively benign tumors. Although it is not known what impact Nf1 heterozygosity has on brain endothelial cell function, Nf1+/− aortic endothelial cells exhibit increased motility and proliferation and likely participate in establishing a permissive environment for peripheral nerve sheath tumors (Munchhof et al., 2006). Similarly, reactive gliosis resulting from abnormal Nf1+/− microglia function might further facilitate the formation of a local microenvironment rich in growth/survival-promoting factors important for glioma formation and maintenance (Giordano et al., 1996; Amankulor et al., 2009).

It is also worth noting several additional features of the Nf1+/− microenvironment. First, it is defined by the presence of cell types and signals unique to a specific region of the brain during a given time of development. In this respect, glial Nf1 loss in the cerebellum occurs in a completely different stromal context than it does in the optic chiasm or brainstem. The gliomagens that facilitate glioma formation and growth as well as the stromagens that promote the establishment of a permissive local environment in one brain region are not equivalent to those present in another brain region. Similarly, the stromagens and gliomagens found in a region at one developmental stage may not be identical to those present later in life. Second, the impact of Nf1 heterozygosity on the evolving tumor microenvironment is unlikely to be the same in all brain locations and at all developmental periods. As different cells in the brain express different molecules in a spatially and temporally regulated fashion, Nf1 heterozygosity may have unique effects on the local brain microenvironment that reflect these regional and developmental conditions. Third, the tumor microenvironment is a dynamic ecosystem, such that the elaboration of specific molecules changes both the cellular and molecular soil in which Nf1-deficient pre-neoplastic/neoplastic cells grow. The panoply of growth/survival factors present at any given time is constantly evolving in response to recruited and modified cell types that, in turn, alter the local environment both spatially and temporally. This state of flux creates a delicately balanced ecological niche as well as a moving target for therapy (Figure 4).

Another level of system complexity results from the influence of the genomic environment. Although not completely elucidated, genetic modifiers likely change the expression of key stromal growth/survival factors or the activity of specific kinases and enzymes. Although these minor alterations by themselves are not sufficient to result in tumor formation, the confluence of these subtle changes in the correct brain region, at the correct time, and in response to specific cancer-initiating genetic changes could significantly influence gliomagenesis and glioma maintenance.

Finally, as we move into an era of personalized medicine, it will become increasingly important to consider developing therapies that specifically target the neoplastic and non-neoplastic cells in the tumor and to conceptualize tumors as evolving ecosystems. Using this approach, we may be able to design treatments that ultimately result in durable clinical responses. Similarly, should genomic polymorphisms identify at-risk patient subpopulations, the ability to disrupt tumorigenesis by impeding neoplastic/non-neoplastic cell interactions during cancer evolution may be possible. Collectively, the emerging data that derives from the study of Nf1 GEM strains may one day inform both chemoprevention...
and chemotherapy management of cancers of the nervous system.

Conflict of interest

The authors declare no conflict of interest.

References


Conflict of interest

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References


Mathematical Modeling of PDGF-Driven Glioblastoma Reveals Optimized Radiation Dosing Schedules

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SUMMARY

Glioblastomas (GBMs) are the most common and malignant primary brain tumors and are aggressively treated with surgery, chemotherapy, and radiotherapy. Despite this treatment, recurrence is inevitable and survival has improved minimally over the last 50 years. Recent studies have suggested that GBMs exhibit both heterogeneity and instability of differentiation states and varying sensitivities of these states to radiation. Here, we employed an iterative combined theoretical and experimental strategy that takes into account tumor cellular heterogeneity and dynamically acquired radioresistance to predict the effectiveness of different radiation schedules. Using this model, we identified two delivery schedules predicted to significantly improve efficacy by taking advantage of the dynamic instability of radioresistance. These schedules led to superior survival in mice. Our interdisciplinary approach may also be applicable to other human cancer types treated with radiotherapy and, hence, may lay the foundation for significantly increasing the effectiveness of a mainstay of oncologic therapy.

INTRODUCTION

Patients suffering from glioblastoma (GBM), the most common and malignant primary brain tumor, have very poor survival. The standard of care is surgery when possible followed by radiation (Figure 1A) and chemotherapy (Stupp et al., 2005). This regime has seen little change over the past 50 years, as has the overall survival for this disease. Radiation is used in adjuvant therapy globally and provides a significant increase in the survival of GBM patients (Walker et al., 1980). Dose escalation studies demonstrated that survival improvements are observed up to an overall dose of 60 Gy (Walker et al., 1979). Beyond this point, there are little, if any, improvements in survival at the cost of increased toxicity (Bleehen and Stenning, 1991; Chan et al., 2002; Morris and Kimple, 2009). Typically, the dosing schedule is 2 Gy per day, 5 days per week, for 6 weeks. Several alternative schedules have been attempted, such as hypofractionated dosing of 3–6 Gy per session, hyperfractionated dosing of 1 Gy fractions two to three times per day, and accelerated dosing using multiple 2 Gy fractions a day to shorten the overall treatment time (Laperriere et al., 2002). None of these strategies, however, have resulted in consistent improvements in tumor control or survival and are thus not routinely used in the clinic.

