From Orphan to Global: The Yin and Yang of JC virus in the nervous system

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Disclosures: *Igor Koralnik, M.D.*

*Igor Koralnik, M.D.* has financial interests to disclose. Potential conflicts of interest have been resolved.

<table>
<thead>
<tr>
<th>Research Support / Grants</th>
<th>Biogen</th>
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<tbody>
<tr>
<td>Consulting</td>
<td>Johnson and Johnson, Medimmune (Scientific Advisory Board)</td>
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<tr>
<td>Royalty</td>
<td>Up To Date Chapter, Neuro HIV and PML</td>
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<tr>
<td>Progeny</td>
<td>Son @ Wash U</td>
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Yin and Yang

- Opposite or contrary forces: complementary, interconnected and interdependent
- Each aspect contains elements of its opposite
- Dynamic system: whole greater than the sum of its parts
Why engage in Rare Diseases Research?

- >6,800 “orphan diseases” according to 1983 Orphan Drug Act
  - Conditions affecting <200,000 people in US
  - ~7% of population or ~25 million people in US
- Office of Rare Diseases Research (ORDR)
- National Organization for Rare Disorders (NORD)
- World wide:
  - Limited expertise / Limited competition
- Potential for significant impact
  - Diagnosis, management and therapies
- Potential for major discovery and innovation
  - Novel conditions, variants
- Use as model to understand broader principles
Why Gray and White matter...
Myelinated fibers in gray matter

There is white matter in gray matter!
Progressive Multifocal Leukoencephalopathy
PML revisited: has the disease outgrown its name?
Ann Neurol 2006
PML: destruction of CNS white matter
JC virions accumulate in the nuclei of oligodendrocytes
JC virus capsid is formed by pentamers of the VP1 protein
JC virus has a circular double-stranded DNA
JCV is latent in kidney, bone marrow and brain: reactivation in the setting of immunosuppression

Tan JID 2009 and J Virol 2010

Immunosuppressed Individuals only

Healthy and immunosuppressed individuals

urine
Predisposing conditions for PML
Koralnik  NEJM 2004, Molloy Arthritis Rheum 2009

- natalizumab, rituximab, efalizumab, dimethylfumarate, fingolimod: case series

• **Diagnosis**: brain biopsy or CSF PCR detection of JCV DNA
• **Neurological dysfunction**: based on location of lesions
• **No specific treatment**, 1 year survival ~50%
What do these HIV-seronegative people have in common?

- 50 yo man with alcoholic cirrhosis
- 29 yo woman with untreated dermatomyositis
- 49 yo man with idiopathic CD4 lymphocytopenia
PML in patients with limited or occult immunosuppression

Gheuens JNNP 2009

- 5 cases at BIDMC and 33 cases reports (1966-2009)

- **Summary of 38 cases:**
  - 7 hepatic cirrhosis
  - 5 renal failure (including one with cirrhosis)
  - 2 pregnant women
  - 2 concomitant dementia
  - 1 untreated dermatomyositis
  - 22 (58%): no specific underlying disease
    - 5/22 (23%) idiopathic CD4 lymphocytopenia (ICL)
Diagnostic delay in PML
Miskin  Ann Clin Translat Neurol 2016

- 111 PML pts seen at BIDMC 1993-2015 with complete clinical information about sx onset and dx
- Mean delay to dx: **74 days** (1-1643 d)
- Other dx considered before PML in 64%
- Diagnostic delay > 1 mo in 77%

- No dx delay difference in 45 HIV- vs 66 HIV+
- No difference of dx delay over time 1993-2015
- Better outcome if dx established within 1 mo of sx onset
- Extended dx delay > 3 mo in HIV+ associated with higher CD4+ at sx onset
Death by CTL

- Gheuens J Virol 2011
Too much of a good thing?

