Understanding the JCV and the development of risk stratification

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Disclosures

Research support for PML biomarker research

BiogenIdec
Understanding JCV, PML…?

MS: not known before Natalizumab (VLA-4)

**Therapy**
mAb: anti-VLA4, LFA-1, CD20, TNFa, CD52, Fumarates, Leflunomide

**Host factors**
MS, RA, sLE, Psoriasis, CD4, CD8, CNS immune surveillance

**Viral factors**
Infection, Mutations (regulatory region, VP1: CNS tropism)

PML
History of JC Virus in PML

1959 Cavanaugh et al.
- inclusion bodies in nuclei of oligodendrocytes

1965 ZuRhein et al.
- EM reveals polyoma-like particles

1971 Padgett et al.
- polyomaviruses isolated using glial cell lines

Astrom KE et al., Brain 1958;81:93-111
Padgett BL et al., Lancet 1971;1:1257-60.
The observed clinical trial PML incidence in patients who received a mean of 17.9 monthly doses of natalizumab was 1.00 per 1000 natalizumab-treated patients (95% CI 0.20-2.80) (Yousry TA, et al. N Engl J Med. 2006;354:924-933). The post-marketing rate is calculated as the number of PML cases since reintroduction in patients that have had at least 1 dose of natalizumab.

Incidence estimates by treatment epoch are calculated based on natalizumab exposure through July 31, 2013 and 395 confirmed cases as of August 6, 2013. The incidence for each epoch is calculated as the number of PML cases divided by the number of patients exposed to natalizumab (e.g., for 25 to 36 infusions all PML cases diagnosed during this period is divided by the total number of patients ever exposed to at least 25 infusions and therefore having risk of developing PML during this time). Biogen Idec, data on file.
Take home messages

Natalizumab-PML

β Distinct clinical and MRI presentation
β Early detection is possible, in doubt: STOP Natalizumab
β Aim for rapid immune-reconstitution, be aware of seizures
β IRIS: a double edged sword
β Risk stratification: a moving field
Take home messages

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PML: early case in the postmarketing phase

Wenning et al.,
361:1075-80
Natalizumab PML: clinical presentation

Berger, Cleveland Clin J 2011;78S2
Natalizumab PML: MRI-pattern

- large >3cm
- subcortical
- T2-hyperintense
- T1 hypointense

- FLAIR hyperintense
  - sharp border
  - grey matter
  - ill-defined border
  - white matter

- DWI hyperintense
- Early PML: 41% Gd+

N=22 patients

Yousry et al., Ann Neurol 2012;72:779
Natalizumab PML: MRI pattern

- T1-hyperintensity: during/after PML-IRIS phase, differentiating against PML-phase
- involvement of cortex (50%) and basal ganglia (28%)
- Gd-enhancement: punctate/rimlike

Yousry et al., Ann Neurol 2012;72:779
1) $A_{I_{JCV}}$ increased in Nat-PML (100% Specificity, 63-80% Sensitivity 63-80%)

2) No correlation between CSF JCV DNA and $A_{I_{JCV}}$: additive diagnostic value

*Below limit of quantification

Natalizumab

**Courtesy of C. Warnke, Düsseldorf, presented at DGN 2013**
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**PML: factors associated with prognosis**

Mortality $\geq 20\%$ (HIV: median survival 183 days)

Survivors:
- younger, lower pre-PML EDSS, restricted MRI extension
- but not: Natalizumab exposure, immunosuppressants, CSF viral load

Vermersch et al., Neurology 2011;76:1697
MRI in "preclinical PML"

Ayzenberg et al, J Neurol 2012;76:574

asymptomatic CSF-PCR neg.

Nat continued

PCR positive

Ayzenberg et al, J Neurol 2012;76:574
MRI in “preclinical” PML

- MRI-abnormalities may precede clinical/CSF changes
- Especially frontal lesions a-/oligosymptomatic
- Diagnostic value of repeat MRI
- “radiologically suspected PML”: interrupt treatment until proven otherwise
- MRI-sequences: T2, FLAIR, DWI, T1, T1-post Gd
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Treatment

No EBM-proven treatment

• ?? Mefloquine, Serotonin 5-OH-tryptamine receptor antagonists, CCR5-antagonists

• ?Nucleoside analogues...

