Pathogenesis of progressive multifocal leukoencephalopathy and risks associated with treatments for multiple sclerosis: a decade of lessons learned

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Progressive multifocal leukoencephalopathy (PML) is a rare, devastating demyelinating disease of the CNS caused by the JC virus (JCV) that occurs in patients with compromised immune systems. Detection of PML in systemically immunocompetent patients with multiple sclerosis treated with natalizumab points to a role for this drug in the pathophysiology of PML. Emerging knowledge of the cellular and molecular biology of JCV infection and the pathogenesis of PML—including interplay of this common virus with the human immune system and features of natalizumab that might contribute to PML pathogenesis—provides new opportunities to monitor viral status and predict risk of JCV-associated disease. 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 are needed to guide treatment choices and surveillance of patients with multiple sclerosis.

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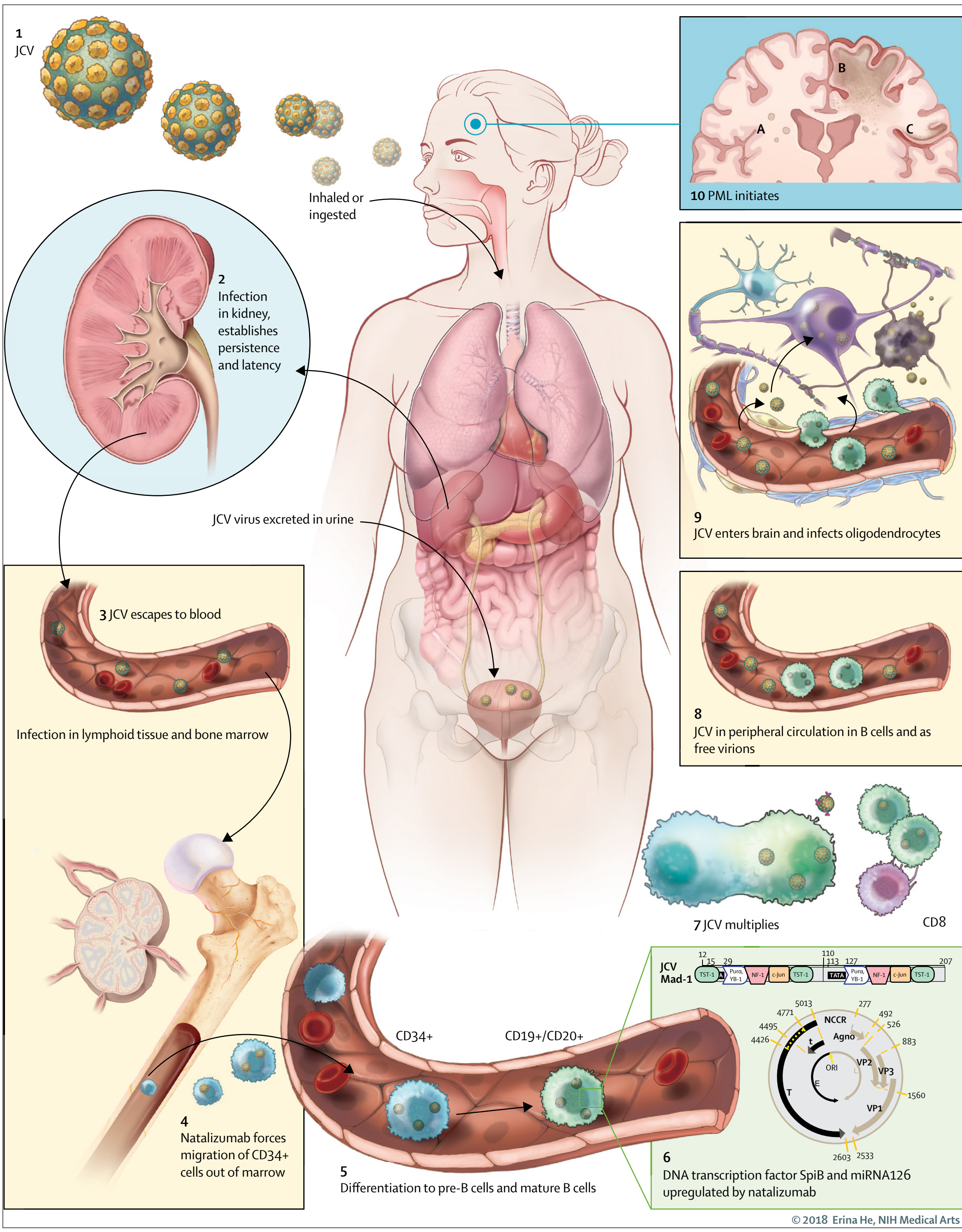
Introduction

More than a decade has passed since the first reports of progressive multifocal leukoencephalopathy (PML) in patients with multiple sclerosis who were taking natalizumab in phase 3 clinical trials.[1–3] Natalizumab is a monoclonal antibody to α4β1 and α4β7 integrins that blocks inflammatory cell entry into the brain and can prevent multiple sclerosis-related clinical relapses. The co-occurrence of PML and multiple sclerosis was unanticipated, because these disorders have little in common except for the destruction of myelin: PML is a JC virus (JCV)-induced lytic brain infection, whereas relapsing-remitting multiple sclerosis is an autoimmune disorder. PML was quickly associated with natalizumab treatment because patients with multiple sclerosis had been treated with other immune therapies for decades without reports of PML.[1–4] The initial prevalence of natalizumab-associated PML in patients with multiple sclerosis was estimated to be one in 1000.[4] More than 750 PML cases have now been confirmed among natalizumab-treated patients, with a fatality rate higher than 20% and substantial morbidity in survivors.[5] The prevalence of PML among patients treated with natalizumab for more than 24 months, with antibody evidence of JCV and previous immunosuppressant exposure, has reached at least one in 70—much higher than the prevalence of any other opportunistic infection in this setting.[6,7] Risk-profiling analyses of patients who are positive for anti-JCV antibody gave an estimated cumulative PML probability over 6 years of 1·7% (95% CI 1·4–2·1).[8] A few reports of PML in patients taking other treatments for multiple sclerosis, such as dimethyl fumarate and fingolimod, have been published.[9–13] However, the prevalence of PML in patients with multiple sclerosis who are taking other immune-modulating therapies is much lower than that associated with natalizumab, perhaps one in 10 000 to one in 100 000. Although outcomes for patients with PML have improved with early detection and initiation of immune reconstitution,[5] PML is a serious and sometimes lethal disorder, and the clinical management of patients with multiple sclerosis—including PML risk assessment and surveillance—remains challenging.

Investigations in patients with multiple sclerosis have contributed to improved understanding of the pathogenesis of PML. This knowledge is crucial in recognising therapy-associated risks of PML, establishing evidence-based monitoring strategies for patients, and informing the selection of effective treatments for individuals with multiple sclerosis. In this Review, we explore several areas of progress. First, we discuss molecular aspects of PML pathogenesis and the cell-specific involvement of JCV infection leading to PML, which are generally applicable to all cases of PML regardless of underlying diseases. Second, we consider the central role of MRI in the diagnosis of PML and outline how treated patients can be monitored to minimise morbidity and advance our understanding of aspects of pathophysiology. Third, we highlight new insights of clinical value in early PML detection.

