The Evolving Pathogenesis of Progressive Multifocal Leukoencephalopathy (PML): Implications for future treatments for Multiple Sclerosis

Carlo Tornatore, M.D.
Professor and Vice Chairman, Department of Neurology
Director, Multiple Sclerosis Center
Medstar Georgetown University Hospital
Washington, DC

PML: Historical Perspective

• 1958- Astrom and E.P. Richardson first describe PML and its pathology
  – Multifocal demyelination
  – Enlarged nuclei of oligodendrocytes
  – Astrocytes with bizarre nuclei in areas of demyelination
  – Cerebellar granule cells with hyperchromatic nuclei

PML: Historical Perspective

- 1965-ZuRhein and Chou identify viral particles in the brain of a patient with PML
  - 40 nm nonenveloped icosahedral viral particles
  - Seen only in oligodendrocytes
  - Categorized as:
    - Genus *Polyomavirus*
    - Family *Papovaviridae* (along with SV40, BK virus)

PML: Historical Perspective

- 1971 - Padgett and Walker cultivate a papova-like virus from the brain of a patient with PML
  - Passed autopsy tissue onto cell culture of human fetal brain tissue
  - Cultured fetal oligodendrocyte precursors ("spongioblasts") and fetal astrocytes found to have viral particles in nuclei as seen by EM
  - Virus is named “JC” virus after initials of PML patient (John Cunningham)

Fig. 4. Empty and full particles of a virus from a patient suffering from progressive muscular dystrophy. Detail of expression arrangement and number is visible, which together with the size and shape of the virus, is of great help in identification and classification.

× 145,000
PML: Historical Perspective

- Mid-1970s Epidemiologic surveys using HI to determine exposure to JCV
  - 65% of adolescence positive for anti-JCV antibodies
  - Increases to 80% by age 50
  - No sex difference
  - Primary infection?


JC Virus Life Cycle

- Humans are the only known reservoir
- Infection is thought to occur in early childhood
- ~60-80% of people carry JCV-specific antibodies

Initial infection

Latency

Hematogenous dissemination

Infection of oligodendrocytes

Demyelination

Kidneys

Lymphoid tissue

Immune system

Compromise

Pathogenesis
Initial infection is with archetype virus which only infects kidney

Dissemination of JCV to the Brain

• 1983  Grinnell found JCV DNA in spleen, lymph nodes, lung and liver of 3 PML patients
  – Concluded that JCV is hematogenously disseminated from the kidneys

• 1987  Houff found that JCV DNA and protein in bone marrow and spleen of two PML patients
  – JCV disseminated by lymphocytes

J Infect Dis. 1983 Apr;147(4):669-75
Dissemination of JCV to the Brain

- 1992 Tornatore found JCV DNA in PBLs of:
  - 89% of PML patients
  - 38% of HIV infected patients
  - 0% of Parkinson’s patients

  Concluded that JCV latent in bone marrow emerged in PBLs during immunosuppression and were hematogenously disseminated


- 1993 Dories found 100% of leukemia patients had JCV DNA in PBLs and bone marrow

Virology 198:59 –70. 1993
Viral genome rearranges to become neuro-glial tropic

JC Virus Life Cycle

- Humans are the only known reservoir
- Infection is thought to occur in early childhood

Initial infection

- ~80% of people carry JCV-specific antibodies

Latency

- Infection of Oligodendrocytes
- Demyelination
- Activation
- Hematogenous dissemination
- Immune System Compromise
- Kidneys
- Bone marrow
- Lymphoid Tissue
- PML Pathogenesis
• BIOGEN IDEC AND ELAN ANNOUNCE VOLUNTARY SUSPENSION OF TYSABRI®

