Natalizumab and Progressive Multifocal Leukoencephalopathy

What Are the Causal Factors and Can It Be Avoided?

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Natalizumab (Tysabri) was the first monoclonal antibody approved for the treatment of relapsing forms of multiple sclerosis (MS). After its initial approval, 3 patients undergoing natalizumab therapy in combination with other immunoregulatory and immunosuppressive agents were diagnosed with progressive multifocal leukoencephalopathy (PML). The agent was later reapproved and its use restricted to monotherapy in patients with relapsing forms of MS. Since reapproval in 2006, additional cases of PML were reported in patients with MS receiving natalizumab monotherapy. Thus, there is currently no convincing evidence that natalizumab-associated PML is restricted to combination therapy with other disease-modifying or immunosuppressive agents. In addition, recent data indicate that risk of PML might increase beyond 24 months of treatment.

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The initial section of this review focuses on the scientific rationale for natalizumab in MS treatment. In the second part, our understanding of PML will be outlined. Third, recent results on altered immune surveillance while undergoing natalizumab treatment are reviewed. In the fourth section, the link of viral reactivation and very late activation antigen 4 (VLA-4) antagonism will be discussed. Finally, this review will address the potential impact of our current knowledge on the use of natalizumab in clinical practice.

THE NATALIZUMAB EXPERIENCE

Multiple sclerosis is an inflammatory demyelinating disorder of the central nervous system (CNS) and one of the most common causes of sustained neurological disability in young adults.1 The presence of leukocytes in cerebral perivascular spaces (CPVS) in areas of disease activity is one of the pathological hallmarks.2–4 An absolute requirement for the influx of leukocytes from the peripheral blood into the CNS is their expression of adhesion molecules, called integrins, and their interaction with counter-receptors from the immunoglobulin supergene family proteins on endothelial cells. α4β1 Integrin (VLA-4) is 1 of the 4 main integrins required for the firm arrest of leukocytes following their rolling adhesion.5

Natalizumab (Tysabri) is a recombinant humanized monoclonal IgG4 antibody that binds, among others, to the α4 subunit of the α4β1 integrin and interferes with the α4-mediated binding to its natural ligands of the extracellular matrix and endothelial lining, vascular cell adhesion molecule 1, and fibronectin.6,7 Although inhibition of leukocyte migration and extravasation is believed to be the leading mode of action of natalizumab, additional mechanisms might modulate the therapeutic and adverse effects of this antibody. Lindberg et al8 recently showed that natalizumab has a direct effect on gene ex-
pression relevant for function and differentiation of T lymphocytes, B lymphocytes, neutrophils, and erythrocytes.

In vivo, antibodies against VLA-4 interfere with the binding of leukocytes to cerebral blood vessels and effectively prevent signs of experimental autoimmune encephalomyelitis, an animal model of MS. Although natalizumab is highly immunogenic in mice, it reduces the influx of T cells and monocytes into the CNS and substantially ameliorates the clinical course of experimental autoimmune encephalomyelitis.10

The efficacy of natalizumab in experimental autoimmune encephalomyelitis led to clinical trials for the treatment of MS. Following very promising results in phase 2 studies,11-13 phase 3 clinical trials were performed that compared natalizumab alone vs placebo (AFFIRM trial14) and natalizumab plus interferon beta-1a vs placebo plus interferon beta-1a (SENTINEL trial15). Both studies showed significant advantage for the natalizumab-treated groups with respect to the primary clinical end points. In the AFFIRM monotherapy trial, natalizumab reduced the rate of clinical relapses at 1 year by 68% and the risk of sustained progression of disability by 42% over 2 years. Post hoc analysis of the AFFIRM trial showed disease remission defined as no activity on clinical (no relapses and no sustained disability progression) and radiological measures in 37% of the natalizumab group compared with 7% of the placebo group.16

