Drug Discovery Today: Disease Models
Vol. 32, 2020

Neurovirology

Natalizumab related progressive multifocal leukoencephalopathy

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Progressive multifocal leukoencephalopathy (PML), is an opportunistic brain infection that is caused by the JC virus. It usually occurs in patients with an underlying disease and therapies used to treat such diseases that inhibit normal immune system function. For example, multiple sclerosis patients treated with natalizumab have been identified at risk for PML. This serious adverse event has been very instructive in improving understanding of PML pathogenesis, biomarkers and patient management with this disease in recent years.

Mechanism of viral pathogenesis of PML and the immune system

Progressive multifocal leukoencephalopathy, PML, is an opportunistic brain infection that is caused by the JC virus. The initial site of JCV infection has not been documented but assumed to be through the respiratory tract or direct oral route. Sero-epidemiology studies showed that the majority of the global population by their 3rd decade has been exposed to JCV [1]. The passage of JCV from an initial site of infection ultimately to the brain is shown in Fig. 1 [2]. Data that built the 'puzzle piece' collective mechanism came from many collaborative investigators over years of work [3–7]. Since there is no animal model of PML due to the restricted host range of JCV to specific human cells, critical observations on JCV pathogenesis have largely come from cell culture studies and investigations of clinical samples from PML patients.

JCV can establish a latent or persistent infection in the kidney uroepithelium. In some patients, however, JCV enters the peripheral circulation so these individuals become viremic. The frequency of viremia at any time in the population maybe 2–3% [8]. Most viremic individuals seem to clear the infection. In some rare circumstances, perhaps with a lack of effective immune clearance, JCV establishes another latent or persistent infection, this time in bone marrow or other lymphoid organs. There is some evidence that the brain may harbor JCV in these states but that has not been rigorously determined. In fact, JCV was not found in nearly 100 autopsied MS brain tissues nor in over 200 autopsied glial tumors [9,10]. Once in the brain, however, JCV selectively infects the myelin, producing cell in the brain, the oligodendrocyte, and to a lesser degree, the

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 https://doi.org/10.1016/j.ddmod.2020.02.002