• Cambridge, MA and Dublin, Ireland (February 28, 2005) -- Biogen Idec (NASDAQ: BIIB) and Elan Corporation, plc (NYSE: ELN) announced today a voluntary suspension in the marketing of TYSABRI® (natalizumab), a treatment for multiple sclerosis (MS). The companies are suspending supply of TYSABRI from commercial distribution and physicians should suspend dosing of TYSABRI until further notification. In addition, the companies have suspended dosing in all clinical trials. This decision is based on very recent reports of two serious adverse events that have occurred in patients treated with TYSABRI in combination with AVONEX® (Interferon beta-1a) in clinical trials. These events involve one fatal, confirmed case and one suspected case of progressive multifocal leukoencephalopathy (PML), a rare and frequently fatal, demyelinating disease of the central nervous system. Both patients received more than two years of TYSABRI therapy in combination with AVONEX.
• 454 (452 MS, 2 CD) confirmed PML cases as of April 3, 2014 (147 US, 267 EEA, 40 ROW).

Biogenidec, Medical Information Bureau April 2014

• As of December 31, 2013, approximately 123,000 patients received natalizumab in the post-marketing setting worldwide.

• As of April 3, 2014, the overall incidence of PML in natalizumab-treated patients was 3.56 per 1000 patients (95% CI 3.24 to 3.90 per 1000 patients)

Biogenidec, Medical Information Bureau April 2014
Why would JCV escape from the kidneys and lymphatic tissue?

• Virus-related phenomena
  – Change in regulatory region to make it more neuro-glial tropic
  – Change in viral capsid, making it more likely to bind to a receptor on the cellular elements in the CNS

• Immune-related phenomena
  – Loss of immune surveillance
  – Increased hematogenous trafficking of JCV infected lymphocytes
Sequencing and analysis of JC virus DNA from natalizumab-treated PML patients

- Analyzed viral DNA sequences in blood, CSF and/or urine of natalizumab-treated PML patients.
  - Analysis of JCV from multiple biofluids revealed that individuals were infected with a single genotype.
  - Multiple PML-associated NCCR rearrangements and VP1 mutations were present in CSF and blood, but absent from urine-derived virus.

CONCLUSIONS:
- These data confirm that JCV in natalizumab-PML patients is similar to that observed in other PML patient groups, multiple genotypes are associated with PML, individual patients appear to be infected with a single genotype, and PML-associated mutations arise in patients during PML development.

Why would JCV escape from the kidneys and lymphatic tissue?

- Virus-related phenomena
  - Change in regulatory region to make it more neuro-glial tropic
  - Change in viral capsid, making it more likely to bind to a receptor on the cellular elements in the CNS

- Immune-related phenomena
  - Loss of immune surveillance
  - Increased hematogenous trafficking of JCV infected lymphocytes
### Selected Treatments Associated With Reports of PML

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral glucocorticoids</td>
<td>• Cyclophosphamide</td>
</tr>
<tr>
<td>Alkylating agents</td>
<td>• Camstine</td>
</tr>
<tr>
<td>Purine analogs</td>
<td>• Fludarabine (Fludara&lt;sup&gt;*&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Antimetabolite</td>
<td>• Methotrexate (Trexall™)</td>
</tr>
<tr>
<td>Monoclonal antibodies</td>
<td>• Rituximab (Rituxan&lt;sup&gt;*&lt;/sup&gt;)</td>
</tr>
<tr>
<td></td>
<td>• Infliximab (Remicade&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td></td>
<td>• Etanercept (Enbrel&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td></td>
<td>• Natalizumab (Tysabri)</td>
</tr>
<tr>
<td></td>
<td>• Basiliximab (Simulect&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td></td>
<td>• Fludarabine (Fludara&lt;sup&gt;*&lt;/sup&gt;)</td>
</tr>
<tr>
<td></td>
<td>• Cladribine (Leustat&lt;sup&gt;*&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>


---

### Changes in JC virus-specific T cell responses during natalizumab treatment and in natalizumab-associated progressive multifocal leukoencephalopathy.