On November 24, 2004, the Food and Drug Administration approved natalizumab for the treatment of relapsing forms of MS. On February 28, 2005, the manufacturers of natalizumab announced the voluntary withdrawal from the market after 2 patients with MS in the SENTINEL trial (combination therapy with interferon beta-1a) and 1 patient with Crohn disease were diagnosed with PML.17,10

In summer 2006, natalizumab was reapproved in the United States and approved in the European Union as monotherapy for the treatment of relapsing forms of MS. In the United States, recommendations were made to limit the use of natalizumab to highly active (more than 2 severe relapses per year) relapsing-remitting MS and for patients not responding to or tolerating first-line treatment (interferon beta-1a, interferon beta-1b, and glatiramer acetate). This restricted approval was the result of a risk-benefit analysis. The initial diagnosis of PML in 2 patients from the combination therapy trial17,18 led to the restricted approval and to risk minimization plans (Tysabri Outreach: Unified Commitment to Health [TOUCH], Tysabri Global Observation Program in Safety [TYGRIS], and Crohn’s Disease–Investigating Natalizumab through Further Observational Research and Monitoring [CD INFORM]). Systematic review of all patients treated in those studies (more than 3700 patients) showed no additional cases; therefore, calculated risk of PML was 1:1000 after an average treatment time of about 18 months.20 Because there is no increased risk of JC-polymavirus replication and spread throughout the CNS in patients with MS per se,21 there is little doubt that treatment with natalizumab is linked to these cases of PML.

Not unexpected, new cases of PML occurred since reapproval and, because of the restricted use of natalizumab, all of these patients received natalizumab in mono-therapy.22-25 Based on reports of 31 confirmed PML cases receiving natalizumab therapy by January 21, 2010, the Food and Drug Administration announced that the risk of PML might increase beyond 24 months of treatment, overall confirming the calculated risk of 1 in 1000 patients from clinical trial experience.26 Thus, in the nearer future, additional cases of PML in patients receiving natalizumab therapy are most likely to occur. This development highlights the necessity to further investigate mechanisms that predispose certain patients to an increased risk of developing PML while receiving natalizumab therapy.

PML: PRIMARY INFECTION AND PLACES OF PERSISTENT INFECTION

Progressive multifocal leukoencephalopathy is an opportunistic demyelinating disease of the brain. It is caused by lytic infection of oligodendrocytes by JC virus (JCV), a double-stranded, not enveloped DNA virus belonging, together with the well-known BK virus and 3 additional viruses just recently defined (K1 virus, WU virus, and Merkel cell polyomavirus), to the group of human polioviruses.27-31 Progressive multifocal leukencephalopathy has almost exclusively been reported in immunocompromised patients, in particular in patients with reduced cellular immunity, including patients with human immunodeficiency virus (HIV), patients with hematological diseases, or patients receiving immunosuppressive medication.32-35 Therefore, detection of JCV-specific cytotoxic CD8+ T lymphocytes in patients with PML is associated with a favorable outcome and early disease control36 and assays measuring T-cell immune functions are currently being investigated as monitoring tools for immunocompromised populations to estimate their risk of polyomavirus infections.37,30

