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Beyond progressive multifocal leukoencephalopathy: expanded pathogenesis of JC virus infection in the central nervous system

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Abstract

Progressive multifocal leukoencephalopathy (PML) is a rare but often fatal brain disease caused by the reactivation of the polyomavirus JC (JCV). Characteristics of PML have expanded considerably since the onset of the HIV epidemic with the advent of combination antiretroviral therapy (cART), and the development of immune reconstitution inflammatory syndrome in PML lesions (PML-IRIS). Recently, the monoclonal antibodies natalizumab, efalizumab and rituximab used for treatment of multiple sclerosis, psoriasis, hematologic malignancies, Crohn's and rheumatic diseases, have been associated with PML. In addition, JCV can also infect neurons, leading to novel neurological disorders JC virus granule cell neuronopathy (JCV GCN) and JC virus encephalopathy (JCVE), and it may also cause meningitis. The newly observed features of PML, the increasingly diverse populations at risk, and the recently discovered grey matter involvement by JCV invite us to reappraise the expanded pathogenesis of this virus in the central nervous system.

Rationale for the review

The human polyomavirus JC, JC virus (JCV) is well known for causing progressive multifocal leukoencephalopathy(PML) [1], an often lethal disease of the brain resulting from lytic infection of glial cells in severely immunosuppressed patients. We review herein new features of PML, and recently discovered clinical entities resulting from neuronal infection by JCV, which should help clinicians in their differential diagnosis of patients with central nervous system (CNS) disorders.

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Epidemiology of PML

PML is a demyelinating disease of the CNS occurring in the setting of severe immunosuppression. Prior to the era of HIV, PML remained a relatively rare disease seen in few immunosuppressed patients, including individuals with hematological malignancies, organ transplant recipients and people with chronic inflammatory conditions. The incidence of PML in the general population was estimated to be 4.4 cases per 100,000 insured persons, using a medical service and outpatient prescription claims database [2]. However, PML prevalence dramatically increased during the AIDS epidemic, where up to 5% of AIDS patients developed the disease. Mortality related to PML has also increased from 1.5 per ten million persons in the pre HIV era to 6.1 deaths per ten million persons in the post HIV era [3]. We reported that of 61 PML patients, 48 (78.7%) had AIDS, 11 (18%) had hematologic diseases, including 3 (4.9%) recipients of bone marrow transplantation, 1 (1.6%) had history of thymoma and 1 (1.6%) had dermatomyositis[4]. More recently, a study based on national inpatient diagnosis codes analyzed a total of 9,675 cases of PML from 1998-2005, including 82% with HIV, 8.4% with hematologic cancers, 2.83% with solid organ cancers, and 0.44% with rheumatologic diseases[5]. Several classes of medications which suppress the host cellular immune response have been associated with PML. Recently, a new category of PML patients has emerged among patients treated with immunomodulatory medications for autoimmune diseases, including those treated with natalizumab for multiple sclerosis and Crohn's disease [6-8], rituximab for lupus [9], and efalizumab for psoriasis [10-11].

JC virus pathogenesis

JC virus infection is species-specific and it is only found in humans. Therefore, research on JC virus pathogenesis has been hampered by the lack of an animal model. While all JCV infected oligodendrocytes appear to sustain a productive infection, some astrocytes do also harbor late JCV genes and are destroyed, while other may sustain an abortive infection and appear transformed. The cellular receptors for JC virus include the N-linked glycoprotein with an alpha (2,6)-linked sialic acid [12], which is present on many human cells. In addition JC virus can bind to the serotoninergic $5HT_{2a}$ receptor in permissive astroglial cell cultures. Infection of these cells *in vitro* is blocked by pharmacologic agents targeting the $5HT_{2a}$ receptor [13]. This receptor is present in several organs, including in the kidney, on epithelial cells, in the blood, on B lymphocytes and platelets, and in the CNS, on glial cells and neurons [14-16]. Unlike other polyomaviruses, JCV infection has a narrow host cell range. Although JCV receptors are present in multiple organs and JCV DNA has been detected in oligodendrocytes, astrocytes, lymphocytes[17], kidney epithelium cells, tonsil stromal cells[18], and plasma cells[19], it has been very difficult to propagate JCV in human cell culture systems.

JC virus is a small ubiquitous DNA polyomavirus with a 5.13 Kb circular enclosed double stranded DNA (Fig 1). The JCV coding region, covering ~ 90% of the viral sequence, confers the genotype which is associated with the geographic origin of the patient. JCV transcription occurs on both strands of DNA. The early genes for the large T and small t antigens, responsible for viral transformation, gene regulation, and replication, are encoded counterclockwise. Conversely, the non coding regulatory region (RR) and the late genes for the agnoprotein and the viral capsid proteins VP1, 2, and 3, are encoded clockwise. The coding region is well conserved and has not been convincingly associated with disease pathogenesis. However, the RR sequence of JCV is hypervariable and contains determinants for neurotropism and neurovirulence [20]. After asymptomatic primary infection which occurs in childhood, the virus remains quiescent in the kidneys, bone marrow, and lymphoid tissue [21-23]. Indeed, JCV can be detected by PCR in the urine of one third of healthy or immunosuppressed individuals alike with or without PML, on cross sectional studies [24-25]. However, JCV is usually not found in the blood of immunocompetent people [26]. JCV RR most often detected in the urine has a stable