- MS patients with natalizumab-associated PML were distinguished from all other subjects in that they either:
  - had no detectable JCV-specific T cell response or
  - had JCV-specific CD4 T cell responses uniquely dominated by IL-10 production.

PLoS Pathog. 2012;8(11)
Why would JCV escape from the kidneys and lymphatic tissue?

- **Virus-related phenomena**
  - Change in regulatory region to make it more neuro-glial tropic
  - Change in viral capsid, making it more likely to bind to a receptor on the cellular elements in the CNS

- **Immune-related phenomena**
  - Loss of immune surveillance
  - Increased hematogenous trafficking of JCV infected lymphocytes

**CD34+ progenitor cells mobilized by natalizumab are not a relevant reservoir for JC virus**


- Viral DNA was not detectable in CD34+ haematopoietic progenitor or peripheral blood mononuclear cells from any sample. Two plasma samples from patients with MS while undergoing natalizumab treatment were JCV-positive. In one case clinically manifest PML developed 8 months thereafter.
JC virus Reactivation During Prolonged Natalizumab Monotherapy for Multiple Sclerosis.

- JCV DNA was detected in blood of 12/43 (27.9%) and in urine of 11/43 (25.6%) subjects without difference between natalizumab-treated patients and controls

- JCV DNA was detected in the CSF of 2/27 (7.4%) natalizumab-treated MS patients who had no symptoms or MRI lesions consistent with progressive multifocal leukoencephalopathy.

- JC viral load was higher in CD34+ cells and in monocytes compared to other subpopulations


• JC Virus in CD34+ and CD19+ Cells in Patients With Multiple Sclerosis Treated With Natalizumab.

-13/26 patients (50%) with baseline and follow-up blood samples had detectable viral DNA in at least 1 cell compartment at 1 or more points.

-10/ 23 patients (44%) receiving treatment for more than 24 months and 3 of the 18 healthy volunteers (17%) also had detectable viral DNA in 1 or more cell compartment

-15/49 MS patients (31%) were confirmed to harbor JCV in CD34+ cells and 12 of 49 (24%) in CD19+ cells.

-CONCLUSION: “JC virus DNA was detectable within cell compartments of natalizumab-treated MS patients after treatment inception and longer. JC virus DNA may harbor in CD34+ cells in bone marrow that mobilize into the peripheral circulation at high concentrations. Latently infected cells initiate differentiation to CD19+ cells that favors growth of JCV. These data link the mechanism of natalizumab treatment with progressive multifocal leukoencephalopathy”

JAMA Neurol. 2014 Mar 24. [Epub ahead of print]
Lymphocyte Gene Expression and JC Virus Noncoding Control Region Sequences Are Linked with the Risk of Progressive Multifocal Leukoencephalopathy.

-Spi-B is upregulated in developing B cells in response to natalizumab

-Progressive multifocal leukoencephalopathy (PML)-derived noncoding control region (NCCR) sequences permitted greater early viral gene expression than kidney-associated NCCR sequences, driven in part by binding of the transcription factor Spi-B to unique PML-associated Spi-B binding sites.

-Naturally occurring JCV sequence variation, together with drug treatment-induced cellular changes, may synergize to create an environment leading to an increased risk of PML


Biomarkers for Risk Stratification

• Markers for absence of JCV infection

• Markers for resistance to JCV infection

• Markers for susceptibility to JCV infection
Biomarkers for Risk Stratification

• Markers for absence of JCV infection

• Markers for resistance to JCV infection

• Markers for susceptibility to JCV infection

Anti-JC virus antibodies: implications for PML risk stratification.


RESULTS:

• In our evaluation of natalizumab-treated MS patients, 53.6% tested positive for anti-JCV antibodies

• Notably, we observed anti-JCV antibodies in all 17 available pre-PML sera samples, which was significantly different from the 53.6% seropositivity observed in the overall MS study population (p < 0.0001).
• As of March 6, 2014, there are 212 natalizumab-treated MS PML patients with known pre-PML anti-JCV antibody status who had samples tested for anti-JCV antibodies, all of which were collected at least 6 months prior to PML diagnosis (range 6 to 187 months).