JCV infection and PML pathogenesis

PML is usually characterised as a rare disease caused by JCV, a common polyomavirus named from the initials of the first patient from whom the virus was isolated.[14] PML develops almost exclusively in patients with a compromised immune system, particularly when cell-mediated immune responses are involved. For example, PML was initially reported in patients with underlying neoplastic diseases, mostly lymphoproliferative diseases, and in patients with organ transplants who had undergone immune suppression for graft protection.[15] In the mid-1980s, HIV-1 infection became the main risk factor, with up to 5% of AIDS-related deaths associated with PML; early initiation of effective antiretroviral therapy to avert severe immunodeficiency has decreased the risk in HIV-infected patients to less than 1%.[16,17] We searched PubMed for papers published from Jan 1, 2005, to Dec 31, 2017 and found that the number of reports of PML associated with multiple sclerosis and other underlying diseases, and therapies to treat them, has increased by an order of magnitude, suggesting greater recognition of PML on the basis of clinical evaluation, MRI, and use of laboratory tests to detect JCV DNA and



anti-JCV antibody. It might therefore be time to consider PML not just as a rare disease, but as a substantial neurological complication in certain high-risk populations.

Biology of JCV infection

The pathophysiology of JCV leading to PML in human hosts is outlined as ten key steps in figure 1; additional details are provided in table 1.[19–26,29,30,35–43] JCV has a narrow cellular host range and a variable effect on the organs it infects. Infection in human endothelial cells in the kidney,[23,24,35,37] and in cells of haematopoietic lineage, such as CD34+ cells, B-cell phenotypes, CD19+ cells, and CD20+ cells, has no known pathological effects, making host infection a silent event.[38] In the brain, however, multiplication in oligodendrocytes is lytic and causes PML with devastating clinical consequences, including progressive motor dysfunction, cognitive impairment, and visual deficits. Infection of neurons in the granular cell layer of the cerebellum can also cause symptomatic neuronopathy.[44]

Molecular regulation of JCV infection

To be susceptible to JCV infection, host cells need to express binding proteins that recognise the viral DNA genome non-coding control region (NCCR; also referred to as the regulatory region) that initiates viral RNA transcription and DNA replication for synthesis of viral proteins (figure 1, steps 6, 7; table 1). The NCCR, which has several transcription factor binding sites that are crucial for JCV multiplication, can have one of two sequence arrangements. Archetype NCCR consists of about 200 linear nucleotides and is found in roughly 30% of the population (in virions excreted in urine; figure 1, step 2). This variant is generally considered to be non-pathogenic in kidney and, if present, in other compartments such as plasma and serum, and even brain. Virus isolated from the brains of patients with PML, such as the index patient JC, became known as the pathogenic prototype variant (also referred to as JCV Mad-1).[43] Unlike the archetype variant, the roughly 200 nucleotides in prototype NCCR are not arranged linearly, but in direct tandem repeats of 98 nucleotide base pairs or other arrangements, always showing duplications. Indirect evidence supports the proposal that the prototype variant is derived from the archetype variant by deletion and duplication.[18] However, no such rearrangement has yet been shown in cell culture or in patients. The tissue compartment or cell type in which such a rearrangement might occur is unknown, but lymphoid cells are a probable host (figure 1, steps 4, 5).[25,26,29,45] New evidence implicates Epstein-Barr virus coinfection as a possible catalyst in the nucleotide transition of the archetype to the prototype variant.[20]

Figure 1: **Proposed stages of PML pathogenesis in patients treated with natalizumab**

(1) Initial infection with JC virus (JCV) through ingestion or inhalation of virion particles might lead to subacute infection and stimulation of antiviral antibody. (2) JCV can infect the uroepithelium of the kidney and establish a persistent or latent infection. (3) JCV might escape into the peripheral circulation, spreading virions into lymphoid tissues (including bone marrow) and establishing a latent infection that can be reactivated at times of immune suppression or modulation. Nucleotide rearrangement of the viral DNA non-coding control region (NCCR) from the less pathogenic archetype variant to the pathogenic prototype variant associated with progressive multifocal leukoencephalopathy (PML) might start at this stage, possibly continuing through stages 4–6. (4) CD34+ cells in the bone marrow can become infected. Natalizumab forces consistent migration of CD34+ cells to the peripheral circulation, which continues for years during treatment. (5) Some migrated CD34+ cells differentiate in a lymphocyte pathway, predominately in the B-cell lineage. Some cells undergoing differentiation can become hosts for viral multiplication. (6) DNA transcription factors in the NCCR, such as SpiB in the POU2A domain, and miRNAs are temporally regulated by natalizumab and favour JCV multiplication in latently infected cells. NCCR nucleotide rearrangement might occur at this stage. DNA transcription factors for viral multiplication include TST-1, Pur alpha/YB1, NF-1, and cJun.[18] (7) JCV multiplication takes place in these cell phenotypes, which might be recognised by CD4+ and CD8+ T-cell-mediated immune clearance with contributions from anti-JCV antibody. Some infected cells escape immune clearance. (8) JCV can remain in circulating B cells, perhaps pre-B cells, or circulate as non-cell-associated free virions in the blood, and traffic to the brain. (9) JCV can enter the brain via haematogenous routes and initiate infection in the target oligodendrocyte. Mechanisms of viral entry are not well documented. (10) PML initiates and progresses as JCV begins lytic, necrotic oligodendrocyte infection. With gradual spreading of the virus, lesions grow in a multifocal pattern: (A) multiple sclerosis lesions in PML patients treated with natalizumab; (B) cortical white matter lesions with punctate lesions just below that are typical in PML; (C) PML lesions in U-fibres near the cortex.

Immunology of JCV infection: antibodies to JCV

The initial route of JCV infection is not known, but is thought to be ingestion or respiratory inhalation (figure 1, step 1). Contact with JCV most commonly results in a subclinical infection that triggers antibody production and cell-mediated immune responses (table 1).[21,22] Findings from sero-epidemiological studies in which anti-JCV antibodies have been measured show a worldwide distribution of JCV. More than 50% of the adult population is estimated to have been exposed.[46] A correlation has been reported between percentage of the seropositive population and decade of life, from 15% in the second decade to 80% in the seventh and eighth decades of life.[30,47] About 55% of patients with multiple sclerosis are JCV seropositive.[36,48] However, patients can convert from seropositive to seronegative, and vice versa, as antibody concentrations fluctuate over time. The rate of seroconversion in either direction is estimated to range from 3% to as high as 10% of the natalizumab-treated population over the course of a year or more.[34] A summary of methods used for the measurement and analysis of antibody to JCV is provided in the appendix.[24,30,34,46–49]

See Online for appendix

Anti-JCV antibodies are directed to different regions of the primary capsid protein VP1, which functions in cell attachment. As with viral NCCR, the VP1 gene can be hypervariable, coding for VP1 proteins with different primary aminoacid sequences, which has led to the typing system for JCV variants. Thorough descriptions of JCV type have linked geographical locations with independent VP1 genes and protein variants.[50] It is therefore not surprising that a single patient with PML can have multiple