• Failure of clinical studies after successful pre-clinical studies?
  - CNS-penetration
  - Late treatment initiation
  - Phase of the disease?

  v Immune-reconstitution
Serum Natalizumab concentration before and after PLEX

Khatrie et al., Neurology 2009
When serum natalizumab concentration decreased <1-2 mcg/ml, de-saturation of α4 integrin was observed.
CD4-cell count vs. function after PLEX

Haghikia et al., PLOSone 2011
Supportive measures: Bochum experience

- The first 2-4 weeks after PLEX are like 'honeymoon'

- Not evidence based: mirtazapine (to block 5HT receptors, >30 mg) and mefloquine 250 mg/week or zidovudine 5 mg/kg (nephrotoxic)

Dahlhaus et al., JNNP 2013, 84:1068
Hoepner et al., Ther Advances Neurol Dis 2013, epub ahead of print
Natalizumab PML: Seizures

N=15 (39.5 a, SD 6.9), single center

- 8 of 15 (53%) with seizures
  - Often manifesting as status epilepticus
  - 61 days after diagnosis of PML
  - 5 cases: Gd-enhancement MRI (IRIS)

<table>
<thead>
<tr>
<th></th>
<th>PML+Seizure (%)</th>
<th>PML-Seizure (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AED prophylaxis</td>
<td>1 (16.7)</td>
<td>5 (83.3)</td>
<td></td>
</tr>
<tr>
<td>No AED prophylaxis</td>
<td>7 (77.8)</td>
<td>2 (22.2)</td>
<td>0.041</td>
</tr>
<tr>
<td>Total</td>
<td>8 (53.3)</td>
<td>7 (46.7)</td>
<td></td>
</tr>
</tbody>
</table>

*Current algorithm in Bochum: preventive antiepileptic treatment*

Hoepner et al., Ther Advances Neurol Dis 2013, epub ahead of print
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Ca. 4 weeks after PLEX, worsening...
**Immune reconstitution inflammatory syndrome**

- Often 2-6 weeks after PLEX/IA

**Features of PML-IRIS**

- Paradoxical worsening of clinical or radiographic finding with recovery of the immune system
- New or increased neurologic deficits
- Increase in the number or size of lesions on neuroimaging
- Contrast enhancement of brain lesions
- Brain edema
- Concurrent with diagnosis of PML

**cave: seizures, „early PML-IRIS“**

**No specific biomarker of IRIS**

Berger, Cleveland Clin J 2011;78S2
IRIS: CD4⁺/CD8⁺ T-cells

- JCV-specific CD4⁺ and CD8⁺ cells: associated with survival, control/elimination of JCV-infected oligodendrocytes

- Bystander damage: Glucocorticosteroids?

Du Pasquier et al., Brain 2004; 127:1970
Aly et al., Brain 2011, 134: 2687
Effects of Glucocorticosteroids on JCV-specific T-cells

IFN-γ-secreting, TNF-α-secreting CD8+ T-cells

T-cell proliferation (CD4/CD8)

- No "preventive" GC-therapy
- Only, if clinical (or MRI) evidence of IRIS
- Mild forms?

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Antoniol C et al. Neurology 2012;79:2258-2264
# Functional Status in PML Survivors with at Least 6 Months of Follow-up Time (N=47)

<table>
<thead>
<tr>
<th>Mild</th>
<th>Able to carry on normal activity and to work; no special care needed</th>
</tr>
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<tbody>
<tr>
<td>100</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
</tr>
<tr>
<td>BOCHUM</td>
<td>13% (n=6)</td>
</tr>
<tr>
<td></td>
<td>20% (n=3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Moderate</th>
<th>Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed</th>
</tr>
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<tbody>
<tr>
<td>70</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
</tr>
<tr>
<td>47% (n=22)</td>
<td>67 % (n = 10)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Severe</th>
<th>Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>17 of 19 cases of severe disability (89%)</td>
<td>40% (n=19)</td>
</tr>
<tr>
<td>75-80% survival</td>
<td>100 % survival</td>
</tr>
</tbody>
</table>

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Long-term follow up

N=15 (39.5 a, SD 6.9), single center, median 21.5 months

- CSF free of JCV DNA: 4.5 months (median, 8/15 patients, 1.5-9 months)
- New Gd-MRI activity: approximately 9 months
- New relapse: approximately 15 months
- Treatment: GLAT, IFN, FTY
- 5 stable without immunotherapy (median 2 years after PML diagnosis)

Dahlhaus et al., JNNP 2013; 84:1068
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Current risk stratification strategy

Anti-JC virus antibody status

- Negative
- Positive

? Specific immunosuppressants
? Interval seroconversion
? >48 months

Blomgren et al., NEJM 2012
**PML-risk stratification: Anti-JC-Virus Antibodies**

Generation 1 Assay (Stratify™)

- Anti-JCV antibodies in all (n=17) PML-patients, 16 - 180 months prior to diagnosis → High-negative predictive value

Gorelik et al, Ann Neurol 2010

Bochum cohort (anti-Tysabri antibody biobank)

- N=2,782 samples, 2,253 patients
  - 58.8% seropositive
  - M (64.4)>F (56.2) seropositive
  - Independent from: disease/treatment duration

Trampe et al., Neurology 2012
JCV-antibodies: age-dependent but independent of pretreatment

Age

Immunosuppressants

Trampe et al, Neurology 2012
JCV-antibodies in pre-PML sera: Bochum cohort (generation 1 assay)