• Of these 212 patients, 210 (99%) tested anti-JCV antibody positive prior to diagnosis and 2 (1%) tested anti-JCV antibody negative.

<table>
<thead>
<tr>
<th>Anti-JCV Antibody Negative</th>
<th>Natalizumab Exposure</th>
<th>Anti-JCV Antibody Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Prior immunosuppressant</td>
<td>Prior Immunosuppressant</td>
</tr>
<tr>
<td>&lt;1/1,000</td>
<td>&lt;1,000</td>
<td>1/1,000</td>
</tr>
<tr>
<td>1-24 months</td>
<td>3/1,000</td>
<td>13/1,000</td>
</tr>
<tr>
<td>25-48 months</td>
<td>7/1,000</td>
<td>9/1000</td>
</tr>
<tr>
<td>49-72 months</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
JC virus antibody status underestimates infection rates.

-67 patients enrolled in a single-center, retrospective cohort study.

-Forty (59.7%) of the 67 patients were JCV seropositive.

-Of 27 JCV seronegative patients, 10 (37%) had JCV viruria.

-Urine JCV DNA copy numbers were significantly higher in the seropositive group (mean log copy number = 5.93, range = 1.85-9.21) than the seronegative group (mean log copy number = 2.41, range = 1.85-5.43; p = 0.0026).

The false-negative rate of the JCV serology in this study was 37%; therefore, JCV serostatus does not appear to identify all patients infected with JCV. Thus, a negative JCV antibody result should not be conflated with absence of JCV infection.

Biomarkers for Risk Stratification

• Markers for absence of JCV infection

• Markers for resistance to JCV infection

• Markers for susceptibility to JCV infection
JC Polyomavirus Infection Is Strongly Controlled by Human Leucocyte Antigen Class II Variants

- HLA class II restricted immune responses, and hence CD4+ T cell immunity is pivotal for JCV infection control.

- Alleles within the HLA-DR1*15 haplotype are associated with a protective effect on JCV infection.

- Alleles within the DQB1*06:03 haplotype show an opposite association.

- These associations between JC virus antibody response and human leucocyte antigens supports the notion that CD4+ T cells are crucial in the immune defense to JCV and lays the ground for risk stratification for PML and development of therapy and prevention.


Biomarkers for Risk Stratification

- Markers for absence of JCV infection
- Markers for resistance to JCV infection
- Markers for susceptibility to JCV infection
L-selectin is a possible biomarker for individual PML risk in natalizumab-treated MS patients.

- The percentage of L-selectin-expressing CD4+ T cells was significantly lower in patients treated long-term with natalizumab (40.2%) when compared with patients not receiving natalizumab treatment (47.2%; p = 0.016) or healthy controls (61.0%; p < 0.0001).

- An unusually low percentage (9-fold lower; 4.6%) was highly correlated with the risk of developing PML in the patient group with available pre-PML samples when compared with non-PML natalizumab-treated patients (p ≤ 0.0001).


Lancet Neurol. 2010 Apr;9(4):438-46

• The presenting symptoms of PML most commonly included
  – changes in cognition and personality
  – motor performance
  – but several cases had seizures as the first clinical event.
Magnetic resonance imaging pattern in natalizumab-associated progressive multifocal leukoencephalopathy.

Ann Neurol. 2012 Nov;72(5):779-87

The most frequent lesion pattern in early scans from PML patients was that of
- large (>3 cm, 15 of 18), subcortical (18 of 18), T2 or fluid-attenuated inversion recovery hyperintense (18 of 18)
- sharp border toward the gray matter and an ill-defined border toward the white matter (18 of 18) on T2-weighted images.
- T1-hypointense (17 of 18)
- diffusion-hyperintense (15 of 15) lesions
- Could detect contrast enhancement in 41% (7 of 17) of the cases on the first scan at clinical presentation.
Microcystic appearance on FLAIR and T2WI
Magnetic resonance imaging pattern in natalizumab-associated progressive multifocal leukoencephalopathy.