	Urine*		Blood†		CSF‡	
	Antibody§	DNA¶	Antibody	DNA	Antibody	DNA
Primary infection[19,20] (ingestion or inhalation)	Not measured	Not symptomatic so rarely measured	Seropositive test result in 15% of people in the second decade of life, increasing to 80% in the seventh and eighth decades; seroconversion in 3–10% of the population	Not measured	Not measured	Not measured
Latency established in kidney[17,18,21,22]	Not measured	Can be undetected to low, or $>10^6$–10^7 copies per mL; sporadic or continual release	Variable concentrations, from low to high titres	Can be transiently detected, generally $<10^2$ copies per mL	Not present	Not present
Escape from kidney to circulation; might enter lymphoid organs such as bone marrow[23–28]	Not measured	Can be undetected to low, or $>10^6$–10^7 copies per mL; sporadic or continual release	Variable concentrations; prevalence of seropositive individuals increases with age	Can be transiently detected, generally $<10^2$ copies per mL	Not present	Not present
Escape into circulation; brain entry in cells or as free virions; infection of oligodendrocytes[29–33]	Not measured	Can be undetected to low, or $>10^6$–10^7 copies per mL	Detected at variable concentrations; titre increase during onset of PML	Transiently detectable; variable levels, commonly from 50 copies per mL to more than 500 copies per mL	High titre or index indicates intrathecal antibody production	Generally detectable at 10–10^7 copies per mL

JCV=JC virus. NCCR=non-coding control region. PML=progressive multifocal leukoencephalopathy. *Urine samples can be tested for JCV DNA (NCCR archetype variant) to show a latent or persistent infection. †The presence of anti-JCV antibody in serum or plasma indicates prior exposure to the virus. High or increasing concentrations of antibody, reported as a titre or index, usually indicate active infection from reactivation of latency or a new infectious episode. A small number of individuals who are seronegative have JCV infection but do not show or make antibody, as in viruria or viraemia.[34] ‡CSF samples with detectable JCV DNA (prototype variant) are used for laboratory-based confirmation of PML diagnosis; the most sensitive assay has a detection limit of 10 genome copies per mL.[35] Usually, the lower the copy number, the better the prognosis.[18] §Measured by ELISA assay (appendix) using viral major capsid protein VP1 derived from the prototype variant as antigen. Almost all patients with PML who have ELISA-confirmed antibody to JCV have the JCV NCCR prototype variant in brain and CSF. ¶Measured using quantitative PCR. 50 genome copies per mL is a low copy number; ≥500 copies per mL is a high copy number. In patients with multiple sclerosis and PML, the median is greater than 100 to less than 500 copies per mL and the range is 10 to 10^7 copies per mL.[36] For full footnotes, see appendix.

Table 1: **Detection of JCV antibody and DNA by stage of JCV infection leading to PML**

representations of VP1 protein at any one time. This observation led to the hypothesis that VP1 gene rearrangement could generate a neurovirulent variant that causes PML.[51,52] To ascertain the extent to which gene rearrangement occurs, deep sequencing studies are warranted.[31] Patients with PML seem to be infected with the prototype VP1 protein, since this is the antigen used in the anti-JCV antibody ELISA assays of commercial, academic, and government laboratories (appendix). The possibility that immune escape of JCV VP1 variants might occur, because of persistent JCV in cell compartments or mutations in the VP1 gene that evade immune recognition,[52] is the subject of ongoing studies.

Not all antibodies to JCV are necessarily neutralising antibodies that protect against PML development. Data from in-vitro studies show that antibody against JCV blocks virion adsorption by target cells, which limits attachment and entry, thereby reducing viral multiplication. However, little clinical evidence from healthy people or patients exists to suggest that JCV infection can be controlled by antibodies.[32] In fact, nearly all individuals who persistently shed JCV in their urine are seropositive. Some seropositive individuals can even be viraemic, and patients with PML can have very high concentrations of antibody in their CSF in the presence of high copy numbers of viral DNA.[53,54]

Cell-mediated immunity to JCV

CD4+ and CD8+ cytotoxic cell recognition of viral antigens probably has a more important role against JCV infection than does anti-JCV antibody. CD8+ cytotoxic T cells to JCV prototype VP1 have been identified in patients with and without PML for many years (figure 1, step 7).[27,55] CD4+ T cells directed against the four major JCV proteins (T antigen, VP1, VP2, and Agno) have been identified as crucial to the control of JCV.[56] Low numbers of CD4+ cells and of cell types releasing interleukin 10 were reported in natalizumab-treated patients with multiple sclerosis, including one of the index cases in whom CSF remained persistently JCV positive for years.[56] CD4+ T cells directed to potentially more neurotropic viral capsid proteins that are not identified by CD4+ T cells in the periphery have also been cultured from brain tissue of patients with PML. These CD4+ cells seemed necessary to stimulate cytotoxic CD8+ cells to function for clearance of JCV from the brain, and so perhaps were lacking in patients with PML.[52] In efforts to further define risk factors for PML, identification of CD4+, CD8+, and other immune system cells with activity to JCV antigens would be informative in high-risk patients.

JCV genome changes during infection

A case can be made that common pathophysiological pathways explain the steps leading to PML, irrespective of the underlying risk that allowed it. For example, some patients with compromised T cells might harbour latent JCV in their kidney, lymphoid organs (such as bone marrow), and possibly brain. If periodic JCV release from latency or even a persistent infection is poorly managed by the immune system, virus might enter the brain as free virions or through an infected cell (figure 1, step 9; table 1). CD4+ T cells that do not adequately recognise JCV antigens are now considered to be an important component of poor immune surveillance,[40] whereas cells in the B-cell lineage have been implicated as possible carriers, since JCV has been identified in CD19+ and CD20+ cells.[41] The brain is not the initial site of JCV infection, and data on latency in the brain are very limited. JCV DNA has been identified in brain tissues of patients who do not have PML; however, no evidence that the entire viral genome was present to initiate and sustain viral multiplication was reported.[57] Only one study specifically investigated the presence of JCV DNA in the brain tissue of patients with multiple sclerosis, and found it to be absent.[28] A multicentre study using blinded samples and controls of positive and negative brain tissues should be considered to determine the existence of latent JCV in the brain. However, at present, it seems likely that release of latent JCV in the periphery from persistently or latently infected lymphoid cells, in which genome rearrangement might take place,[33,58] is a key factor in the development of PML. The variant derived from the kidney or urine is considered to be non-virulent or at least less neurotropic than the prototype variant, so the best candidate site for latency is probably lymphoid cells (figure 1, steps 4, 5; table 1).[59] These cells might be hosts for rearrangement of the viral NCCR from archetype to prototype, and perhaps rearrangement of the VP1 gene. Lymphoid cells would be subject to factors that activate viruses (eg, Epstein-Barr virus) and might even promote JCV NCCR gene rearrangement and insertion, as well as being potential targets for RAG1 and RAG2 enzymatic mechanisms, best known for their role in immunoglobulin diversity.[27,54]