Three recent advances provide insights into GBM biology that may impact therapy. First is the realization that GBM falls into several molecular subgroups that appear to be dominated by specific signaling pathways (Brennan et al., 2009; Phillips et al., 2006; Verhaak et al., 2010). These subgroups include proneural GBM that is related to abnormal platelet-derived growth factor (PDGF) signaling, classical GBM with canonical epidermal growth factor receptor (EGFR) amplification, and mesenchymal GBM with common loss of NF1 function. The second advance is the development and use of genetically engineered mouse models of GBM that provide genetically and histologically accurate models of these molecular subtypes of GBM (Hambardzumyan et al., 2011; Huse and Holland, 2009; Sharpless and Depinho, 2006). The third development is a series of work describing a subset of glioma cells that share many characteristics with stem cells (Galli et al., 2004; Ignatova et al., 2002; Singh et al., 2004). These cells are preferentially resistant to radiation and temozolomide and are considered an underlying cause of disease recurrence (Bao et al., 2006; Chen et al., 2012; Liu et al., 2006).
Figure 1. Human and Murine Gliomas Display Similar Recurrence Patterns in Response to Radiation
(A) Representative MRIs showing human and mouse gliomas that are resolved by radiation treatment but then recur.
(B) Representative images and quantification of a radiation dose response assayed in E2f1-Luc glioma-bearing 24 hr after a given radiation dose. Error bars are SD.
(C) Schematic of the mathematical model used to describe the radiation response. The tumor is modeled as two separate cellular components: the stem-like resistant cells (SLRCs) and the differentiated sensitive cells (DSCs). SLRCs can repopulate the tumor, and some DSCs cells, represented by $g$, are able to revert to SLRCs in response to radiation.
(D) Flow-chart summarizing the workflow described in the paper. See also Figure S1.
The PDGF-induced mouse model of GBM accurately mimics the 25%–30% of human GBMs in which aberrant PDGF signaling is present (Breiman et al., 2003; Shih et al., 2004; Verhaak et al., 2010). This model also contains a subpopulation of tumor cells that have similarities to stem cells (Barrett et al., 2012; Bleau et al., 2009; Charles et al., 2010). Stem-like cells are thought to reside in the perivascular niche and are maintained in that state at least partly by nitric oxide (NO) that signals through cyclic guanosine monophosphate, PKG, and NOTCH (Calabrese et al., 2007; Charles et al., 2010; Eylar et al., 2011). Within as little as 2 hr, NO can induce tumor cells to acquire a stem-like phenotype resulting in enhanced neurosphere and tumor formation upon transplantation (Charles et al., 2010). Other niche factors, such as hypoxic conditions, have also been shown to induce stemness (Heddleston et al., 2009; Li et al., 2009). Additionally, recent work has demonstrated that there are multiple tumorigenic cell types within a given tumor and that terminally differentiated astrocytes and neurons can dedifferentiate under oncogenic stress (Chen et al., 2010; Friedmann-Morvinski et al., 2012). These observations suggest that GBMs possess a dynamic heterogeneity of differentiation states that may allow them to rapidly and dynamically acquire a more resistant phenotype.

We hypothesized that mathematical modeling of this dynamic plasticity could be used to enhance radiation therapy. In the past few decades, the vast majority of mathematical modeling of the effects of radiation on cells has been based on the linear quadratic model. This model is widely accepted in the radiation literature due to its close agreement with experimental results for almost all radiation values of clinical interest (Hall and Giaccia, 2012). Several previous studies have specifically investigated the impact of radiotherapy on glioblastoma (Dionysiou et al., 2004; Harpold et al., 2007; Rockne et al., 2008; Stamatakis et al., 2006). These studies range from purely computational experiments to models fitting clinical data and have been utilized in predicting the outcomes of accelerated hyperfractioned schedules. Other recent work has successfully utilized mathematical modeling of cellular in vitro or rat-based in vivo systems to describe glioma behavior (Gao et al., 2013; Massey et al., 2012). Despite the multitude of work that has been done on optimal fractionation schedules, there has been very little success against aggressive gliomas in the clinic (Gupta and Dinshaw, 2005).