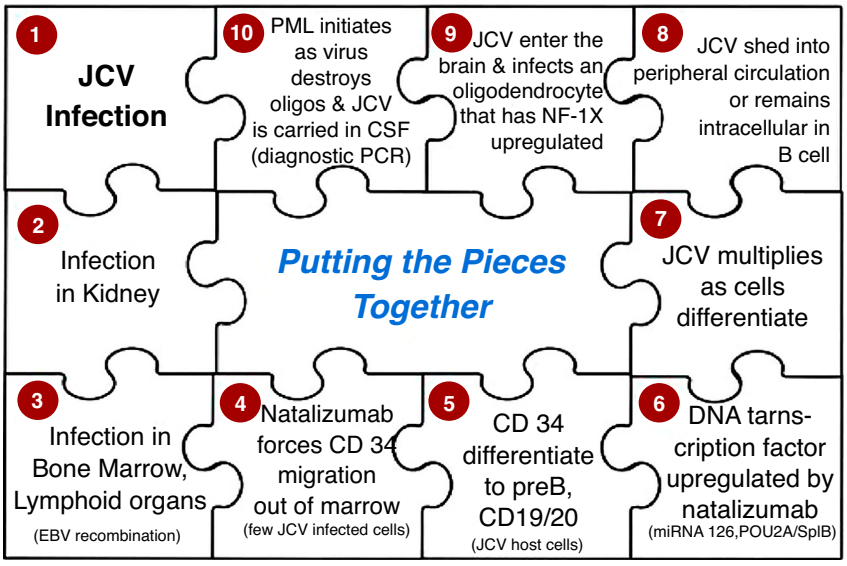

Fig. 1. JC Virus pathogenesis of progressive multifocal leukoencephalopathy (PML).
Steps 1–3 show initiation of JC virus (JCV) infection, and establish latency in kidney that can become persistent in lymphoid organs, notably the bone marrow, in which the viral regulatory region sequences become rearranged from the nonvirulent to a neurovirulent or PML variant. Steps 4–6 involve the biological effects of natalizumab treatment over time, forcing migration of CD 341 and pre-B cells into the circulation since it prevents homing of these cells in the marrow due to blocking of binding to cell adhesion molecules. At these steps, natalizumab is associated with temporal gene regulation of several factors including those that augment JCV replication that may account for the high incidence of PML in patients with multiple sclerosis treated with natalizumab for 24 doses or more. Steps 7–10 show the progression of JCV to the brain and the establishment of PML. The authors thank David Carter, In Tune Communications, Canada, for assembly of the figure. EBV, Epstein-Barr virus.

astrocyte leading to clinical disease identified as PML. All along the pathway of JCV from the kidney to the brain, the cells that support JCV infection have specific DNA binding transcription factors that recognize the viral non-coding regulatory region, NCCR, that uses those factors for successful infection as identified in Fig. 1.

PML usually occurs in patients with an underlying disease and therapies used to treat such diseases that inhibit normal immune system function. A common feature in all PML cases, regardless of the underlying disease or therapies, is that patients continued to demonstrate humoral antibody responses including very high antibody titers to the JCV virus but lacked specific CD 4 and CD 8 responses against JCV antigens [11]. T cell mediated immunity appears to be a key feature in limiting JCV infection. It was assumed that the diminished T cell responses augmented JCV reactivation from a persistent state in the kidney, bone marrow or perhaps other immune system organs, seemingly escaping immune clearance in the absence of specific T cells resulting in possible viral multiplication. If JCV gains access to the brain, virions can infect the myelin-producing cell, the oligodendrocyte, resulting in a lytic, necrotic cell death leading to demyelination [4].

The first descriptions of PML were in leukemia and lymphoma patients, and then reported in organ transplant recipients; both groups treated with immunosuppressive drugs [12]. In AIDS patients in the mid-1980s before antiretroviral (ARVs) treatment, the incidence of PML was approximately 3–5% in HIV-1 seropositive individuals making it an AIDS defining illness. With the widespread use of ARV treatment by the 1990s allowing immune reconstitution and better control of JCV infection, the reported incidence of PML dropped more than ten-fold [13]. Another example is in multiple sclerosis patients specifically treated with natalizumab. This serious adverse event of natalizumab treatment in patients with multiple sclerosis has been very instructive in improving understanding of PML pathogenesis and patient management in recent years [14].

Natalizumab related progressive multifocal leukoencephalopathy

Natalizumab (Tysabri®; Biogen, Cambridge, MA, US) is a humanized neutralizing monoclonal antibody (IgG4κ) directed against α4 integrin expressed on T cells surface that plays a crucial role in leukocytes transmigration across the endothelial barrier in central nervous system and gut. By binding to α4 subunit, natalizumab inhibits their association with endothelium receptors and stops leukocytes trans-migration from blood to the brain, reducing the CNS inflammation in multiple sclerosis (MS) and has been found to be effective in Crohn's disease.

Table 1. PML risk estimates per 1000 patients in anti-JC virus antibody positive patients [21].

Duration of Tysabri use	No prior use of immunosuppressants				Prior use of immunosuppressants
	No index value	Index <0.9	Index >0.9–1.5	Index>1.5	
1–12 months	0.1	0.1	0.1	0.2	0.3
13–24 months	0.6	0.1	0.3	0.9	0.4
25–36 months	2	0.2	0.8	3	4
37–48 months	4	0.4	2	7	8
49–60 months	5	0.5	2	8	8
61–72 months	6	0.6	3	10	6

Natalizumab was approved for the first time in 2004 for MS treatment after promising results obtained from 2 phase III trials: AFFIRM and SENTINEL which showed significant efficacy in reducing MS burden of disease [15,16]. It remained on the market until it was voluntarily removed by Biogen in 2005, because of the appearance of PML in 2 MS patients. In 2006, Natalizumab was reintroduced into the market under a risk evaluation and mitigation (REM) program in the US, known as Tysabri Outreach: Unified Commitment to Health (TOUCH®).