- good immunologic and virologic response to cART: increased CD4 counts, decreased HIV VL
- paradoxical development of an inflammatory form of PML
- manifestation of immune reconstitution inflammatory syndrome (IRIS)
- PML/IRIS: abrupt worsening of neurological function in the setting of immune recovery, different than the natural progression of PML
IRIS vs PML progression?
Metabolic profile of PML lesions

- 42 patients, 100 MRI and 99 $^1$H-MRS
- 17/42 (40%) IRIS:
- Lesions of PML-IRIS:
  - higher Cho/Cr, ml/Cr, LIP1/Cr and LIP2/Cr
  - lower NAA/Cr
- Lip/Cr: triglycerides, lactate, anaerobic metabolism

**HIV- PML IRIS**

**HIV+ PML**
Diagnostic algorithm of IRIS: contrast enhancement and Lipid1/Cr

Gheuens Neurology 2012

Logistic regression model to evaluate IRIS predictors
Parsimonious selection of best predictors

<table>
<thead>
<tr>
<th>P(IRIS)</th>
<th>CE- (95% CI)</th>
<th>CE+ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lip1/Cr &lt;= 1.5</td>
<td>13 % (7-18)</td>
<td>56 % (37-76)</td>
</tr>
<tr>
<td>Lip1/Cr &gt; 1.5</td>
<td>24 % (15-33)</td>
<td>79 % (68-89)</td>
</tr>
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</table>

- Help differentiate IRIS from natural progression of PML
- Guide management re: corticosteroid administration
Natalizumab (Tysabri) prevents the migration of mononuclear cells into CNS
JC virus goes Global:
PML in 2 MS patients on natalizumab

Share Price Bounded by TYSABRI Uncertainty

JC virus cliff
PML in 792 MS and 3 Crohn’s patients rx with natalizumab (Tysabri) (9/5/2018)

- 1 new NTZ/PML case in the world every 3 days
- Risk of PML 1/90 after 24 mo of NTZ in JCV seropositive patients with prior immunosuppressive rx
PML-IRIS in a natalizumab-treated MS patient  Gheuens Neurology 2012

FLAIR

T1+ Gad
Rush MS clinic 2010-2017: 17 Natalizumab-PML MS patients

- Mean of 50 infusions (4.2 y)
- Uni/multilobar
- All treated with Granulocyte-Colony stimulating factor (G-CSF)
- 16/17 alive 2 y post PML onset (1 suicide)
- Only 17% MS relapse Y1 and 29% Y2 post PML
- Less than 45% MS relapse Y1 post NTZ discontinuation in other studies
- *PML may improve the clinical course of MS!*
JCV does not only affect the **WHITE** matter: discovery of 3 novel syndromes involving **GRAY** matter
Granule cell neurons (GCN)
HIV+ patient with cerebellar dysfunction and atrophy
1) JCV granule cell neuronopathy ($JCV_{GCN}$): infection of cerebellar granule cell neurons

Koralnik Ann Neurol 2005
Molecular basis of GCN tropism


- deletions in JCV VP1 gene resulting in change of the C terminus of the major capsid protein
JCV GCN in MS patients with cerebellar atrophy after 4-5 y of NTZ

Switzerland
Schippling
Ann Neurol
2013

Arizona
Agnihotri
Neurology 2014
Every truth goes through three stages
(Arthur Schopenhauer 1788-1860)

- It is ridiculed
- It is violently opposed
- It is accepted as being self-evident
- It is listed by Biogen on the Tysabri black box warning

**JC virus infection of granule cell neurons in the cerebellum (i.e., JC virus granule cell neuronopathy [JCV GCN]) has been reported in patients treated with TYSABRI. JCV GCN can occur with or without concomitant PML. JCV GCN can cause cerebellar dysfunction (e.g., ataxia, incoordination, apraxia, visual disorders), and neuroimaging can show cerebellar atrophy. For diagnosis of JCV GCN, an evaluation that includes a gadolinium-enhanced MRI scan of the brain and, when indicated, cerebrospinal fluid analysis for JC viral DNA, is recommended. JCV GCN should be managed similarly to PML.**
Worldwide recognition of JCV GCN