Here, we aimed to model a dynamic radiation response with the goal of identifying optimal schedules capable of improving radiation efficacy in a mouse model of PDGF-driven glioma. Our model considers two separate populations of cells: the largely radioresistant stem-like glioma cells and the radiosensitive differentiated glioma cells. We hypothesized that, after exposure to radiation, a fraction of the radiosensitive cells could rapidly revert to the radioresistant state. The inclusion of this dynamic hierarchical population structure and its plasticity induced by exposure to ionizing radiation is a key feature of our framework. Based on this model, we described an optimized schedule that was predicted to prolong survival. Crucially, when tested in a clinically relevant glioma mouse model, this schedule markedly improved survival compared to a standard schedule. The fidelity of the model was improved by adding nonlinear temporal constraints to the acquisition of radioresistant properties based on the time since the previous radiation treatment. This second iteration of the model was able to generate a second optimized schedule that also improved survival in glioma-bearing mice. The mathematical model identifies the fraction of cells capable of acquiring radioresistance and the temporal constraints under which this process occurs as sensitive parameters for predicting radiation response. Specifically, our model predicts that if tumors were unable to rapidly acquire radioresistance, there would be no benefit to any of the optimum schedules. Our data support the functional importance of dynamic radioresistance to therapy and suggests that, at least in PDGF-driven glioma, the standard radiation schedule used may not be optimal. These findings may have broad implications for improving radiation therapy and provide a framework for future optimization of cytotoxic treatment delivery.

RESULTS

Initial Characterization of Radiation Dosing Using an Animal Model for PDGF-Driven GBM

We first performed a dose-response study to determine the effectiveness of various single-fraction doses of radiation (Figure 1B). We generated PDGF-B-induced tumors in Nestin-tv-a;E2f1-Luc mice using the replication-competent ASLV long-terminal repeat (LTR) with a splice acceptor (RCAS)/t-v-a mouse-model system (Uhrbom et al., 2004). These mice express firefly luciferase driven by the E2f1 promoter (E2f1-Luc), allowing for a noninvasive readout of cellular proliferation. This model is similar to human gliomas, in that glioma-bearing mice transiently respond to radiation treatment but ultimately succumb to disease recurrence (Figure 1A). We irradiated glioma-bearing mice with a variety of single doses: 2 Gy (approximately the daily dose used in humans), 4 Gy, 10 Gy, and 15 Gy. Twenty-four hours after irradiation, we found a progressive decrease in E2F1-drive bioluminescence activity with increasing radiation dose that appeared to plateau around 10 Gy (Figure 1B). For this reason, we chose a 10 Gy dose for further investigations.

Mathematical Modeling of GBM Cell Dynamics Predicts Treatment Response

We designed a mathematical model of GBM cell dynamics in response to radiation therapy. The model considers two distinct subpopulations of cells: stem-like/resistant cells (SLRCs) and differentiated/sensitive cells (DSCs) (Figure 1C). SLRCs reproduce symmetrically at rate $r_s$ to give rise to two SLRCs and asymmetrically at rate $a_s$ to produce a SLRC and a DSC. Initially, the ratio of DSCs to SLRCs is given by $R$. Our model incorporates a bidirectional flow of cells between the SLRC and DSC states. In addition to SLRCs converting to a differentiated sensitive state, our model assumes that a fraction of DSCs may be capable of reverting to become SLRCs after exposure to ionizing radiation (Bleau et al., 2009; Charles et al., 2010; Chen et al., 2012; Li et al., 2009; Pistilli et al., 2010). The rate at which DSCs revert to a stem-like state is given by $v$, and the fraction of DSCs that can revert is given by $\gamma$. 

\[
\text{Cell 156, 603–616, January 30, 2014 ©2014 Elsevier Inc. 605}
\]
SLRCs are relatively radioresistant, whereas DSCs respond to radiation therapy via cell-cycle arrest, mitotic cell death, and apoptosis (Bao et al., 2006; Chen et al., 2012; Hambardzumyan et al., 2008). We modeled the cell population response to radiotherapy using the linear quadratic model, which is widely accepted in the radiation literature due to its close agreement with experimental results (Dale, 1985; Fowler, 2010). The basic linear quadratic model states that the fraction of cells that survives a radiation dose of d Gy is given by \( \exp[-\alpha d - \beta d^2] \). The parameters \( \alpha \) and \( \beta \) are specific to the type of tissue that is being irradiated; the parameter \( \alpha \) represents cell killing resulting from a single radiation track causing damage to a specific chromosomal locus, whereas \( \beta \) represents cell killing via two tracks of radiation causing damage at the same locus. Within our mathematical framework, the parameters \( \alpha_s \) and \( \beta_s \) characterize the response of SLRCs to radiation, whereas the parameters \( \alpha_d \) and \( \beta_d \) denote the response of DSCs. In order to simplify the model, we considered the increased radiosensitivity of DSCs to radiation. At the time of diagnosis of the disease, there are \( ND \) cells. When these cells are exposed to the first dose of \( d \) Gy, there occurs a change in their numbers according to the linear quadratic model, producing \( ND_0 e^{-\alpha d - \beta d^2} \) SLRCs and \( ND_0 e^{\alpha_d d - \beta_d d^2} \) DSCs. Additionally, there are \( gh ND_0 e^{-\alpha d - \beta d^2} \) DSCs that are capable of reverting to the SLRC state. Using this description, we can then calculate the number of cells present at time \( t \) after exposure of the cell population to a dose of radiation. The number of DSCs is given by the number of cells that survived radiation and do not have the potential to revert to SLRCs plus any new growth and conversion from SLRCs since treatment; in addition, there are DSCs in the process of reversion. Similarly, the number of SLRCs is given by the population of cells that survived the dose of radiation plus any growth and reversion that has occurred since then:

\[
N_1^t = ND_0 e^{-\alpha d - \beta d^2} \left[ 1 - \gamma e^{\alpha_d (t - L) - \beta_d (t - L)^2} \right]^+ + \gamma ND_0 e^{-\alpha d - \beta d^2} \int_0^t e^{\alpha_d (t - s - M) - \beta_d (t - s - M)^2} ds
\]

where we use the notation \( x^+ = x, x \geq 0, \) and \( x^- = 0, x < 0 \). Further, note that, for the sake of readability, we have assumed that the rates \( \lambda_s, \lambda_d, \) and \( \nu_d \) are sufficiently large so they can be ignored; for the optimization described below, however, these terms were included (values listed in Table 1). For the full model without this assumption, see Equations 7 and 8 in the Supplemental Information available online.

We can use the analytic description above to predict the response of the tumor to any course of radiation therapy.

### Determination of an Optimal Radiation Schedule

To evaluate the response to a given radiation schedule in the context of our mathematical model, we considered the number of tumor cells present 2 weeks after treatment conclusion as an endpoint. To implement the optimization algorithm, an initial set of parameter values was derived from preliminary data (Figure 1B), previous studies (Galbán et al., 2012; Hambardzumyan et al., 2009; Pitter et al., 2011), or estimates (Table 1; Supplemental Information). We then predicted the survival outcomes for 10 Gy either administered as a single dose or in a clinically standard treatment (5 days of 2 Gy), finding that a standard fractionation schedule would perform significantly better than a single dose (Figures 2A and 2D).

We then aimed to identify an optimal fractionation schedule, with the goal of finding those schedules that minimized the number of tumor cells 2 weeks after the treatment conclusion. Mathematically identifying the global optimal schedule was not computationally feasible due to the complexity of our model, as well as the uncertainty of some of the parameters. Because of this, we utilized simulated annealing, a Monte-Carlo-based method (Kirkpatrick et al., 1983; Van Laarhoven and Aarts, 1987), to identify the best treatment strategies (see Supplemental Information; Table 2).

A clinically motivated constraint set for our schedules is presented in the Supplemental Information. With this constraint set and using our initial set of parameters (Table 1), we identified an optimal schedule, “optimum-1,” that was predicted to do significantly better than standard treatment. We also created a control schedule by generating a scrambled sequence with a similarly clustered dosing scheme that was predicted to not perform significantly better than standard treatment (Figures 2A and 2D; Table 2).

### An Optimized Radiation Schedule Significantly Improves Survival in a Mouse Model of PDGF-Driven Glioma

We then returned to the RCAS/tv-a mouse system to test the model’s predictions in a survival assay. We performed survival experiments using PDGF-B-driven gliomas in Nestin-tv-a; Linaka/Arf−/− mice. The genetic background of these mice is similar to human PDGF-driven tumors (Verhaak et al., 2010). As mice developed symptoms of glioma, such as lethargy, weight loss, seizures, etc., they were randomized into either the mock-treated group or one of the various 10 Gy radiation treatment groups, which consisted of a single dose, standard fractionation, optimum-1, and a scramble control (Table 2). The
endpoint of survival was defined as the time point at which the animal had to be sacrificed because of excessive tumor burden: greater than 10% weight loss, lethargy, or seizure. Mock-treated mice quickly succumbed to their disease, with a median overall survival of 5 days after the onset of symptoms (Figure 2B).

Animals in the single-dose and the clinical-standard groups had respective median survivals of 28.5 and 33 days after the onset of symptoms, which was significantly longer than the mock-treated group (p < 0.0001; Figure 2B). Although the median survival of the single-dose-treated animals was shorter than the standard treatment group, there was no significant difference between treatments (hazard ratio [HR] [95% CI] = 1.613 [0.7453–4.863]; p value = 0.1742; Figures 2D and 2E). Mice treated with optimum-1 had a median survival of 50 days (Figure 2B), which was significantly longer than the clinical standard schedule (Figures 2D and 2E; HR [95% CI] = 0.3015 [0.04708–0.3760]; p value = 0.001). Due to the increase in median survival observed with the optimized schedule, we next compared the optimized schedule to 2 weeks of clinical standard therapy; in the latter, mice were treated with 20 Gy, delivered in ten fractions given over 12 days, with a 2-day weekend break. The 20 Gy treatment group had a median survival of 53 days (Figure 2B), which was significantly greater than the 10 Gy clinical standard schedule (Figures 2D and 2E; HR [95% CI] = 0.2084 [0.01295–0.1319]; p < 0.0001), but not significantly different from optimum-1 (Figure 2E, HR [95% CI] = 1.429 [0.6230–3.698]; p value = 0.3907).