Natalizumab and the risk of PML

What unique features does natalizumab have that no other drug-associated PML risk shares? Patients with natalizumab-associated PML are not systemically immune suppressed. Other opportunistic infections are not prominent, suggesting that PML is a specifically enhanced problem rather than the result of broad immunosuppression. Furthermore, years of treatment seem to be necessary for the risk of PML to be manifest. These two factors highlight the need to understand PML pathogenesis beyond pure immunosuppressive explanations. To suggest that inadequate immune surveillance is the major underlying mechanism of PML in natalizumab-treated patients with multiple sclerosis might be oversimplistic. Even with immune reconstitution inflammatory syndrome (IRIS), some natalizumab-treated patients with PML continue to have detectable virus in CSF for months to years.[36]

	Pathology	Duration	Blood		CSF		MRI
			Antibody	DNA	Antibody	DNA	
Presymptomatic PML	Unknown, probably as in classic PML	Estimate of 3–6 months from viral entry into brain to onset of neurological symptoms*	Anti-JCV antibody increases; dynamic increase supports PML diagnosis	Transient, 50–500 copies per mL	Detectable, titre increasing	Generally low titre detectable, 10–10^7 copies per mL	New lesion on surveillance MRI; generally small; DWI bright
Classic symptomatic PML without immune responses†	Demyelinating plaques, bizarre astrocytes, oligodendrocytes with nuclear inclusions, notably absent inflammatory response	3–6 months from onset of symptoms to death if no immune reconstitution	Marked increase typical of PML	Transient, 50–500 copies per mL	Detectable, titre increasing	10–10^7 copies per mL, rarely undetectable	Typical brain lesions‡, enlarging; rare if any contrast enhancement§; no mass effect
PML with IRIS§	Classic pattern plus inflammatory response with variable mix of CD8+ and CD4+ lymphocytes; might have declining levels of JCV	1–5 months after immune reconstitution¶, associated with potential for survival of PML; might be present at diagnosis in natalizumab-associated PML	Increases	Transient, 50–500 copies per mL	High titre	10–10^7 copies per mL; might increase then decrease during course of disease	Typical brain lesions;‡ contrast enhancement usual but not required for IRIS diagnosis§; punctate pattern and T1 bright cortical line indicate this stage of PML
Post-PML in survivors||	Atrophy, fibrosis, rare JCV-infected cells	Years, depending on underlying disease; fixed lesion might support clinical improvement 6–12 months after diagnosis but is then clinically stable in most cases	Few data exist but probably relatively stable at high titres	Transient, 50–500 copies per mL	High titre	Often undetectable, but can remain detectable**	No contrast enhancement; brain atrophy in region of prior lesions

DWI=diffusion-weighted imaging. JCV=JC virus. IRIS=immune reconstitution inflammatory syndrome. PML=progressive multifocal leukoencephalopathy. *Duration of the presymptomatic stage depends on lesion location in the brain. †Typical of PML that develops in patients with untreated HIV or AIDS or in other highly immune-deficient settings in which virtually no immune response is seen. ‡Four key features suggest a PML lesion in asymptomatic patients: subcortical location (involvement of U-fibres), T1 hypointensity, DWI hyperintensity, and the presence of punctate T2-hyperintense lesions. Evolution of the lesion on subsequent scans is important in substantiating a diagnosis. §PML with IRIS can also occur at the presymptomatic and symptomatic phase when partial immune deficiency occurs (common at onset in patients with multiple sclerosis and natalizumab-associated PML). Punctate lesions and T1 cortical bands probably indicate inflammation with or without contrast enhancement. Previous use of corticosteroids reduces the chance of contrast enhancement without eliminating the inflammatory response. ¶PML with IRIS persists for up to 5 months or longer and might necessitate repeated treatment for suppression. ||Death from PML typically occurs within 6 months of diagnosis, whereas survivors die of other causes months to many years later, often hastened by underlying diseases. **JCV DNA copy number typically decreases and is often undetectable in survivors of PML. However, virological cure does not occur and low levels of detectable virus in the CSF can persist indefinitely. For full footnotes, see appendix.

Table 2: **Clinical stages of PML**

Two unique features of natalizumab might contribute to its special risk. The first is that natalizumab forces migration of hematopoietic stem cells, CD34+ cells, and precursors of B cells from the bone marrow (figure 1, step 4). Natalizumab shares this feature with efalizumab, the other monoclonal antibody associated with a high risk of PML. JCV can be latent or persistent in CD34+ cells or pre-B cells in the bone marrow.[24,38,57] In culture models of similar cell types, DNA-binding factors act on the JCV transcription sites.[28] These DNA-binding transcription factors can also be found in CD19+ and CD20+ cells in the peripheral circulation. The high percentage of such cells forced out of the bone marrow for long periods might result in the release of some cells with latent infection (figure 1, step 5). In natalizumab-treated patients, immune cells might not completely clear newly released virions, particularly if they remain intracellular like Epstein-Barr virus. The second feature of natalizumab is evident in the temporal relation between initiation of treatment and occurrence of PML. Natalizumab upregulates gene products—POU domain DNA transcription factors, particularly SpiB, which binds JCV NCCR—in a pathway that is crucial for B-cell maturation. The time course of natalizumab's effect on POU domain regulation is consistent with PML incidence—after 2 or more years of dosing.[42,60]

These two characteristics of natalizumab—forced migration of cells from the bone marrow and temporal upregulation of factors that highly favour JCV growth—focus attention on JCV cellular interactions leading to PML (figure 1, steps 4–6; table 1). Although perhaps still premature, it is worth considering how laboratory analysis of these modulated transcription factors in immune cells and immune-cell antiviral function might help to identify patients at high risk of PML before oligodendrocyte infection is initiated.[61,62]

Early detection, diagnosis, and management of PML

Brain imaging makes a vital contribution to the diagnosis of PML, which also routinely requires the identification of active CNS pathology and JCV in the brain.[63] Indeed, PML diagnosis cannot be verified without an MRI lesion. The sensitivity of MRI in identifying PML lesions has made it the modality of choice in monitoring natalizumab-treated patients with multiple sclerosis for early detection of PML. MRI has also contributed to our understanding of the clinical stages of PML, which depend on the degree of brain infection and the status of the immune response to this unique infection (table 2). We define onset of PML as the time at which JCV enters the brain and infects oligodendrocytes, which ultimately leads to a clinically serious brain injury that is not initially detectable on MRI. This early cellular, presymptomatic period is followed by a period, probably 3–6 months in duration, during which an MRI lesion is evident before symptoms are observed (table 2).[64] This time course accounts, at least in part, for the low risk of PML in early months of therapy and the roughly 6-month interval during which PML is most likely to be identified after stopping natalizumab treatment and transitioning to a low-risk therapy. The substantial variation in symptomatic disease state depends on whether or not immune reconstitution is achieved. Without immune reconstitution, classic PML (as was seen in patients with untreated HIV or AIDS) is generally fatal because no effective immune response is generated. Alternatively, as generally occurs in natalizumab cases, successful immune reconstitution precipitates an inflammatory syndrome that can arrest the disease. This IRIS response must come quickly enough to avert death from disease progression. The viral disease is generally controlled when IRIS occurs and the patient survives for more than 6 months, albeit with a fixed brain lesion (table 2).