Ann Neurol. 2012 Nov;72(5):779-87

The most frequent lesion pattern in early scans from PML patients was that of
- large (>3 cm; 15 of 18), subcortical (18 of 18), T2 or fluid-attenuated inversion recovery hyperintense (18 of 18)
- sharp border toward the gray matter and an ill-defined border toward the white matter (18 of 18) on T2-weighted images.
- T1-hypointense (17 of 18)
- diffusion-hyperintense (15 of 15) lesions
- Could detect contrast enhancement in 41% (7 of 17) of the cases on the first scan at clinical presentation.

“Moth eaten appearance on T1”
Magnetic resonance imaging pattern in natalizumab-associated progressive multifocal leukoencephalopathy.

Ann Neurol. 2012 Nov;72(5):779-87

The most frequent lesion pattern in early scans from PML patients was that of
- large (>3 cm, 15 of 18), subcortical (18 of 18), T2 or fluid-attenuated inversion recovery hyperintense (18 of 18)
- sharp border toward the gray matter and an ill-defined border toward the white matter (18 of 18) on T2-weighted images.
- T1-hypointense (17 of 18)
- diffusion-hyperintense, (restricted diffusion) (15 of 15) lesions
- Could detect contrast enhancement in 41% (7 of 17) of the cases on the first scan at clinical presentation.

New PML lesions have restricted diffuse on DWI
Magnetic resonance imaging pattern in natalizumab-associated progressive multifocal leukoencephalopathy.

Ann Neurol. 2012 Nov;72(5):779-87

The most frequent lesion pattern in early scans from PML patients was that of
- large (>3 cm; 15 of 18), subcortical (18 of 18), T2 or fluid-attenuated inversion recovery hyperintense (18 of 18)
- sharp border toward the gray matter and an ill-defined border toward the white matter (18 of 18) on T2-weighted images.
- T1-hypointense (17 of 18)
- diffusion-hyperintense (15 of 15) lesions
- Could detect contrast enhancement in 41% (7 of 17) of the cases on the first scan at clinical presentation.

Gadolinium enhancement

- Enhancement in acute PML is heterogeneous
- Enhancement typically involves less than the total lesion area
- Morphology can be subtle, speckled, or linear
Hyperintense cortical signal on magnetic resonance imaging reflects focal leukocortical encephalitis and seizure risk in progressive multifocal leukoencephalopathy

Clinically silent PML and prolonged immune reconstitution inflammatory syndrome in a patient with multiple sclerosis treated with natalizumab.

Reported the case of a woman with natalizumab-treated multiple sclerosis (MS) and clinically silent progressive multifocal leukoencephalopathy (PML) with an unusually long preclinical phase, followed by acute symptoms due to development of immune reconstitution inflammatory syndrome (IRIS).

Mult Scler. 2013 Aug;19(9):1226-9
CSF Testing for JC Virus

- PCR analysis of the CSF for JC virus is the best test for confirmation of PML caused by JC virus\textsuperscript{1,2}

  - Analysis of CSF for JCV DNA is very specific for diagnosis of PML\textsuperscript{2}
  - The other parameters of spinal fluid analysis are typically normal\textsuperscript{1}

\textsuperscript{1} Aksamit AJ. Neurologist. 2006 Nov;12(6):293-298.
\textsuperscript{2} Kappos L et al. Lancet Neurol. 2007;6:431-441.