**Mathematical Modeling of Other Clinically Relevant Fractionation Schedules Leads to Iterative Updating of the Model**

We then set out to investigate other fractionation schedules that have been clinically tested in GBM. Hyperfractionation schedules consist of a large number of smaller-dose treatments in an attempt to minimize damage to surrounding normal tissue, but according to clinical trials, this approach has not improved overall survival (Coughlin et al., 2000; Laperriere et al., 2002). Hypofractionation schedules involve a larger fraction size with fewer treatments, resulting in a shorter overall treatment time that again yields similar survival to conventional therapy (Laperriere et al., 2002). Surprisingly, our initial model predicted that both hypo- and hyperfractionated schedules would perform significantly differently than standard therapy: the hypofractionated schedule was predicted to perform as well as or slightly better than optimum-1 (Figures 3A and 3G).

We also analyzed two different mathematically predicted schedules: optimum-1 and the scrambled control sequence (Figures 2A and 2D; Table 2). The median survival of mice treated with the scrambled control schedule was 30 days (Figure 2B), which was not significantly different from the standard schedule (HR [95% CI] = 1.613 [0.7453–4.863]; p value = 0.2346; Figures 2D and 2E). Mice treated with optimum-1 had a median survival of 50 days (Figure 2B), which was significantly longer than the clinical standard schedule (Figures 2D and 2E; HR [95% CI] = 0.3015 [0.04708–0.3760]; p value = 0.001). Due to the increase in median survival observed with the optimized schedule, we next compared the optimized schedule to 2 weeks of clinical standard therapy; in the latter, mice were treated with 20 Gy, delivered in ten fractions given over 12 days, with a 2-day weekend break. The 20 Gy treatment group had a median survival of 53 days (Figure 2B), which was significantly greater than the 10 Gy clinical standard schedule (Figures 2D and 2E; HR [95% CI] = 0.2084 [0.01295–0.1319]; p < 0.0001), but not significantly different from optimum-1 (Figure 2E, HR [95% CI] = 1.429 [0.6230–3.698]; p value = 0.3907).

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We tested this prediction for both schedules by overall survival in mice. Mice were randomized as described above into either a hyperfractionated group or hypofractionated group and compared to standard therapy (Table 2). Mice treated with these schedules had a median survival of 37.5 days and 36 days, respectively (Figure 3B). Similar to results observed in human clinical trials, neither of these schedules was significantly different from the clinical standard schedule (Figure 3D; HRhyper [95% CI] = 0.5237 [0.1708–1.167]; p value = 0.1383; HRhyp [95% CI] = 0.3427 [0.1123–1.046]; p value = 0.0599). These results, and the hyperfractionated schedule in particular, highlighted a
weakness in our model that we addressed with a second iteration of the model.

**Time-Dependent Acquisition of Radioresistance Improves the Mathematical Model**

To address the inaccurate predictions of the original model, we iteratively updated our model such that the fraction of cells rapidly acquiring resistance, $g$, now depends on the time elapsed since the previous dose of radiation. Whereas the initial model treated $g$ as a time-independent constant following radiation, the updated model stipulates that $g$ varies over time and that there is a time where a maximum number of cells are prone to reversion in response to subsequent exposure to ionizing radiation. The updated model thus describes the acquisition of resistance with two additional time-dependent parameters: the time of maximal reversion after radiation, $m$, and the width of the window during which reversion can occur after radiation, $s^2$. The model stipulates that, after the first dose of radiation, $\gamma_0$ cells are capable of reversion; for a later dose given $t$ hr after the previous dose of radiation, the fraction of cells capable of reversion is given by $\gamma(t) = \gamma_0 e^{-(t-m)/s^2}$. Other than these additional parameters added to further describe $g$, the updated model is the same as the original model.

To investigate this time-dependent model, we first tested its predictions against volumetric time series data of mice after treatment with 2 weeks of standard therapy (Figure 3E). This comparison allowed us to identify parameter values capable of recapitulating the time-series data. Based on these model parameters, we found a closer concordance between predicted mouse survival times and observed experimental survival times of the optimum-1, hyperfractionated, hypofractionated, and standard schedules (Figures 3F and 3G). In addition to more accurately predicting the survival response, the model also makes significantly different predictions with regard to the
enrichment of the SLRC population after radiation. Both models similarly predict that 1 day after the last dose, optimum-1 will lead to a larger number of SLRCs relative to standard therapy. However, the models offer differing predictions for the hyperfractionated schedule. The original model predicts that the hyperfractionated schedule maximally enriches the SLRC population among all schedules tested (Figure 4A), whereas the time-dependent model predicts that optimum-1 enriches the SLRC population to a greater extent than the hyperfractionated schedule (Figure 4B).