sequence which is found in healthy and immunosuppressed patients alike, with and with out PML. The structure of this RR has been called the "archetype", as it is thought that it is the one from which all other forms have evolved [27]. Conversely, JCV RR most often isolated from the CSF and brain tissues from PML patients have rearrangements, including duplications, tandem repeats, insertions and deletions (Fig 1) [20]. Therefore, these rearranged RR most likely arise in the setting of immunosuppression, and are necessary for reactivation of JC virus to result in PML. Indeed, JCV replication and transcription is dependent on binding of nuclear factors such as NF-1 to specific sites in the RR. The presence of additional NF-1 binding sites in rearranged RR forms was directly proportional to the level of viral transcription in glial cell lines [20]. In addition, detection of rearranged JCV RR in the plasma correlated with poor clinical outcome in patients with PML [28].

Host Immune Response

Host humoral immune response to JCV has been extensively studied. The first test used in the seventies to estimate JCV seroprevalence was the hemagglutination inhibition assays (HI) which is based on the ability of JCV to agglutinate human type O erythrocytes in vitro. The presence of antibodies in serum is revealed by the ability to prevent this agglutination. Using whole JC virions, this test detected a seropositivity of 60% in the 20-29 years age group in the US [29]. More recently, with an HI assay based on virus-like particles containing the JCV VP1 major capsid proteins, Knowles et al found JCV seroprevalence reaching 50% in the 60-69 years age group in England and Wales [30]. Using recombinant VP1 protein, quantitative enzyme immuno assay (EIA) could detect IgG antibodies in up to 86% of healthy German subjects, without specification of age group [31]. These results contrast with another US study, where JCV seroprevalence by EIA was only 39% in the 24-30 year old group, and 65% in the 65-74 year old group[32]. Other reported JCV antibodies in 72% in 26-31 year old pregnant women in Finland [33] and 68% in Swiss healthy blood donors in 50-59 years old group [34]. Comparing both HI and EIA, one study has determined a higher EIA titer than HI titers in a Japanese and US population [35]. Since there is no recognizable clinical event associated with JCV primary infection, and no clearly defined JCV seronegative population, the variability of these results may be explained by differences in the technology used and the populations tested.

Elevated JCV-specific serum antibody titers are found in both HIV-positive and HIV-negative PML patients, and a detectable intra-thecal synthesis of JCV-specific antibody in HIV-positive PML patients after combined antiretrovial therapy (cART) paralleled JCV clearance from CSF [36-37]. Therefore, the humoral immune response alone is not sufficient to prevent reactivation of JCV, leading to PML. Thus, the cellular immune response is necessary for prevention of viral reactivation and proliferation. Such response may be mediated by JCV-specific CD4 T cells, which have been detected in the blood of PML survivors and correlated to JCV clearance from the CSF [36, 38]. The role of CD8+ cytotoxic T lymphocytes(CTL) has been studied in detail, using recombinant vaccinia viruses expressing the JCV T antigen or VP1 protein [39], or pools of overlapping peptides covering the entire VP1 protein [40-42]. CTL recognize 9 amino acid epitopes of viral proteins presented on the class I human leukocyte antigen (HLA) molecules of infected cells. These cells are destroyed by the CTL, thereby preventing further spread of the virus. We have mapped several CTL epitopes of JCV T antigen and the VP1 capsid protein restricted by HLA A*0201, the most common class I allele present in the North American population [40, 43-44]. Although the regulatory protein T is transcribed early in the viral cycle, we and others have detected a stronger immune response against the VP1 protein [36, 39, 43]. JCV-specific CTL are usually detected in the blood of PML survivors, and rarely in PML progressors, who have a fatal outcome within one year from disease onset [40, 44]. A prospective study showed that 87% PML patients with demonstrable JCV-specific CTL early after PML onset have an inactive disease upon follow up, while 82% of those who are not able to mount such response continue to have an active disease over time [45]. Furthermore, the CTLs,

present in the CSF of PML survivors, display effector memory phenotype in the early phase of the disease. In addition, CD8+ T-cells are the major inflammatory cells found in PML lesions, where they aggregate around JCV-infected cells [46]. Further studies aiming at characterizing other T cell epitopes of all of the JCV proteins restricted by different HLA alleles are needed for elucidating the breath of JCV cellular immune response in diverse populations.

Classic PML

Clinical presentation—Typically, PML results from productive infection of oligodendrocytes and to a lesser extend astrocytes. Therefore, neurologic deficits correspond to areas of demyelination in the brain. The presenting symptoms can vary and include weakness, sensory deficit, hemianopsia, cognitive dysfunction, aphasia, or coordination and gait difficulties. The disease usually does not involve the optic nerves or the spinal cord. However, we have observed incidental spinal cord demyelination in a post mortem study [47]. Interestingly, 18% of PML patients also suffer from seizures [48]. This is somewhat surprising because seizures are considered to arise from the cortical grey matter, whereas PML is a white matter disease. However, PML patients presenting with seizures frequently had demyelinating lesions immediately adjacent to the cortex.