PML therapy has been reviewed in detail elsewhere.[65] No antiviral therapies, including widely used mirtazapine and mefloquine hydrochloride,[66] have been shown to improve outcomes, but immune reconstitution does improve the course of PML. The concept of using plasma exchange to hasten immune reconstitution in natalizumab-related cases is thus a rational approach that has been widely adopted and associated with improved PML outcomes.[67] However, the potential augmentation of damaging IRIS remains a concern that clinicians must weigh against the dangers of PML.[68,69] Similarly, active use of corticosteroids or maraviroc[70] to blunt IRIS remains controversial. Active immune reconstitution seems likely to contribute to better outcomes, at least in more advanced disease.

Early MRI detection of PML lesions

Gathering informative data to more clearly articulate recommendations for surveillance and management of this rare and serious disease remains extremely challenging. The aim of early diagnosis of PML (table 2; table 3),[79–86] preferably before the onset of clinical symptoms, is to limit brain damage and thus disability. Recommended MRI parameters are widely available.[81,84,87] Annual scans of the brain are increasingly recommended to monitor the efficacy of disease-modifying treatments for multiple sclerosis (table 3). More frequent brain scans are recommended for early detection of PML in higher-risk settings. Findings from a retrospective analysis[64] of patients with PML who had frequent scans showed that lesions develop months before symptoms. It is now recognised that PML symptoms might only develop months after JCV enters the brain and forms a visible lesion on MRI. We are aware of 19 publications,[2,64,78,86–99] reporting on 48 patients with PML who were asymptomatic at the time of a detectable lesion. 21 of these patients developed symptoms within 41 weeks after lesion visualisation; natalizumab was withdrawn before the development of symptoms in 13 patients, and four patients remained symptom-free. Disabling outcomes, including mortality, appear to be reduced in patients who are diagnosed before symptom onset.[99]

It is essential to be aware that PML lesions actively evolve on repeated imaging, either because the JCV-induced disease progresses or because the inflammatory response controlling the infection results in evolution of the image characteristics. Thus, stable appearances on repeated MRI helps to rule out PML, whereas evolving lesions are consistent with a PML diagnosis. PML cannot be diagnosed on a single MRI scan without additional clinical and virological confirmation.

Despite the increasing number of reported PML cases, the low frequency and sporadic appearance of PML in patients treated with natalizumab, and the variable regulatory control of the global distribution and use of natalizumab, make a prospective assessment of the sensitivity, specificity, and accuracy of imaging difficult. The four most distinguishing imaging features of a PML lesion (applicable to lesions in asymptomatic patients) are suggested to be its subcortical location (involvement of U-fibres), T1 hypointensity, diffusion-weighted imaging hyperintensity, and the presence of punctate T2-hyperintense lesions (figure 2).[97,100] Unlike PML associated with HIV or AIDS, gadolinium contrast enhancement is often seen even at presentation of PML in the setting of treated multiple sclerosis. Occasional cortical and deep grey matter involvement can occur, but white matter distribution is predominant in PML.

	PML risk estimate (per 1000)*	Monitoring steps			Brain MRI sequences †			
		Anti-JCV antibody	MRI		FLAIR/T2	DWI	T1	T1 with Gd enhancement
			Frequency	Indication				
Immunomodulatory drug treatment, including natalizumab	··	··	Annually	Multiple sclerosis activity	Recommended‡	Optional§	Optional¶	Optional‖
Natalizumab treatment, anti-JCV negative	··	Every 6 months	Annually	Multiple sclerosis activity	Recommended‡	Optional§	Optional§	Optional‖
Natalizumab treatment, anti-JCV positive, no prior immunosuppression								
Anti-JCV antibody index <0·9								
Treatment duration 1–72 months	0·1–0·6	Every 6 months	Annually	Multiple sclerosis activity	Recommended‡	Optional§	Optional§	Optional‖
Anti-JCV antibody index 0·9–1·5								
Treatment duration 1–36 months	0·1–0·8	Every 6 months	Annually	Multiple sclerosis activity	Recommended‡	Optional§	Optional§	Optional‖
Treatment duration 37–72 months	2–3	··	Every 3–4 months	PML surveillance	Recommended**	Recommended**	··	··
Anti-JCV antibody index >1·5								
Treatment duration 1–24 months	0·2–0·9	··	Annually	Multiple sclerosis activity	Recommended‡	Optional§	Optional§	Optional‖
Treatment duration 25–72 months	3–10	··	Every 3–4 months	PML surveillance	Recommended**	Recommended**	··	··
Natalizumab treatment, anti-JCV positive, prior immunosuppression								
Treatment duration 1–24 months	1–24	0·3–0·4	··	Annually	Multiple sclerosis activity	Recommended‡	Optional§	Optional§
Treatment duration 25–72 months	25–72	4–8	··	Every 3–4 months	PML surveillance	Recommended**	··	··

Multiple sclerosis disease activity is monitored in all patients for optimum clinical management; the aim is to provide disease-modifying therapy to achieve a status of NEDA. Risk for PML in patients with multiple sclerosis who are treated with natalizumab is stratified by JCV antibody index and duration of exposure, and those with a risk estimate greater than 0·9 per 1000 patients are recommended for intensive PML surveillance. DWI=diffusion-weighted imaging. EMA=European Medicines Agency. FLAIR=fluid-attenuated inversion recovery. Gd=gadolinium. IR=inversion recovery. JCV=JC virus. NEDA=no evidence of disease activity. PD=proton density. PML=progressive multifocal leukoencephalopathy. *Risk estimates per 1000 natalizumab-treated patients, from a report of the EMA (Feb 26, 2016).[74] †Spine MRI is not recommended for monitoring of PML or multiple sclerosis disease activity; it is indicated if a neurological examination suggests possible spinal cord localisation of pathology.[71–73] ‡Follow-up sequences recommended by Wattjes et al:[72] (1) axial PD and/or T2-FLAIR/T2-weighted, (2) 2D or 3D contrast-enhanced T1-weighted; optional sequences: (1) unenhanced 2D or high-resolution isotropic 3D T1-weighted, (2) 2D and/or 3D dual IR, (3) axial DWI. Core sequences recommended by Traboulsee et al:[73] (1) anatomical 3D IR-prepared T1 gradient echo, (2) 3D sagittal T2-weighted FLAIR, (3) 3D T2-weighted, (4) 2D axial DWI, (5) 3D fast low-angle shot (non-IR prepared) post-Gd; optional sequences: (1) axial proton attenuation, (2) pre-Gd or post-Gd axial T1 spin-echo, (3) susceptibility-weighted imaging. Core sequence recommended by Traboulsee et al:[75] axial FLAIR; additional sequences: (1) axial T2-weighted, (2) sagittal FLAIR, PD, or T2-weighted, (3) axial T1-weighted pre-Gd and post-Gd; optional sequences: (1) 3D T1-weighted, (2) DWI. Sequences recommended by Vågberg et al:[76] (1) axial T2-weighted, (2) 3D T2-FLAIR, (3) 3D T1-weighted post-Gd. §Optional in Wattjes et al;[72] recommended by Traboulsee et al.[73] ¶Optional in Wattjes et al.[72] ‖Recommended by Wattjes et al,[72] Traboulsee et al,[73] and Vågberg et al;[76] recommended (useful) by Traboulsee et al,[75] but not deemed to be essential for clinically silent disease activity; McGuigan et al[71] recommended Gd according to local protocol. In view of the recent statement by the EMA (July 21, 2017)[77] that "healthcare professionals should use gadolinium contrast agents only when essential diagnostic information cannot be obtained with unenhanced scans", some groups are reassessing the role of Gd in the management of patients with multiple sclerosis, especially when monitoring patients with NEDA. **McGuigan et al,[71] Traboulsee et al,[73] and Vågberg et al[76] recommended FLAIR; Wattjes et al[72] recommended FLAIR, T2-weighted, and DWI; Yousry et al[78] recommended FLAIR or T2-weighted; no data exist to suggest superiority of one sequence over another in this scenario.