To test the effects of various schedules on the enrichment of SLRCs, we then treated mice with the standard, hyperfractionated, and optimum-1 schedules. Glioma tissue was harvested for side-population (SP) analysis on the sixth day, i.e., 1 day after the last dose of radiation. Stem-like cells are frequently identified from a variety of normal and malignant tissues by flow cytometry as the SP based their ability to efflux Hoechst dye via the ABC transporter, ABCG2 (Greve et al., 2012). Previous work has demonstrated that, in PDGF-driven murine gliomas, SP cells are enriched for canonical cancer stem cell properties, such as stem-marker expression, enhanced tumor-sphere formation, and enhanced tumorigenicity (Bleau et al., 2009). We generated tumors using a previously described RCAS vector that expresses both PDGF-B and enhanced GFP, which results in gliomas with GFP-positive tumor cells (Fomchenko et al., 2011). This system allowed us to limit the SP analysis to bona fide tumor cells (Figure 4C). We observed that tumors treated with the optimized schedule have a 3.55-fold enrichment when compared to standard therapy (p value = 0.0265; Figure 4D). Although optimum-2 was predicted to enrich the SLRCs further than optimum-1, we saw no significant difference in the SP between the two groups (p value = 0.3805). We also tested the optimum-2 schedule using overall survival in mice and observed a significant improvement in survival compared to standard treatment (hazard HR [95% CI] 0.2720 [0.04074–0.2967] ratio; p value < 0.0001; Figure 4E). Optimum-2 was also predicted to have longer survival than optimum-1 (Figure S3). The median survival of the optimum-2 group was longer than that of the optimum-1 group; this difference, however, did not reach statistical significance (Figure 4F; HR [95% CI] = 0.8788 [0.4572–1.689], p value = 0.1768).

To further improve the predictive accuracy of the model, we performed a final iteration by reparameterizing the model using the experimental survival data (Figures 5A and 5B). Performing this calculation led to a further confirmation that the time dependence of \( \gamma \) was essential to the model: fitting the time-dependent model to the survival data led to a smaller minimal mean square error as compared to the original model. The time-dependent model was able to fit the observed data to within an error of 5.2 days, in contrast to the original model, which could only fit the data to an error of 16.32 days. Thus, including time-dependent dedifferentiation increases the model’s ability to match the survival data. We therefore concluded that the time-dependent form of \( \gamma \) is necessary to accurately explain the observed survival data and it is likely that any cell reversion due to ionizing radiation occurs in a time-dependent fashion.

Lastly, we created a simplified version of the model that was more suitable for analysis and interpretation. The simplified model predictions for the tumor cell populations prior to dose \( i+1 \) (assuming \( t \) hours between doses \( i \) and \( i+1 \), and \( t_0 \) hours between doses \( i–1 \) and \( i \)) are given by

\[
\begin{align*}
N_{i+1}^d & = (1 – \gamma(t_b))e^{-(t_0 + t)} + N_i^d e^{-\mu d t} \\
N_{i+1}^s & = N_i^d e^{-\mu d t} + \gamma(t_b)N_i^s e^{-\nu d t}
\end{align*}
\]

According to this simplified form of the model, the optimized therapies optimum-1 and optimum-2 increase survival by converting cells from the fast-growing radiosensitive population to the slow-growing radioresistant population. Notably, sensitivity analysis of the simplified model identifies the parameters that describe reversion as novel sensitivity parameters (Figure 5C; Supplemental Information).

Finally, as a thought experiment, we considered this model in a setting where there is no reversion (\( \gamma(t_b) = 0 \)) and therefore no ability to rapidly acquire radioresistance. Under these conditions, the model reduces to the standard linear quadratic model, which highlights two important observations. First, in this scenario, all fractionation schedules would result in the same ratio of stem-like to differentiated cells (Figure 5D). This finding is in clear contradiction to the observations of our SP analysis (Figure 4D).
Second, if there was no reversion, the model would predict that all fractionation schedules result in the same survival (Figure 5E), which is also contradicted by the observations from mouse survival experiments (Figures 2B and 4E). Taken together, these observations provide significant evidence for the fact that ionizing radiation encourages rapid reversion of a subset of glioma cells to a radioresistant stem-like state.

In sum, our iterative mathematical modeling approach, informed and validated by mouse modeling, allowed us to determine not only a radiation delivery schedule that prolonged survival in mice, but also to identify parameters of the biological processes guiding cellular behavior in gliomas that are responsible for radioresistance. This validated mathematical model can be used, in future work, to investigate the effectiveness of alternative schedules and test their effects on GBM cell populations.

**DISCUSSION**

Standard radiation delivery schemes are based on decades-old data that mostly predate recent findings on cancer stem cells. In GBM patients, many different radiation schedules have been tried in the clinic based on classic radiobiological data, but thus far all have had roughly the same effectiveness. Here, we adopted a combined experimental and theoretical approach with the goal of identifying treatment schedules that would lead to better survival in animal models of the disease by accounting for dynamic transitions of cells between relatively radiosensitive and radioresistant pools. Our approach was based on the assumption that the tumor has a kinetic response to radiation causing some of the surviving cells to acquire resistance by adopting a more stem-like quiescent state over a matter...
of hours. Based on this approach, we successfully identified two treatment schedules that significantly extended survival in glioma-bearing mice, whereas a control schedule failed to do so, as predicted. The fact that optimized schedules clearly outperformed other schedules suggests that the response to radiation is dynamic and that the schedule of a given total dose of radiation can affect its ultimate efficacy.