As of March 2019, approximately 196,600 patients received natalizumab with a total of 725,272 patient-years of exposure in the post-approval setting worldwide with the global overall incidence of PML in natalizumab treated patients being 4.14 per 1000 (95% Confidence Interval [CI] 3.86–4.43 per 1000 patients) with 814 confirmed PML cases [17].

Three factors were demonstrated to increase the risk of natalizumab related PML – positive anti-JCV antibody status in ELISA assay (STRATIFY JCV® DXSelect ™Antibody Assay; Focus Diagnostics, Cypress, CA, USA); prior immunosuppressive therapy; and duration of therapy with natalizumab over 24 months [18]. Plavina et al. went further to correlate the incidence of PML to the level of anti JCV antibodies in serum, measured as ''index'' [19]. As seen in Table 1, among patients with positive anti-JCV antibodies index value between 0.9 and 1.5, the estimated risk of PML was 0.1 per 1000 patients, while patients with index $\geq$1.5 had an increased risk of 1/1000 without previous immunosuppressive therapy and 24 months natalizumab treatment and reached an incidence of 10/1000 over 61–72 months [19,20].

PML in MS patients is not unique to NTZ since it has been reported with other disease-modifying treatments, such as dimethyl fumarate and fingolimod [22], but the risk is significantly lower than what is seen with NTZ. The ability of natalizumab to prevent effective CNS immunosurveillance was initially considered to be the underlying explanation for the occurrence of PML with this agent [23]. While impaired neuroimmune-surveillance almost certainly contributes to the development of PML in patients treated with NTZ, the antibody has other effects that may predispose to the development of the disease. For example, JCV can infect CD34 hematopoietic stem cells, pre B and more mature B cells so if released into the peripheral circulation, the virus undergoes replication of its genome DNA and multiplication of new virions [24]. Interestingly, in NTZ treated MS patients, NTZ mobilizes CD 34 cells from the bone marrow at levels 3–10 fold higher than normal allowing it to be more likely for the JCV to enter the brain in these cells or as free virus. Search for biomarkers to use for risk stratification of NTZ related PML to help with more accurate individualized risk analysis to allow a higher number of patients to utilize NTZ safely has also led to a better understanding of PML pathogenesis.

Biomarkers

At the moment, the anti-JCV antibody index measurement is the only humoral parameter used for risk stratification of PML disease to help with more accurate benefits/risk analysis of NTZ.

Some of the potential biomarkers are listed in Table 2.

For example, L Selectin (CD62L) is a cell adhesion molecule expressed on T lymphocytes, which plays an important function in their adhesion and migration along the vascular endothelium and re-entry in secondary lymph nodes. Because of its involvement in T cell trafficking, L selectin could have a crucial role in brain inflammation status. A dramatic reduction of L selectin expression was annotated in natalizumab treated patients who will then develop PML. A study done by Schwab et al. found that a patient had a 55 fold

Table 2. Potential Biomarkers for Progressive Multifocal Leukoencephalopathy.

- L – selectin (CD62 L) [25–27]
- Host microRNA-320, 320b, 126, and 10b [5,28,29]
- JCV-specific effector T memory cells [11,30,31]
- usCRP [32]
- Agnoprotein [33]
- Urinary JCV DNA detection [34]

JCV, JCPyV, human polyomavirus; PML, progressive multifocal leukoencephalopathy; usCRP, ultra-sensitive C-reactive protein.

higher risk to develop PML when CD62L expression was low [25]. However, concerns for differences in technique and variability in CD62L led to the consideration that this marker is too variable to be considered a good biomarker for PML risk stratification, although work is ongoing [26,27].