Primary infection by JCV is still not well characterized. In current concepts, initial infection takes place in childhood and is asymptomatic. JCV infection is a common infection, although exposition rates measured as JCV seroprevalence vary throughout the literature, ranging from 33% to more than 80%.40-44 This can be explained by different methods used, resulting in differences in specificity (eg, because of cross-reactivity between different polioviruses) and sensitivity (eg, because of differences in affinity of the antibody to the targeted epitope), but also by epidemiological evidence for variation in geographical exposure.45 Route of primary infection is unknown, although high seroprevalence argues for a common route, such as oropharyngeal or respiratory. Not yet proven, initial infection could possibly take place in tonsillar tissue.46 Lymphocytes are generally believed to be a carrier of JCV to other places of persistency. B cells are the most discussed candidates. These cells do not necessarily need to establish a replicating infection but could possibly carry the virions cell surface associated.47 There is good evidence that persisting and replicating asymptomatic infection of renal tissue results in periodic shedding of JCV in urine. This shedding has been reported in healthy as well as in immunosuppressed individuals.48-50 A recent study suggests that shedding of JCV in the urine increases at 12 months of treatment with na-
talizumab, followed by JC viremia in peripheral blood mononuclear cells at 18 months,37 prompting the hypothesis that viruria might precede viremia, ultimately causing CNS infection with JCV and subsequently PML. However, these results remain controversial, as neither increase in viruria nor positive JCV DNA findings in peripheral blood mononuclear cells have been confirmed in large collectives.51-53 Consequently, JCV load in urine, peripheral blood mononuclear cells, or plasma might correlate with immunosuppression but may have limited predictive value as a screening tool for PML.59

Currently, our knowledge about transmission of JCV infection and its replication cycle in the healthy human population is limited. The transmittable form of JCV is commonly referred to as the JCV archetype, as it is thought that all other genotypes originate from it. The JCV archetype is detectable in the urine. In contrast, the JCV-PML type appears more neurotropic and can be isolated from brains of patients with PML. Classification schemes on nomenclature and characteristics for different genomic JCV variants are evolving. Pathological JCV-PML-type variants are characterized by deletions, duplications, and point mutations in the noncoding regulatory region and/or the coding region, defining pathogenicity and neurotropism.55

There is growing evidence on the persistency of JCV in the CNS.50-58 A more recent study investigated viral protein and DNA load in the brains of immunocompetent patients without PML. No viral proteins were expressed in any of these cases. Nevertheless, fragments of the viral DNA were present in various regions of the normal brain. JCV DNA was found in oligodendrocytes and astrocytes, but not in neurons.56 These findings suggest that JCV has access to the brain in immunocompetent individuals. In the setting of immunosuppression, it is conceivable that either the passing of virus during JC viremia or resident virus persisting in the normal brain may express its genome and initiate its lytic cycle in oligodendrocytes.36

The prognosis of PML is poor. Notwithstanding that mortality has substantially dropped since the introduction of highly active antiretroviral therapy (HAART) for treatment of AIDS, survival of patients with PML 1 year after diagnosis ranges only from 38% to 62%.34 No pathognomonic initial symptoms of PML have been defined, which often makes an early clinical diagnosis of this disorder very challenging. Some of the classic clinical signs and symptoms of PML include rapidly progressive dementia, motor dysfunction, and vision loss, which can be difficult to differentiate from MS relapses.34,59,60

Critical for the diagnosis of PML is the demonstration of virus by JCV polymerase chain reaction (PCR) in the CSF and surrogate disease markers by magnetic resonance imaging (MRI). Before the introduction of HAART, JCV PCR had a sensitivity of 72% to 93% and a specificity of 92% to 100%. Since HAART therapy, sensitivity is reported to be lower (about 58%), most likely because of an improved immune response and lower copy levels of JCV in CSF while undergoing antiretroviral treatment.34 A recent study showed positive JCV PCR results in some patients with MS without any clinical or radiological evidence for PML. Thus, low numbers of JCV copies within the CSF might also be part of the physiological replication process.60 Because there are only a limited number of cases in natalizumab-treated patients so far, sensitivity and specificity of CSF JCV PCR for this subpopulation is unknown, although cases of false-negative PCR in recently reported patients with PML proved limited sensitivity and gives reason for concern.22

Magnetic resonance imaging is a sensitive detection method, but many surrogate disease markers are not specific for PML. Typically, there are hyperintense, multifocal asymmetric signal abnormalities throughout the supratentorial subcortical white matter on T2-weighted and fluid-attenuated inversion recovery sequences.62,63 While there is a relative absence of gadolinium uptake in PML, in cases of immune reconstitution inflammatory syndrome, contrast enhancement is frequently observed. This syndrome is defined as a combination of clinical worsening in a patient with PML despite recovery of the immune system, explained by an overwhelming inflammatory immune response.64 This syndrome was first seen in patients with AIDS after initiation of HAART but has also been reported in natalizumab-treated patients.22,23