Although the mathematical model presented here offers complexity, it does not include several potentially important biological factors, such as the immune system, stromal-tumor interactions, nutrient gradients, and others. For example, the work by Stamatakos et al. (2006) developed a sophisticated four-dimensional model for the response of high-grade gliomas to ionizing radiation. Based on their computational model, the authors are able to discuss the effects of cell-cycle time, reoxygenation times, and cell density on tumor response to therapy. Whereas these factors are important, using a simplified model focusing on a single factor, such as dynamic radioresistance, is a powerful way to isolate and better study that phenomenon. Additionally, it has previously been shown that working with a simplified model allows for a more thorough exploration of the mathematics behind the specific parameter, which often uncovers nonobvious predictions (Michor et al., 2005; Norton, 1988). Lastly, simplified models are amenable to more complex mathematical analysis, such as optimization of treatment schedules.

Glioma stem cells are functionally defined by their capacity to self-renew and to generate heterogeneous tumors upon transplantation (Vescovi et al., 2006). As stem-like cells are more therapeutically resistant and ultimately give rise to recurrent disease, it is commonly believed that decreasing the stem-like population will increase overall survival (Cheng et al., 2010; Scopelliti et al., 2009). However, our model predicts an improved overall survival for fractionation schedules that enrich the SLRC population. The side population, which is enriched for quiescent stem cells (Bleau et al., 2009; Deleyrolle et al., 2011; Harris et al., 2008), was elevated in the two optimized schedules that increased overall survival. However, the success of our model is driven by these cells acquiring a quiescent state and slower proliferation rate and therefore is not dependent on a complete dedifferentiation. Further characterization of the ability of radiation to induce other stem-like properties remains an exciting area for future studies.

While eradicating all glioma cells, including the stem-like population, is essential for ultimately curing the disease, our model describes a phenomenon whereby utilizing alternatively fractionated schedules can increase the SLRC population and still result in a slower-growing residual tumor and prolonged time to recurrence. In this regard, our model joins a growing body of evidence suggesting that the relationship between cells with stem-like character and clinical outcomes might not be as straightforward as previously thought. A recent theoretical paper modeling tumor growth kinetics argues that, whereas cancer stem cells are necessary for tumor growth, the kinetics of growth are best described by the nonstem compartment (Morton et al., 2011). Additionally, a recent human GBM study compared the percentage of CD133+ glioma stem cells in patient-matched primary and recurrent samples (Pallini et al., 2011). Patients whose gliomas contained an increased percentage of CD133+ at recurrence demonstrated a significantly longer survival than those with decreased CD133+ cells at recurrence. These studies support our finding that a relative enrichment in the resistant stem-like population might prolong survival by increasing the time to recurrence.

### Translating Optimized Schedules to Human Patients

There are some clear hurdles and open questions in regards to translating our findings from the mouse to the clinic. One measure of predicted toxicity and lethality of different fractionation schedules is given by the biologically effective dose (BED) (Fowler, 2010; Hall and Giaccia, 2012). This measure is frequently used to compare the effectiveness and toxicity of different schedules. It is difficult to use BED to compare the optimized schedules tested here, as the spacing of our doses is inconsistent. However, if we use the common assumption that doses separated by more than 6 hr are independent, then the optimum-1 schedule had one of the lowest BED values of all schedules tested. Because of this, it might even be possible to increase the dosage levels while keeping the toxicity of the schedule at or below the level of the standard therapy. An important avenue for extending these results to the clinical setting will be to consider optimizing fractionation schedules while stipulating that the schedule has an equal or lower BED than that of standard therapy.

Note also that this treatment approach enriches a slow-growing glioma stem cell (GSC) population and therefore would not be curative. However, previous studies have shown that cancer stem cells are dependent on the NOTCH signaling pathway (Androutsellis-Theotokis et al., 2006; Charles et al., 2010; Eyer et al., 2011), and further studies have shown depletion and therapeutic sensitization of GSCs when treated with gamma-secretase inhibitors (Gilbert et al., 2010; Hovinga et al., 2010; Wang et al., 2010). Future studies that combine optimized radiation with therapeutics that specifically target GSCs, via NOTCH or other pathways, might further improve outcomes.