***Table 3:* Protocol for PML surveillance in patients with multiple sclerosis, by treatment group**

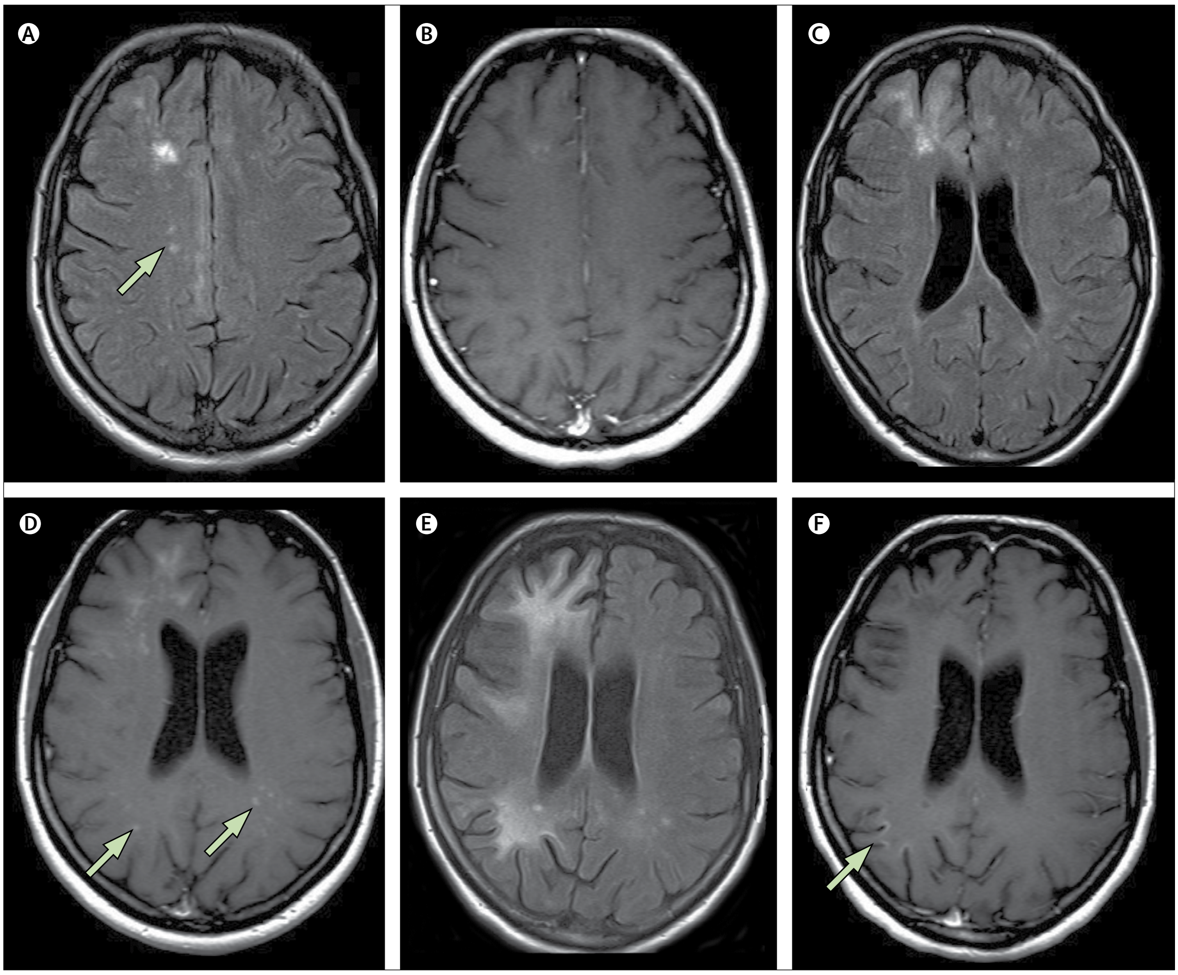


Figure 2: **MRI of natalizumab-associated PML in a patient with multiple sclerosis**
Fluid-attenuated inversion recovery (FLAIR) images (A, C, E) and enhanced T1-weighted images (B, D, F) from a patient with multiple sclerosis who developed natalizumab-associated progressive multifocal leukoencephalopathy (PML). (A–B) Asymptomatic PML. An enhancing right frontal lesion is shown, with multiple smaller non-enhancing punctate lesions (green arrow). (C–D) PML with immune reconstitution inflammatory syndrome. The lesion has enlarged on FLAIR and the enhancing area has increased; note the enhancing punctate lesions bilaterally (green arrows). (E–F) Post-PML. Further enlargement of the lesion on FLAIR and presence of T1 hyperintense cortex (green arrow) can be seen .

Punctate lesions, suggesting an inflammatory response in the lesion, might offer some insight into the pathophysiology of PML. This finding has emerged in settings where partial immune response to JCV is common, and was not noted in the era when most cases were HIV/AIDS-associated and an inflammatory response was absent on pathological examination. Punctate lesions appear to develop in perivascular spaces within the brain, where JCV has been identified in mononuclear cells and infected glial cells.[45] Histological examination has shown that inflammation to JCV that is typical of IRIS is associated with a marked infiltration of CD8+ T lymphocytes, especially in the perivascular spaces.[101] This pattern might therefore be a marker of IRIS, and is consistent with early evidence of contrast enhancement (suggesting IRIS) in many natalizumab-associated cases of PML. Although punctate lesions often enhance with gadolinium, their unenhanced presence on T1 imaging suggests that they might instead specifically reflect an inflammatory response.[86,100] The alternative interpretation that these are the smallest islands of demyelination in early infection is plausible, but their early enhancement favours their location in relation to blood vessels with increased permeability to gadolinium. If these lesions reliably represent PML with IRIS, they could direct clinicians to focus on anti-inflammatory therapy for these patients. Another interesting type of MRI lesion that highlights probable inflammatory responses is a T1 bright subcortical lesion, which is often associated with seizures and inflammatory PML lesions (figure 2).[71]