Restoring the immune system is the only proven intervention for PML.65 The downside of restoring an immune response against JCV is the risk of immune reconstitution inflammatory syndrome. There is some evidence that immune reconstitution inflammatory syndrome can be attenuated through the administration of corticosteroids.23,66 While immune reconstitution is realized by HAART therapy in patients with AIDS, early cessation of immunosuppressive medication was associated with favorable clinical outcomes in transplant recipients.67,68 Plasma exchange has been proposed as a therapeutic tool that may allow a faster restoration of immune effector function in natalizumab-treated patients.23,65,66 Unfortunately, it appears that the pharmacological half-life of natalizumab far exceeds its biological half-life.70 Therefore, even accelerated clearance of treatment may not have a beneficial effect in all affected individuals. Currently, a randomized multicenter trial is under way for the treatment of PML with meloquine hydrochloride. The primary outcome measure is the JCV PCR product in the CSF over up to 24 weeks.71 The trial is based on positive in vitro data on the efficacy of meloquine on JCV replication.72

**IMPAIRED IMMUNE SURVEILLANCE IN NATALIZUMAB-TREATED PATIENTS WITH MS**

Reactivation of a latent infection in natalizumab-treated patients is likely linked to its therapeutic principal of action. Natalizumab was specifically designed to reduce trafficking of lymphocytes into peripheral tissues; therefore, it was postulated that treatment with natalizumab results in reduced immune surveillance of the CNS. Indeed, CSF from natalizumab-treated patients with MS contained significantly fewer white blood cells, CD4+ T cells, CD8+ T cells, CD19 B cells, and CD 138 plasma cells compared with patients with MS not treated with natalizumab.52 Furthermore, the CD4:CD8 ratio in the CSF of natalizumab-treated patients was significantly reduced...
to levels comparable with those of patients with human immunodeficiency virus.\textsuperscript{73} In contrast to patients with human immunodeficiency virus, there was no reversed CD4:CD8 ratio in peripheral blood. It was also shown that CD4\textsuperscript{+} cells express significantly less unbound α\textsubscript{4} integrin before and after natalizumab therapy compared with CD8\textsuperscript{+} cells.\textsuperscript{73} Therefore, an absolute threshold of unbound VLA-4 may be required for migration across the endothelial barrier, perhaps partially explaining reduced migration of CD4\textsuperscript{+} T cells into the CNS. Surprisingly, reduced cell counts were still detectable 6 months after cessation of natalizumab treatment.\textsuperscript{70} This was unexpected, as natalizumab has a biological half-life of 11 days and its biological activity therefore cannot be expected to continue beyond 6 weeks after cessation.\textsuperscript{74}

The preferential and prolonged effect of natalizumab on CD4\textsuperscript{+} T cell number in the CNS could possibly be explained by a decrease in the number of antigen-presenting cells and the expression of major histocompatibility complex class II antigens in CPVS. Autopsy material from a patient who died of PML while receiving natalizumab treatment\textsuperscript{17} was investigated for major histocompatibility complex expression and the number of antigen-presenting cells within the cerebrovascular spaces\textsuperscript{29} and compared with anatomically matched healthy brain tissue from patients with MS not treated with natalizumab and tissue from patients with PML not associated with natalizumab treatment. Major histocompatibility complex II expression and the number of antigen-presenting cells in CPVS were significantly reduced compared with control brains. In addition, not a single CD4\textsuperscript{+} T cell was detectable in the CPVS of natalizumab-treated patients with PML, whereas CD8\textsuperscript{+} T cell numbers were not reduced in the CPVS as compared with controls. The latter might not be surprising, because major histocompatibility complex I was shown to be significantly upregulated in patients with PML treated with natalizumab compared with all other controls. While it is difficult to detect JCV-responsive CD4\textsuperscript{+} T cells in patients with PML,\textsuperscript{70} JCV-specific CD8\textsuperscript{+} T cells are detectable in the peripheral blood of patients with PML infected with human immunodeficiency virus, and their presence has been associated with a more favorable outcome.\textsuperscript{36,77,78} CD8\textsuperscript{+} T cell responses are directed toward an HLA-A\textsuperscript{0}0201–restricted JCV epitope, VP1\textsubscript{39}.\textsuperscript{70} However, initiation and perpetuation of antigen-specific CD8\textsuperscript{+} T cell responses are likely to require the help of CD4\textsuperscript{+} T cells in the form of cytokines and other inflammatory mediators.\textsuperscript{80}