• JCV GCN in the setting of:
  – AIDS
  – Hematologic malignancies
  – Idiopathic lymphocytopenia
  – Tysabri treatment of MS
74 yo HIV-negative lady with hx of lung cancer presenting with encephalopathy
2) JCV Encephalopathy (JCVE): infection of cortical pyramidal neurons

Wuthrich
Ann Neurol
2009
JCV$_{\text{CPN1}}$: agno gene deletion

Dang PLOS One 2012, Ellis PLOS One 2013, JNV 2014
67 yo HIV-neg lady with head ache, cognitive deficit and gait imbalance

CSF OP: 30 cm H20
10 WBC (91% Ly)
61 mg/dl protein

CSF JC virus PCR: 48x10^6 cps/ml
3) JCV Meningitis (JCVM): infection of leptomeninges and choroid plexi

Agnihotri Ann Neurol 2014
Implications of infection of neurons and meningeal cells by JCV variants

Gheuens Annual Rev Pathol 2013

- Mutation in the coding region affects cellular tropism of the virus in the CNS
- Site of latency and possible reactivation
- Evasion of the immune response
- CNS dysfunction: overt vs cryptic
- Different JC variants or yet unknown polyomaviruses associated with other neurological diseases?
The Great Circle of JCV Neuropathogenesis

Miskin Curr Op Neurol 2015
When to include JC virus in the differential diagnosis?

• White matter lesion(s) that do not respect vascular territory with focal neurological dysfunction (PML)
• PML patients with new neurological symptoms despite immune recovery (PML IRIS)
• Gray matter lesions and encephalopathy (JCVE)
• Cerebellar atrophy (JCV GCN)
• Aseptic meningitis and hydrocephalus (JCVM)
• Immunosuppressed hosts:
  – HIV, Tx, Cancer, lymphocytopenia, MS drugs (Tysabri, Tecfidera, Gilenya), “others”…
• All of the above with new onset seizures
• Dx: JCV CSF PCR, brain biopsy
Sequencing then... (circa 1990)
High Throughput Sequencing now...
(Deep sequencing, *Next Gen* sequencing)

- DNA sheared into short fragments (~250bp)
- Ligation of adapters
- PCR amplification and sample identification
- Multiple copies of a gene yields overlapping DNA fragments
How Deep is Deep enough?

- Millions of reads/sample: increases coverage
- Coverage: length of the genome and depth of each region
- Depth: necessary to distinguish low frequency sequences
Scale of Human Genomic DNA per cell vs average Viral genome
ViroFind
A novel platform for virus detection and discovery

• Background
  – Viruses often suspected in aseptic meningitis/encephalitis/myelitis
  – Viral variants associated with novel neurologic diseases or presentation: JCV, Zika
  – Viruses implicated in pathogenesis of MS, ALS, Alzheimer’s, Parkinson’s etc
  – Limitation of PCR technology: one virus-one test

• Knowledge Gap
  – Characterization of the entire “VIROME” in human samples
  – Association vs causality vs biomarkers?
  – Integration of viral sequence data with genomics, immunomics, metabolomics etc
  – “Viromics” field underdeveloped
ViroFind: workflow

- 131,706 viral probes (8.415 Mbp)
- Mean coverage of 89.39% genomes of 561 selected DNA and RNA viruses that can infect humans or could cause zoonosis
- Potential to detect virus variants and yet undiscovered viruses
ViroFind vs Deep sequencing for JCV detection in 5 PML brains