Confirming the diagnosis of presymptomatic PML

The success of frequent brain MRI will be measured by the identification of an increased proportion of asymptomatic lesions determined to be PML. Imporvements of the American Academy of Neurology diagnostic criteria[63] require symptoms for a definite diagnosis of PML, yet the disorder would ideally be detected through close MRI monitoring of high-risk patients and arrested without the occurrence of symptomatic brain damage. Verification of a PML diagnosis without symptoms is challenging. At a very early stage, CSF viral load might be low or undetectable, and the dynamic nature of PML cannot be confirmed by a single scan. MRI lesions might be characteristic of PML, but no MRI features have been described as being pathognomonic. Small lesions can be difficult to differentiate from multiple sclerosis lesions, especially when the lesion load is high.[86]

A crucial clinical point is that in natalizumab-treated patients at risk, new MRI lesions consistent with PML should be assumed to be PML, and active longitudinal diagnostic and therapeutic steps—including repeated CSF sampling if required, repeated MRI, and serial JCV antibody titres—should be done to help to establish the diagnosis. During these procedures, clinical management should be pursued as if PML were present. Such an approach was successfully implemented in at least three patients who had PML-compatible MRI changes but negative CSF JCV PCR results.[87,89] In two of these patients, managed as if the diagnosis were established, JCV was subsequently detected in CSF on repeat sampling. In all patients, the MRI pattern evolved to one compatible with the development of PML with IRIS, strongly supporting the diagnosis. Asymptomatic patients often later develop symptoms associated with IRIS, ultimately fulfilling traditional diagnostic criteria.

To date, serial quantitative determinations of JCV antibody titres have too rarely been used in the consideration of difficult cases of potential PML. Active JCV disease, including PML, typically drives an increase in JCV antibody titres, which is used to confirm JCV-related disease. Thus, even if viral DNA is not detected in CSF, compatible and evolving MRI lesions associated with increasing systemic JCV antibody titres should provide substantial support for PML diagnosis.[102] However, this approach might not work in patients who have previously received immunotherapy, in which case biopsy or presumptive diagnosis without confirmation becomes necessary. Brain biopsy remains the ultimate criterion when a definite diagnosis is needed and viral DNA in the CSF has not been detected. However, biopsy is difficult at the earliest disease stages, when preclinical lesions are small, and this approach should be used judiciously only when certainty about the diagnosis is clinically critical.

Towards successful risk-mitigation strategies

A risk-mitigation strategy was developed with the aim of protecting patients from PML in the setting of natalizumab therapy.[103] The fundamentals have been actively discussed and variably applied.[6,7,104–108] However, the ideal of witnessing plummeting incidence of PML cases has not yet materialised.[109] We summarise our own suggestions, which are based on a recent algorithm[79] and a review of available data (table 3). We propose that surveillance be guided by estimated risk—derived from anti-JCV antibody index, duration of exposure, and prior immune therapies—with patients dichotomised into two groups: (1) regular surveillance if PML risk is less than or equal to 0·9 per 1000 patients; or (2) intensive surveillance if risk is above 0·9 per 1000 patients. This approach allows simple adjustments when the estimated risks change or new risks are identified (panel).

Panel: Improvements for risk assessment and surveillance of patients with multiple sclerosis

- Risk biomarkers for progressive multifocal leukoencephalopathy (PML) must be expanded and made more accurate
- Enhanced global data collection on cases of PML, including patients with multiple sclerosis and PML associated with natalizumab or other disease-modifying therapies, should be pursued to inform risk assessment and outcome analysis
- Recommendations for surveillance of patients treated with natalizumab or other disease-modifying therapies should be geared to risk profile
- Patients at low risk (<0·9 cases of PML per 1000 exposed to natalizumab) should receive routine assessment for multiple sclerosis disease activity as part of disease-modifying therapy selection and refinement, as well as PML surveillance
- Patients at higher risk (>0·9 cases of PML per 1000 exposed) should undergo enhanced PML monitoring with more frequent MRI and antibody index assessments
- Updated risk assessments should be available as output from the recommended global data collection surveillance network to allow refinement of best practice
- Patients with escalating risk factors should change therapy before PML detection

Shortcomings of risk-stratification elements

The three key risk-stratification elements for PML—JCV antibody status, duration of treatment with natalizumab, and previous immune suppression—are known to be flawed, which might explain their suboptimal effect on PML prevalence. First, although the detection of JCV antibody indicates infection with the virus that causes PML, JCV viraemia and viruria can be present in patients who are antibody-negative.[110] Moreover, seroconversion from positive to negative, and vice versa, can complicate testing for anti-JCV antibody as part of a risk-mitigation programme for PML.[34] An increase in antibody titre or index indicates a history of active infection resulting from a persistent infection or reactivation of latent infection. However, results of quantitative antibody analysis, while suggestive of more active infection with increased risk, are not predictive after prior immunotherapy.[49] Although overall production of antibodies correlates inversely with disease risk, some evidence that antibodies might have a role in controlling the virus is emerging, which is reviving interest in vaccination strategies for JCV or PML management.[32,111] Thus, JCV antibody status falls far short of an ideal biomarker.

Second, duration of therapy as a risk parameter is flawed because of uncertainty about the timing of PML development. The measured variable is duration from the start of natalizumab therapy to clinical diagnosis of PML, which might be a considerable amount of time after the first symptoms emerge.[112] The actual biological interval of interest is time to brain infection with the virus. With more intense monitoring of presymptomatic high-risk populations, including observation of pre-symptomatic lesions on MRI and presymptomatic increases in immunoglobulin concentrations leading up to PML diagnosis, infection seems likely to take place at least 6 months before the clinical manifestations of disease.[46,99] However, the presymptomatic interval is probably even more variable, depending on the clinical expression of lesions in different brain regions. For example, brainstem lesions are likely to lead to symptoms more rapidly than do frontal lobe lesions. Risk estimates for the effect of infection duration become even less meaningful when the biological imprecision of the measure is considered more critically.[107]

Third, the effect of previous immune suppression on risk is poorly described in the scientific literature. It seems fundamentally untenable that the specifics of type and duration of prior immunotherapy are of little consequence in determining risk for PML. At present, a dose of azathioprine would receive equal weight to long-term cyclophosphamide therapy, yet the effect of each drug on the immune system must be very different. In view of these shortcomings, negative commentary on the precision of present risk-mitigation strategies is unsurprising, but these particular uncertainties are perhaps not critical clinically when considered in the overall context of a flawed framework.[6,72,74,107]