**VLA-4 ANTAGONISM, VIRAL REACTIVATION, AND MALIGNANCY**

There are 2 possible mechanisms of JCV reactivation discussed in the literature. Either the persisting virus within the CNS or passing virus during JC viremia is responsible for JCV reactivation in the setting of immunosuppression or impaired immunosurveillance. Interestingly, CD34\textsuperscript{+} cells are susceptible to JCV infection,\textsuperscript{84} and JCV DNA is detectable in the bone marrow from patients with and without PML.\textsuperscript{82} In addition, CD34\textsuperscript{+} hematopoietic precursor cells express high levels of VLA-4 on their surface\textsuperscript{83,85} and CD34\textsuperscript{+} bone marrow cells have, in comparison with circulating cells in the peripheral blood, a higher VLA-4 expression rate and VLA-4 avidity.\textsuperscript{86} Bonig and coworkers\textsuperscript{87} and our group recently showed that natalizumab mobilizes CD34\textsuperscript{+} hematopoietic progenitor cells.\textsuperscript{88} Binding of natalizumab to α\textsubscript{4} integrin may block the VLA-4–mediated interaction of CD34\textsuperscript{+} cells of the bone marrow with its ligands in the extracellular matrix, eg, vascular cell adhesion molecule 1, and may lead to mobilization of CD34\textsuperscript{+} cells to the peripheral blood. Antibody-mediated blockage of CD34\textsuperscript{+} cell homing into the bone marrow could play an additional role.\textsuperscript{89,90} Mobilization of hematopoietic progenitor cells by monoclonal antibodies against VLA-4 in primates and mice was also demonstrated.\textsuperscript{91-93}

As outlined earlier, natalizumab upregulates transcription factors important for the differentiation of B lymphocytes.\textsuperscript{8} Thus, during natalizumab-induced B cell differentiation, JCV-infected bone marrow cells might be activated, leading to JC viremia and PML as a consequence of natalizumab therapy.\textsuperscript{94-96} In this context, rearrangement of archetype JCV to PML-type genotypes could occur.\textsuperscript{97} This hypothesis could also help to explain PML cases undergoing treatment with monoclonal antibodies against CD20 and CD52. After initial depletion, reconstitution of the B cell line might cause JC viremia and PML.\textsuperscript{98} However, so far there is no conclusive evidence for an increased incidence of JC viremia in natalizumab treatment. Furthermore, given the fact that natalizumab significantly reduces the extravasation of cells that express VLA-4, the presence of CD34\textsuperscript{+} cells in peripheral tissues while receiving natalizumab therapy needs to be verified.