<table>
<thead>
<tr>
<th>Pat. 1</th>
<th>Pat. 2</th>
<th>Pat. 3</th>
<th>Pat. 4</th>
<th>Pat. 5</th>
<th>Pat. 6</th>
<th>Pat. 7</th>
<th>Pat. 8</th>
<th>Pat. 9</th>
<th>Pat. 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (F/M)</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Sample collection prior to PML diagnosis (months)</td>
<td>2.9</td>
<td>a) 29.5</td>
<td>20.6</td>
<td>17.7</td>
<td>37.6</td>
<td>a) 33.6</td>
<td>a) 26.3</td>
<td>19.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b) 25.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) 24.5</td>
<td>b) 2</td>
<td></td>
<td>b) 31.7</td>
</tr>
<tr>
<td>Age at PML diagnosis</td>
<td>35</td>
<td>40</td>
<td>35</td>
<td>45</td>
<td>42</td>
<td>44</td>
<td>58</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td>No. of natalizumab infusions at PML diagnosis</td>
<td>31</td>
<td>29</td>
<td>n.a.</td>
<td>24</td>
<td>n.a.</td>
<td>39</td>
<td>31</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>Immunosuppressive pretreatment (yes/no)</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Anti-JCV-antibodies (detected/not detected)</td>
<td>detected</td>
<td>detected</td>
<td>detected</td>
<td>a) detected</td>
<td>detected</td>
<td>detected</td>
<td>detected</td>
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</table>

Trampe et al., Neurology 2012
Anti JCV-antibodies: Seroconversion (generation 1 assay)

<table>
<thead>
<tr>
<th>Anti-J CV First analysis</th>
<th>Anti-J CV Last analysis</th>
<th>nOD\textsubscript{450} (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>19/194 initially negative negative patients tested positive ?$\approx$9.8% in 7.7 months</td>
<td><img src="chart1.png" alt="Graph showing nOD\textsubscript{450}" /></td>
</tr>
<tr>
<td>positive</td>
<td>13/276 initially positive positive patients tested negative ?$\approx$4.7% in 7.9 months</td>
<td><img src="chart2.png" alt="Graph showing nOD\textsubscript{450}" /></td>
</tr>
</tbody>
</table>

Trampe et al, Neurology 2012
Second generation ELISA

- Technical improvements: e.g. precoated plates, purification of antigen, cross-reactivity, low titre range

- Same material: Gen2 assay: 57.4%, Gen1 assay 54.7%

- 64 pre-PML samples: 63 positive, 1 negative (15 months), then positive (2 months)

? Independent reproduction: seroconverters, seroreverters, intermittent serostatus
Currently: 6 monthly testing!

Lee et al, J Clin Virol 2013:57:141
High anti-JCV-antibody titers in Pre-PML sera

Trampe et al, Neurology 2012
JCV-antibody reactivity in pre-PML sera: Swedish cohort

Warnke C et al. J Neurol Neurosurg Psychiatry
doi:10.1136/jnnp-2012-304332
L-Selectin is a possible biomarker for individual PML risk in natalizumab-treated MS patients

Schwab / Wiendl Neurology 2013: 81: 1-7

Figure 1: Surface expression of CD62L and its correlation to progressive multifocal leukoencephalopathy development in multiple sclerosis patients receiving natalizumab therapy.

A

B

% CD62L+ of CD4+ T cells

% CD62L+ of CD4+ T cells

Healthy controls (n=21)
MS (untreated) (n=28)
MS (glatiramer acetate, interferon β-1b) (n=21)
MS (natalizumab) (n=22)
PML (natalizumab) (n=9)

A

90th percentile
75th percentile
Mean
Median
25th percentile
10th percentile

B

ρ=0.001

ρ=0.016

ρ=0.0024
High Saturators Cluster with high Concentration, Low Body Weight

RMMSC cohort
n=122 (28-30 day infusion cycle)

Top 30th Percentile

% Saturation
- > 95%
- 90% - 95%
- 85% - 90%
- ≤85%

Mass (kg)

% Saturation
- > 95%
- 90% - 95%
- 85% - 90%
- ≤85%

Top 30th Percentile

Mass (kg)

n=122 (28-30 day infusion cycle)

Courtesy of J. Foley, presented at AAN 2013
Could body weight differences partially explain the US/EU paradox?

Natalizumab-related PML patients appear to have a lower median body mass

* p<0.001
* NS

US clinic is significantly different than PML and Swedish population

**Courtesy of J. Foley, presented at AAN 2013**
Biomarkers: lost in translation?

- Prospective validation for a low-event sADR (ethical, logistic concerns)
- Feasibility of biomarker (stability, technique)
- Approval/incorporation into guidelines
- Translation into clinical practice (complexity of data)
Summary

• Early diagnosis: impact on prognosis
• although not evidence based, immune-reconstitution and supportive treatment
• IRIS: no prophylactic treatment
• current 3-parameter risk stratification, repeat serology every 6 months
• future developments: Ab-index, adhesion molecules, body mass index
  Ë prospective validation warranted