Risk stratification with new disease-modifying treatments for multiple sclerosis

The risk-mitigation strategy developed for natalizumab is probably only truly applicable in relation to this drug. PML risk with other available and emerging disease-modifying treatments for multiple sclerosis—dimethyl fumarate, fingolimod, rituximab, ocrelizumab, and cladribine—is much lower than the risk associated with natalizumab,[104] and although such risk must be acknowledged, it should not severely affect decision making where benefits can be accrued by implementing early and effective treatment for multiple sclerosis. In the case of dimethyl fumarate, monitoring for lymphopenia seems likely to identify a higher-risk group in whom alternative therapy should be sought. Prolonged lymphopenia with absolute lymphocyte counts of less than 750 lymphocytes per mL accounts for most cases of PML associated with dimethyl fumarate treatment, although the risk might reside particularly in the loss of CD8+ cells that are crucial to control of JCV.[73] Measurement of circulating lymphopenia, however, is not universally helpful. For fingolimod, this strategy cannot be applied because circulating lymphocyte numbers decrease while effective lymphocytic function appears largely normal. Similarly, alemtuzumab-associated risk for PML has not been shown in patients with multiple sclerosis, despite a marked effect on lymphocyte profiles. Alternatives to lymphocyte counts might include serial antibody measurements or monitoring for circulating JCV. Multiplex PCR for identification of prototypic virus seems a plausible means of risk stratification, but which needs to be investigated as a contributing factor in a reliable risk assessment.[43] The low overall risk associated with most disease-modifying treatments makes this method difficult to validate and probably impractical to use as a stratification factor in practice. Other alternative PML risk-stratification approaches under investigation in natalizumab-associated PML include measurements of CD62L and lipid-specific IgM bands.[113,114] At present, a similar logic applies to rituximab and ocrelizumab. These monoclonal anti-bodies directed against B cells have not been associated with excess risk of PML in patients with multiple sclerosis, despite a large number of cases associated with rituximab when used in the setting of haematological malignancies and other diseases with greater underlying risk of PML.[75,76] The theoretical risk suggests that clinical vigilance is warranted, but no other risk-mitigating strategy can be recommended for PML at this time when using these emerging multiple sclerosis treatments.

Lessons learned and the future of risk mediation

The identification of additional factors and technology that would aid PML risk assessment and be more predictive of PML risk in patients with multiple sclerosis should be a theme of investigation. The ability to quantitatively define T-cell recognition and response to JCV infection, and to identify the emergence of prototypic virus, could aid the clinician in detecting a small subset of high-risk patients for whom treatment with natalizumab would be foolhardy. The present system has not had a great effect so far on the incidence of new cases of PML. Imprecision of the risk model is probably partly to blame, but it also seems likely that risk monitoring and communication to inform patients are not being done consistently, or that patients and clinicians are choosing to continue with natalizumab treatment even when they identify a substantially increased risk.

Therapeutics for autoimmune diseases and immune disorders or for neoplastic disease of genetic origin are being developed. Optimising these treatment choices to include PML risk will require more detailed data than exist at present. For example, the relative efficacy of multiple sclerosis therapies and totality of their known risks, including risk of PML, must inform prescribing patterns. Estimates of these factors are difficult to substantiate because of a lack of comparative data. The known benefits of treatment must be integrated with the risk of PML and other complications encumbered by available therapies. Quantifying all of these factors and explaining them to a patient, who must fit this evidence into a personal risk-tolerance profile, is a very difficult task. Improved tools need to be developed to provide meaningful information to patients and clinicians so that they can make an ethically sound decision for the patient's management.[77]

Conclusion and future directions

Substantial progress in understanding JCV and PML has been made in the past decade. Close observation of patients with natalizumab-associated PML and additional cases of PML seen in patients with multiple sclerosis have provided an opportunity to learn more about the molecular biology of JCV and to make some progress in understanding the evolution of risk and invasion of the brain. Enhanced identification of high-risk patients has allowed the use of MRI to evolve such that detection of PML lesions before symptom onset is commonplace in this group. Improved use of MRI and interpretation of MRI data have proved to be pivotal for PML. However, the clinical management of patients with multiple sclerosis remains challenging.

Although PML is still a serious and sometimes lethal disease, PML outcomes have markedly improved. Most patients survive in settings where immune reconstitution is possible, and severe disability from PML can often be avoided with early detection of disease. However, we are still unable to ascertain individual risk precisely enough to personalise PML management, and very early diagnosis to minimise injury is the best approach at present.

Meanwhile, practical ways to enhance communication about risk and help patients to select the optimum therapeutic approach, allowing for their own willingness to accept or avoid risk, is an ongoing clinical challenge. It is especially important to ensure that patients do not develop PML because of inadequate monitoring or understanding of known risk. Ultimately, if the choice to use natalizumab or other disease-modifying treatments is to continue to be made by patients with multiple sclerosis and their clinicians, full understanding is needed of the overall difference in outcome between those who accept the risk associated with disease-modifying drugs and do well, and those who develop PML; only with this understanding is it possible to conclude that the benefits of treatment clearly justify the associated risk of PML. If patients choose to continue therapy with full knowledge of the associated risks and benefits, it can be argued that principles of ethical care have been served.

Such an analysis depends on the availability of credible data. PML is not a reportable disease, and detailed retrospective data gathering is laborious and incomplete. Registration of cases with systematic reporting of circumstances of the disease would allow the effect of risk-mitigation concepts to be studied. Development of widespread or universal data collection and consideration of cases could speed up research on risk and outcomes and allow the development of more precise risk-mitigation programmes. We believe that although present mitigation strategies are not perfect, the largest failure is in not implementing changes in therapy when PML risk is known to be increased. With the availability of multiple sclerosis therapies that compare favourably with natalizumab in terms of effectiveness, replacement of natalizumab in high-risk patients should be more uniformly adopted to reduce the burden of this potentially devastating disease.

Search strategy and selection criteria

We searched PubMed for articles published between Jan 1, 2005, and Dec 31, 2017, using the search terms "PML", "progressive multifocal leukoencephalopathy", "JCV", "human polyomavirus", "antiviral antibodies", "PML IRIS", and "natalizumab". Articles were also identified by searches of the authors' own files and the reference lists of selected papers. There were no language restrictions. The final reference list was generated on the basis of relevance to the topic of the Review, with a focus on landmark publications. Preference was given to more recently published works that would provide the latest findings and direct the reader to previously published literature.

Contributors

The authors contributed equally to the literature search, collection of reported data, interpretation of data, and the organisation and writing of the Review. EOM led the design of figure 1. TAY led the development of figure 2.

Declaration of interests

EOM has received consultancy fees as a member of the GlaxoSmithKline Independent Adjudication Board (PML evaluation), the Takeda/Millennium Independent Adjudication Board (PML evaluation), the Roche/Genentech PML Adjudication Board (PML evaluation), and the Dr. Reddy's Laboratories, New Jersey, Science Advisory Board (JCV activation); he is named on a National Institutes of Health patent for Multiplex qPCR (patent number 14/408,919 NIH Ref NO E-088-2012/0-US-03). TAY has served as an investigator in clinical trials funded by Biogen, GlaxoSmithKline, Merck, and Novartis, has received fees for the analysis of MRI data acquired in these studies, and has received consultancy fees from Biogen Idec and Ixico Technologies. DBC has received consultancy fees from Biogen, Millennium/Takeda, Bristol Myers Squibb, Genzyme (Sanofi), Pfizer, Amgen, Roche/Genentech, GlaxoSmithKline, Merck/Serono, Inhibikase, Dr. Reddy's Laboratories, and Protagonist Therapeutics; he has received personal fees as a Data and Safety Monitoring Committee member for Biogen and as a Data and Safety Monitoring Board member for Genzyme (Sanofi), Pfizer, Amgen, Roche/Genentech, GlaxoSmithKline, Merck/Serono, Quintiles, and Shire Pharmaceuticals, and consultancy fees as a member of PML Adjudication Boards for Millennium/Takeda and Bristol Myers Squibb.

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