Thus far, there are only a few reports on the reactivation of CNS latent virus other than JCV in natalizumab-treated patients. Human herpes virus 6 (HHV-6) is a pleiotropic β-herpes virus commonly reactivated in the setting of acute and prolonged immunosuppression.\textsuperscript{98,99} Human herpes virus 6 has also been suggested to be involved in the pathogenesis of MS,\textsuperscript{100,101} and it has also been associated with PML pathogenesis.\textsuperscript{102} Elevated serum HHV-6 IgG levels and HHV-6A DNA in the CSF of a subset of patients treated with natalizumab were recently reported. Also, in vitro superinfection of JCV-infected glial cells with HHV-6 increased JCV expression.\textsuperscript{103} Interestingly, HHV-6 has also been detected in CD34\textsuperscript{+} hematopoietic progenitor cells.\textsuperscript{104,105}

JCV is probably not the only latent virus reactivated in natalizumab-treated patients. Mobilization of virus-infected bone marrow cells might be a natalizumab-associated, but not a virus-specific, adverse effect. Altered immune surveillance combined with potential latent viral reactivation could also enhance the risk of malignancy. Thus far, there have only been 4 reported cases of melanoma in natalizumab-treated patients, which may present association by chance.\textsuperscript{100,107}

**RISK STRATIFICATION IN PATIENTS RECEIVING NATALIZUMAB THERAPY—FOCUS ON SEROLOGICAL TESTING**

Based on reports of confirmed PML cases, risk of PML might increase beyond 24 months of treatment.\textsuperscript{23,26} Thus, attempts to stratify patients receiving natalizumab therapy
at higher or lower risk for developing PML have been undertaken. At the 2010 American Academy of Neurology meeting in Toronto, Ontario, Canada, Gorelik and co-workers108 (Biogen Idec) presented promising data on JCV serological testing as a potential tool for risk stratification. The enzyme-linked immunosorbent assay using JCV-like particles detected anti-JCV antibodies in 54% of patients with MS, with false-negative results in 2.5% of patients.108 In 13 of 13 tested samples, 16 to 180 months prior to developing PML while receiving natalizumab therapy, JCV serological test results by this assay were reported to be positive. Annual seroconversion rate for JCV was 2%.108 Thus, applying this test in clinical practice could help to define a group of around 46% of patients with MS receiving natalizumab therapy with a significantly lower risk of developing PML. Still, when considering applying this test in clinical practice, the following critical issues need to be addressed:

1. Results from prospective testing in different cohorts (high numbers of patients, geographical diversity) are required to confirm sensitivity and specificity of the assay.

2. A variety of methods to detect JC antibodies have been published, resulting in controversial results for seropositivity in the past. Thus, JCV serological testing will need to be performed in a strictly standardized manner at selected centers applying the same protocols. Physicians need to be educated not to rely on unvalidated testing.

3. Differential risk management strategies for both the JCV-seropositive majority and the seronegative minority need to be defined. First approaches are outlined in the following paragraph.

**DIFFERENTIAL RISK MANAGEMENT STRATEGIES FOR JCV-SERONEGATIVE AND SEROPosITIVE PATIENTS WITH MS RECEIVING NATALIZUMAB THERAPY**

Existing data indicate that natalizumab is immunosuppressive and that these properties may be a contributing factor in the susceptibility to developing PML. As outlined earlier, JCV serological testing could be the first tool available to stratify patients at lower or higher risk for this adverse effect. Because this testing proves to be of value when applied in clinical practice, it would result in a seronegative group of around 46% of patients with a low risk of developing PML (“low-risk group”) and in a group of 54% of seropositive patients most likely to be at higher risk than 1 in 1000 of developing PML (“group of higher risk”).

The established 3-step diagnostic algorithm for natalizumab-treated patients with new or worsening neurological signs and symptoms, as proposed by Kappos et al.,54 should certainly remain unchanged for both groups. This also includes early suspension of natalizumab treatment and strategies for clinical, MRI, and laboratory assessments.

Because of evidence for increased risk while receiving treatment with natalizumab beyond 24 months,23,26 annual MRI should be performed in asymptomatic patients beyond this duration of treatment regardless of the serological status. In addition, patients should be informed about the potential risk of PML, and alternative treatment options for the individual patient should be discussed with the well-informed patient. For the low-risk group, annual serological retesting seems to be feasible to exclude seroconversion and transfer into the group of higher risk.