A differential microRNAs (miRNAs) expression has also been recently proposed as a possible biomarker for individual PML risk stratification during natalizumab therapy [28,29]. miRNAs are small non-coding RNA molecules that regulate gene expression. PML patients expressed more miR-320 and miR-320b respect to non-PML patients. Interestingly, both of these miRNA also have a binding site in L-selectin coding region. Low expression of L-selectin on CD4 + T cells was correlated to an over-expression of miR-320 and miR-320b in pre-PML patients compared to non -PML patients [29]. MiR-126 and miR-10b are also possible natalizumab related PML therapy monitoring marker due to its role in the upregulation of its gene targets: POU2AF1 and Spi-B [5,28]. PO2AF1 is a regulator of Spi-B that binds to the promoter of JCV (Fig. 1).

JCV-specific effector T memory cells have also been investigated in evaluating better PML outcomes. Early JCV-specific CD8 + response is higher in PML survivors (100%) than in the PML progressors group (27.3%) within 6 months of PML onset [11]. Early detection of JCV specific CD8+ lymphocytes was associated with better control of PML and longer survival time. In a recent study, the authors proposed a CD4+/CD8+ ratio as a possible monitoring factor for PML risk and natalizumab treatment [30]. They found a close correlation between anti-JCV index, total WBC and lymphocytes by flow cytometry analysis of 52 MS natalizumab treated patients at 24 months.

Most of the research, albeit promising, has been conducted on a small number of patients with PML and further investigation is necessary before these methods can be validated for routine clinical practice. Other issues that need to be considered before introduction to routine monitoring are related to the high degree of complexity of some of the methods and the need for standardization.

Extended interval dosing

Beyond exploring ways to identify ''at risk patients'' for PML, clinicians have also investigated the role of NTZ frequency of infusions, an approach which has become known as, extended interval dosing (EID) schedules (infusion intervals >4 weeks) [35,36]. The approved treatment schedule (300 mg intravenous infusion every 4 weeks, standard interval dosing (SID) was selected to provide >80% saturation of mononuclear cell $\alpha_4\beta_1$-integrin receptors for approximately 1 month after administration [37]. Dosing of natalizumab in the intermediate range – less frequently than every 4 weeks, but more frequently than every 10 weeks may result in acceptable reduction of trough concentration and saturation of natalizumab to allow to maintain NTZ efficacy of MS suppression but reduce the risk of PML [38].

The approach of extended interval dosing in reducing the risk of PML has been explored using the largest database of patients on natalizumab, the TOUCH® program, a risk evaluation and mitigation strategy program mandated by the FDA [39]. Even though PML is an uncommon event, the size of the TOUCH dataset provided sufficient power in evaluating PML risk in anti JCV antibody-positive patients. There was a substantial reduction in PML risk with natalizumab EID compared with SID. Although a robust reduction in PML cases with statistically significant differences was observed, 13 PML cases were seen, suggesting that although the EID regiments are associated with significantly lower PML risk than SID, the risk is not completely eliminated [40].

The biological mechanisms underlying the observed PML risk reduction requires additional research, but partial reversal of natalizumab's pharmacodynamic effects, including decreased receptor saturation, increased soluble vascular cell adhesion molecule expression, and a reduction in natalizumab-induced peripheral lymphocytosis, have been reported to occur 4–8 weeks after the last dose and may allow for the reestablishment of some immune surveillance in the central nervous system [41–44]. Other hypothesis for the observed EID risk reduction may lie with the decreased forced migration of cells from the bone marrow and relative temporal downregulation of factors that highly favor JCV growth [14].

Treatment

Treatment for PML with proven efficacy is lacking. For the most part, the supportive evidence for the variety of strategies entertained to date is derived from case reports or small series evaluated without standardized outcome assessments.

Strategies to directly combat PML have included antiviral therapies, particularly targeting JCV replication and/or cell entry. Since JCV has been noted to use the 5-HT_{2A} receptor for entry into cells, drugs that compete for binding to the receptor and other agents with serotonergic activity, such as mirtazapine, have been examined for the ability to prevent viral spread with mixed results [45,46]. More recently, however, studies have shown that JCV may infect cells independently of the 5-HT_{2A} receptor, which can explain the variable results obtained with blockade of this receptor [47].