GBM is by definition a heterogeneous disease, and it is unclear how robust an optimized schedule developed for proneural glioma would perform across the various other GBM subtypes. The mouse model used in these studies is driven by PDGF signaling, which is characteristic of approximately 25%–30% of human GBMs. Of note, this mouse model might not reflect the biology of other commonly altered signaling pathways, such as EGFR amplification or NF1 loss, and further studies are needed to determine if the optimization will extend to these tumors. Additionally, even tumors with similar molecular underpinnings are likely to exhibit variability in the parameters used to optimize radiation delivery, such as proliferation rate and the fraction of cells capable of rapidly acquiring resistance. This observation brings up the possibility there may be no universal optimum schedule but rather multiple schedules where optimization for a given patient is dependent on detailed pathologic analysis of each resected tumor.

Additionally, the parameter values we used were determined iteratively based on the mouse model, and it is probable that the schedules presented here will not translate precisely to human tumors. Our investigation was performed for 1 week of therapy delivering a total of 10 Gy of radiation. Human patients receive 60 Gy of radiation over 6 weeks, and optimizing that schedule might not simply be six cycles of the 1 week optimized schedules. Nevertheless, our findings suggest that the gliomas...
Figure 4. Optimized Radiation Schedules Enrich the Glioma Stem Cell Population

(A and B) Graph showing predicted SLRC/DSC ratio for hyperfractionated, optimum-1, and optimum-2 schedules, using the original model (A) or the time-dependent model (B). All values are normalized to predictions for standard therapy. Parameters in (A) and (B) are, respectively, from the Original Parameters and Second Iteration in Table 1.

(C) Representative gating strategy for eGFP+ tumor cell side-population (SP) analysis. The upper panel depicts the gate used to identify GFP-positive cells, based on a GFP-negative sample shown in the insert. The lower panel depicts the gate used to identify the SP, based on a Fumitremorgin C-verapamil-treated control shown in the insert.

(legend continued on next page)
respond dynamically and that the response follows kinetics with a timescale of hours, not weeks. It is therefore imperative to include such considerations into a theoretical framework in order to determine optimum radiation administration schedules for human patients.

Finally, our work studied radiation in isolation, whereas in man, radiation is usually administered after neurosurgical resection and with temozolomide. These treatment modalities need to be incorporated into models aimed at identifying dosing strategies for human patients. Nonetheless, our studies suggest that modeling glioma response to radiation as a dynamic heterogeneous process can predict a treatment schedule that improves overall survival. It also suggests that the schedule that patients are currently receiving may not be optimal.

**EXPERIMENTAL PROCEDURES**

**Generation of Tumors Using RCAS/TVA**

All of the animal experiments were conducted using protocols approved by the Institutional Animal Care and Use Committees of Memorial Sloan-Kettering...
Cancer Center, protocol 00-11-189. Tumors were generated as previously described by injecting RCAS-transfected DF1 cells into n-tva mice (Hambardzumyan et al., 2009). Mice were monitored carefully, and treatment began when they displayed neurological symptoms, such as lethargy or head tilt due to tumor burden, at which point they were irradiated for either bioluminescence (BLI) or survival assays. For BLI, mice were analyzed 24 hr after irradiation due to tumor burden, at which point they were irradiated for either bioluminescence or survival assays. For BLI, mice were analyzed 24 hr after irradiation. For survival, mice were monitored until recurrence of symptoms. The various radiation schedules are described in Table 2; further details on mouse work can be found in the Supplemental Information online.

**MRI Reconstruction and Analysis**

Please see the Supplemental Information online.

**SP Analysis**

Hoechst 33342 staining was performed as previously reported (Bleau et al., 2009). Briefly, glioma-bearing mice were treated with standard, hyperfractionated, optimum-1, or optimum-2 schedules. Twenty-four hours after the last treatment, mice were euthanized and tissue was harvested for SP analysis. bona fide tumor cells were identified based on eGFP+ expression. SP was based on Hoechst dye exclusion, and the data were analyzed by FlowJo. Further details on the SP analysis can be found in the Supplemental Information online.

**Statistics**

Please see the Supplemental Information online.

**Mathematical Modeling**

Please see the Supplemental Information online.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Extended Experimental Procedures, four figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2013.12.029.

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This packet was assembled by the Neuro-Oncology Faculty at Washington University. If you have suggestions for additional reading materials or resources, please e-mail Dr. David H. Gutmann (gutmannd@wustl.edu).
Neuro-Oncology Resident Rotation
Resources

National Cancer Institute
  http://www.cancer.gov/cancertopics/wyntk/brain
  http://www.cancer.gov/cancertopics/treatment/brain
  http://www.clinicaltrials.gov/

CBTRUS
  http://www.cbtrus.org/

Radiation Oncology Treatment Group
  http://www.rtog.org/

Society for Neuro-oncology
  http://www.soc-neuro-onc.org/

Children’s Brain Tumor Foundation
  http://www.cbtf.org/

Pediatric Brain Tumor Foundation
  http://www.pbtfus.org/

Brain Tumor Society
  http://www.tbts.org/

American Brain Tumor Association
  http://www.hope.abta.org

National Brain Tumor Foundation
  http://www.braintumor.org/index.html

Accelerate Brain Cancer Cure
  http://www.abc2.org/