JCV PCR of CSF
PML: CSF PCR

• Semiquantitative measurements of JC viral DNA load in CSF have been shown to correlate with survival and may potentially be used as a method of monitoring the disease response to therapeutic interventions

  (Yiannoutos CT, Curfman B et al. Relation of JC virus DNA in the cerebrospinal fluid to survival in acquired immunodeficiency syndrome patients with biopsy proven progressive multifocal leucoencephalopathy. Annals Neurol 1999;45:816-821.)

PML: CSF PCR

• Most studies suggest a sensitivity of $\approx 75\%$ with a specificity of $\approx 90-99\%$ for detection of JC viral DNA in the CSF.

• Low sensitivity may be due to the fact that the virus is mainly contained intracellularly with few virions free in CSF.

• False negative rate of $\approx 25\%$ means that a negative result does not exclude the diagnosis and it may be necessary to repeat the lumbar puncture or carry out a brain biopsy.
Clinical outcomes of natalizumab-associated progressive multifocal leukoencephalopathy.

• OBJECTIVE:
  – Assessed clinical outcomes and identified variables associated with survival in 35 patients with natalizumab-associated PML.

• RESULTS:
  – At the time of analysis, 25 patients (71%) had survived.
  – Survivors were younger (median 40 vs 54 years) and had lower pre-PML Expanded Disability Status Scale scores (median 3.5 vs 5.5) and a shorter time from symptom onset to diagnosis (mean 44 vs 63 days) compared with individuals with fatal cases.
  – Of patients with nonfatal cases, 86% had unilobar or multilobar disease on brain MRI at diagnosis, whereas 70% of those with fatal cases had widespread disease.
  – Gender, MS duration, natalizumab exposure, prior immunosuppressant use, and CSF JC viral load at diagnosis were comparable.
  – Most patients were treated with rapid removal of natalizumab from the circulation. The majority of patients developed immune reconstitution inflammatory syndrome and were treated with corticosteroids.

• CONCLUSIONS:
  – Natalizumab-associated PML has improved survival compared with PML in other populations.
Of 336 PML patients, 254 were alive at least 6 months after PML diagnosis (76% survival rate)

Biogenidec Medical Information April 2014

JCV Viral Life Cycle:
Potential Areas for Intervention
Blocking The Binding of JC Virus to 5HT2A Receptor on Neuroglial elements

- Mirtazepine

- Risperidone

JCV Viral Life Cycle: Potential Areas for Intervention
Blocking JC Viral Replication and Transcription

- Cidofovir

- Interferon-α

JCV Viral Life Cycle: Potential Areas for Intervention

- Mirtazepine
- Risperidone
- Cidofovir
- Interferon-α
- 5HT2A receptor
Blocking JC Viral Assembly

- Mefloquine

JCV Viral Life Cycle: Potential Areas for Intervention
Promoting Viral Clearance

• Promoting immune reconstitution
  – PLEX to remove natalizumab or other monoclonal antibody (if non-complement binding)
  – Neupogen
    • Will increase both lymphocyte and granulocyte counts
  – IL-2

JCV Viral Life Cycle: Potential Areas for Intervention

- Mirtazapine
- Risperidone
- Cidofovir
- Interferon-α
- Mefloquine
- 5HT2A receptor
- PLEX
- G-CSF
- IL-2

To promote viral particle clearance
Combination Therapy?

- Previous studies looking at treating PML were done in immunosuppressed patients and generally with monotherapy. Is this relevant to natalizumab-related PML in MS patients where the immune reconstitution may be more robust?

- Is combination therapy to treat natalizumab-related PML in MS patients rational or are we just combining multiple inadequate medications with potential for adverse side effects?

- One possible protocol based on blocking different stages of the JC viral life cycle would use all of the following together once dx of PML is made:
  - PLEX
  - Cidofovir
  - Interferon-α
  - Mefloquine
  - Mirtazepine
  - G-CSF if leukopenic/lymphopenic

- If IRIS develops:
  - IVIG
  - Steroids
  - maraviroc?