<table>
<thead>
<tr>
<th>Sample</th>
<th>Platform</th>
<th>Mapped JCV Reads</th>
<th>Total Reads</th>
<th>Mapped Reads/Total Reads</th>
<th># Fold Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PML Brain sample 1</td>
<td>ViroFind</td>
<td>1,097</td>
<td>1,023,903</td>
<td>$1 \times 10^{-3}$</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Deep Sequencing</td>
<td>24</td>
<td>738,541</td>
<td>$3 \times 10^{-5}$</td>
<td></td>
</tr>
<tr>
<td>PML Brain sample 2</td>
<td>ViroFind</td>
<td>9,327</td>
<td>1,826,482</td>
<td>$5 \times 10^{-3}$</td>
<td>58</td>
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<tr>
<td></td>
<td>Deep Sequencing</td>
<td>45</td>
<td>515,119</td>
<td>$8 \times 10^{-5}$</td>
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<tr>
<td>PML Brain sample 3</td>
<td>ViroFind</td>
<td>375,653</td>
<td>430,842</td>
<td>$9 \times 10^{-1}$</td>
<td>127</td>
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<tr>
<td></td>
<td>Deep Sequencing</td>
<td>2,940</td>
<td>428,463</td>
<td>$7 \times 10^{-3}$</td>
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<tr>
<td>PML Brain sample 4</td>
<td>ViroFind</td>
<td>584</td>
<td>1,001,280</td>
<td>$6 \times 10^{-4}$</td>
<td>43</td>
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<tr>
<td></td>
<td>Deep Sequencing</td>
<td>7</td>
<td>520,848</td>
<td>$1 \times 10^{-5}$</td>
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</tr>
<tr>
<td>PML Brain sample 5</td>
<td>ViroFind</td>
<td>10,345</td>
<td>1,345,674</td>
<td>$7 \times 10^{-3}$</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>Deep Sequencing</td>
<td>56</td>
<td>899,654</td>
<td>$6 \times 10^{-5}$</td>
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</tr>
</tbody>
</table>

- Increase in number of mapped JCV reads
- Better ratio of mapped/total reads
- Enrichment of viral sequences up to 127 fold
- Detection of HHV6-B sequences in 11/18 control brain samples
ViroFind: genetic distance between each JCV strain and JCV prototype MAD1
(Chalkias PLOS One 2018)

Each red cord: single SNP

JCV genetic diversity in PML brains previously underestimated
• There is White matter in Gray Matter / Gray matter in White Matter
• Orphan Diseases can go Global
• Rare Diseases as models of Neuropathogenesis for common diseases
• Other viral variants associated with other neurological diseases? (MS, Alzheimer’s Parkinson’s, ALS etc.)
Training for a Neurology Career in a Rare Disease: The Role of Cyberconsults

Shruti P. Agnihotri, MD¹ and Igor J. Koralnik, MD²
Global Neurology Research Program in Zambia

• Central African country the size of Texas
  – English official language
  – Politically stable / safe

• 13 million people
  – HIV seroprevalence: 14%
  – Up to 25% adults are HIV+ in urban centers

• Only 4 Neurologists! (vs ~ 370 in MO)
  – Dr. Omar Siddiqi, since 2010: research on CNS opportunistic infections, new onset seizures in HIV and TB meningitis (NINDS K23) Siddiqi Neurol 2013 and 2015, CID 2014
Developing a Successful Global Neurology Program

Omar K. Siddiqi, MD, MPH,1,2 Merritt Brown, MD,3 Christine Cooper, MD,4 Masharip Atadzhanov, MD, PhD,2 Shabir Lakhi, MBChB, MMed, MPH,2 and Igor J. Koralnik, MD1,5

• Blueprint on how to develop a Global Neurology Program
• Testimony of the first residents who participated to the elective
• Foundation for first neurology residency training program to start next week!!!
• Dr. Deanna Saylor (Hopkins): Zambia Neurology educator
Collaborators

- Barbara Hanson
- Dominique Tucker
- Gayathri Rajan
- Xin Dang
- Andrew Cogswell
- Rasha Waheed
- Sarah Corbridge
- Lakshmi Warrior
- Fabian Sierra Morales
- Zach Orban

- Nicholson Lab
- Kordower Lab
- Epilepsy section
- MS section
- Movement Disorders section

- NIH R01 NS047029 and NS074995, R21 NS099787, Biogen

- PML patients and their families
The department of Neurological Sciences salutes you!
THANK YOU