So far, as outlined earlier, there are no reliable tools available to further stratify PML risk in the group of higher risk. Attempts to do so should only be performed in the setting of clinical studies. JCV PCR in CSF, peripheral blood, urine, or methods to assess T-cell immune function in asymptomatic patients are not yet able to predict risk of PML. Thus, only close clinical monitoring by specialized neurologists, possibly combined with short but standardized MRI protocols (eg, every 6 months), might further help to minimize risk in this group. In addition, it has not been shown that prolonged continuous therapy with natalizumab is required to ensure its efficacy. Therefore, alternative treatment paradigms appear feasible: (1) Based on published observations, we know that natalizumab has an immediate effect on the number and composition of leukocytes in the CSF.77 (2) It is also known that the effect of natalizumab on leukocyte numbers in the CSF after cessation of treatment persists for at least 6 months125 and (3) that cell numbers normalize 14 months following the discontinuation of therapy.86 In addition, it was demonstrated that the patients are clinically stable while receiving first-line disease-modifying therapies during the 14-month period after cessation of natalizumab therapy.109 While an increase in T2 lesion load on MRI 15 months after cessation of treatment has been shown in 1 study,110 there was no change of surrogate disease markers on MRI in another.109 Thus, it may be necessary to—at least in less severely affected patients of the higher-risk group—limit the use of natalizumab for a certain period, followed by a treatment holiday during which patients are treated with 1 of the conventional disease-modifying therapies. In patients with very aggressive disease, natalizumab may only be used as an induction therapy. Such treatment algorithms remain speculative at present and controlled clinical trials must be performed to shed further light on these clinically relevant questions. One trial, the Treatment Interruption of Natalizumab (RESTORE) trial, accessing markers of immune function and disease activity during natalizumab treatment interruption and after natalizumab treatment is resumed, is currently recruiting, with an estimated primary completion date in December 2011.111 Thus, clinical trials like the RESTORE trial could be offered to patients considering treatment holiday.

**CONCLUSIONS**

Currently, natalizumab is only recommended as monotherapy in patients with MS not responding to first-line treatment or treatment-naive patients with high clinical disease activity or in those not capable of tolerating conventional therapy. This restricted approval originated from the observation of patients who developed PML while receiving natalizumab therapy in combination therapy...
with interferon beta-1a in the context of clinical studies.\textsuperscript{13} Recently, 5 more cases of PML in patients with MS who had received natalizumab in monotherapy were reported.\textsuperscript{22-25} Thus, there is currently no convincing evidence that monotherapy is safer in this regard than combination therapy with disease-modifying agents. Progressive multifocal leukoencephalopathy is not a natalizumab-specific adverse effect; it has been diagnosed in the context of many other immunomodulatory drugs as well. Clearly, further studies are warranted to understand the immunological effects of natalizumab besides blocking cell migration across the blood-brain barrier.

As outlined earlier, impaired immune surveillance and viral reactivation caused by treatment with natalizumab are yet not fully understood. However, there is growing suspicion that long-term treatment with natalizumab may put patients at risk of PML.

These key findings suggest long-term effects on CNS intrinsic immune system and viral reactivation. Because there is currently no proven treatment for patients with PML receiving natalizumab other than accelerated clearance of therapy,\textsuperscript{26-28} risk stratification for lower and higher risk for developing PML and regimens to establish an early diagnosis are crucial. JCV serological testing using standardized protocols at certain centers might be the first step to stratify risk. This could result in differential risk management concepts for the JCV-seronegative or the JCV-seropositive group, including alternative treatment algorithms for natalizumab administration in the seropositive group. This needs to be evaluated in controlled clinical trials. The major challenge in the near future will be to identify biomarkers associated with an elevated risk of developing JCV infection of the brain to further stratify patients at risk.

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