Other agents that reduce viral replication have been identified by a drug screening study, which yielded the candidate mefloquine. Mefloquine, an antimalarial with an unknown mechanism of action, demonstrated the promise of clinical utility against PML. Combining this with the fact that the drug can penetrate the blood–brain barrier, a clinical trial was initiated but then ended early when an interim analysis showed no difference between treatment groups [48].

Pharmacological cdk inhibitor R-roscovitine has been shown to suppress JCV replication in experimental models and the same group previously showed potential benefit of silencing RNA Ag122 which targets JCV agnoprotein to effectively inhibit JCV infection in vitro in SVG –A cells when introduced into the brains of mice after injecting JCV positive cells and significantly reduced the percentage of JCV infected cells compared with control treatment [49]. Using combination therapy for cell entry plus viral replication inhibition may prove more successful than the use of solitary agents for fighting off JCV but awaits proof of concept.

While a rapid-acting and effective antiviral therapy remains a sought for goal, optimal immune reconstitution to control the JC virus without causing brain damaging immune reconstitution inflammatory syndrome (IRIS) is the most practical approach to treat PML currently. In the case of natalizumab associated PML, plasma exchange to negate the effect of therapy has been adopted, particularly in the case of recent drug infusion. However, although effective in removing NTZ, PLEX has been also reported to be detrimental for patients' outcome, as PLEX may cause an excessively rapid restoration of immune surveillance, eventually worsening clinical symptoms and inflammatory brain damage through IRIS [50].

Programmed cell death protein 1 (PD-1) expression is up-regulated on CD4+ and CD8+ lymphocytes in patients with PML, which acts as a negative regulator of immune responses, may limit successful containment or clearance of JCV. It is hypothesized, that treatment of PML with PD-1 blockade using Pembrolizumab could reinvigorate specific antiviral immune activity [51].

Modulators of the immune response may also be necessary to combat PML IRIS. Glucocorticoids have been used in PML IRIS and are sometimes used as prophylaxis against it [52]. This approach raises the concern for posing a threat to the delicate immune system balance and carries the risk of suppressing the ability to control JCV infection in the brain. Therefore, optimal timing for using steroids is controversial. Expert opinions and preliminary studies suggest postponing steroid administration until full blown PML-IRIS becomes evident [53].

The inflammation inhibitor maraviroc, a CCR5 antagonist, has also been advocated as a treatment of PML IRIS. This drug was developed as a therapy for HIV and is effective and well-tolerated in treating HIV infections. CCR5 is involved in targeting inflammatory responses with several case reports including both HIV- and natalizumab associated PML support the possibility that maraviroc therapy may blunt IRIS in the setting of PML [54,55].

Early diagnosis is also very important to optimal clinical outcomes. Since demyelination due to JC replication in oligodendrocytes causes a serious and generally poorly reversible disability, restoring the immune response at the earliest time is critical. In high-risk patients such as natalizumab treated patients with positive JC virus and 2 years or more of natalizumab therapy, periodic MRI screening has detected asymptomatic PML which has had a much better clinical outcome than has been associated with clinically evident PML [18].

In the absence of an effective treatment for PML, early detection of the disease in patients with multiple sclerosis who are receiving natalizumab or other immunomodulatory treatments is vital to minimize CNS injury and avoid severe disability. Frequent MRI, stratified along a clinical and virus-specific immune risk profile, can be used to detect presymptomatic PML. Improved approaches to PML risk stratification, optimal drug dosing are needed to guide treatment choices and surveillance of patients.

Disclosures

Lana Zhovtis Ryerson has received personal compensation for speaker bureau from Biogen, Genentech, and Teva; served on advisory board for Biogen; and received research support from Biogen.

Eugene O. Major has received consultancy fees for PML evaluation as a member of the Glaxo Smith Kline Independent Adjudication Board, Takeda/Millennium Independent Adjudication Committee, Roche/Genentech PML Adjudication Board, Dr. Reddy's Laboratories, New Jersey Science Advisory Board (for JCV activation), Shire Pharma PML Board (PML risk). EOM is named on the NINDS/NIH patent for Multiplex Ultrasensitive qPCR patent number 14/408, 919.

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