Although PML usually affects individuals with profound cellular immunosuppression, on occasion, it has also been diagnosed in patients with no initial clinically apparent immunosuppression. We have followed 5 such patients at our institution, and found 33 others reported in the literature. Of the total of 38 cases, 22 (57.9%) had no specific underlying diagnosis. Within this group of 22, 5 (22.7%) had low $CD4^+$ T cell counts (80-294cells/μl) and were eventually diagnosed with idiopathic $CD4^+$ lymphocytopenia, and 1 had low $CD4^+$ T cell count of 308cells/μl [49]. PML was fatal in most of these patients. Therefore, PML need to be considered as part of the differential diagnosis in patients with new onset neurological symptoms, even without overt immunosuppressive risk factors.

Radiological findings—Although MRI is the modality of choice in diagnosing PML, both CT and MRI can be employed. The affected brain lesions are usually detected in the white matter and do not correspond to specific vascular territories. These lesions appear as hypodense or patchy on CT, whereas MRI shows areas of hyperintensity on T2-weighted and FLAIR (fluid attenuated inversion recovery) images and hypointensity on T1-weighted images (Figure 2). Often multiple lesions are present in one patient and frequently localize to the subcortical hemispheric white matter or the cerebellar peduncles. Furthermore, PML lesions can also be found in grey matter structures such as the basal ganglia or thalamus, where myelinated fibers reside. Classic PML lesions are devoid of edema, mass effect or contrast enhancement on imaging.

Diagnosis—The diagnosis of PML is established by demonstration of JCV DNA or proteins by in situ hybridization(ISH) or by immunohistochemistry staining (IHC) on brain biopsy, or by detection of JCV DNA in CSF by PCR. Histologically, PML is characterized by a productive, lytic infection of oligodendrocytes and astrocytes by JCV, leading to multiple areas of demyelination in the CNS. In addition, there can be reactive gliosis and giant, bizarre multinucleated astrocytes in affected areas. Furthermore, cases with typical clinical and radiological presentation can be considered as "possible PML" even in absence of JCV detection in the CSF if other causes of infection or tumors have been ruled out[50].

Treatment—Currently, there is no known specific antiviral agent against JCV. A few antiviral medications have been studied for treatment of PML mostly in retrospective case series. Cidofovir, an antiviral agent used in human cytomegalovirus (CMV) infection, initially showed promise in improving survival of HIV-positive PML patients in combination with cART and

independently, in two retrospective studies [51-52]. A multicohort analysis combining data from one prospective[53] and five cohort studies totaling 370 cases for analysis of cidofovir efficacy in treatment of HIV-positive PML patients who were already on cART, did not show a survival benefit in patients taking cidofovir (hazard ratio for death 0.93, 0.66-1.32), nor did cidofovir treatment improve PML-related residual disability by 12 months [54]. Cytarabine, a chemotherapeutic agent which inhibits JCV replication in vitro [55], was associated with stabilization of PML in 7/19 (37%) of HIV-negative patients with leukemia or lymphoma in one retrospective study[56]. However, a randomized controlled clinical trial using cytarabine either intravenously or intrathecally, in HIV positive patients with PML showed no survival benefit in addition to treating HIV with cART with a p=0.85 log-ranked test [57]. Furthermore, intrathecal infusion of cytarabine in 27 PML patients in another study also did not show any survival benefit [58]. More recently, the discovery that JCV enters cultured cells via the serotonin receptor, $5HT_{2a}$, prompted the clinical use of mirtazapine, a serotonin receptor blocker. Although anecdotal case reports describe favorable outcome in PML patients treated with mirtazapine[59-60], solid supporting evidence of efficacy is still lacking. In a recent study, one year survival was 62% among 14 PML patients treated with mirtazapine compared to 45% in 11 untreated PML patients, a difference that was not significant ($p = 0.45$)[61]. Finally, a large screen of chemical agents showed that mefloquine, an anti-malaria medication, could inhibit JCV replication in a cell culture system [62]. A multicenter worldwide clinical trial is now evaluating the use of mefloquine for treatment of PML [63].

Without a specific antiviral agent, the current treatment goal in PML is to restore host adaptive immune response to JCV for control of the infection. In HIV-positive patients, this is accomplished mainly by initiating treatment of HIV with cART. In HIV-negative patients, the main therapeutic objective is to reduce, if possible, immunosuppressive medications, allowing the adaptive immune system to contain the infection. However, in organ transplant recipients, such reduction increases the risk of graft rejection. Therefore, a better strategy may be to augment the cellular immune response to JCV using immunotherapies, such as dendritic cell vaccines[64].

Prognosis—Although PML is still a fatal disease and there is currently no specific treatment, HIV-positive PML patients are living longer in the cART era. Several groups have defined factors associated with PML prognosis in HIV positive patients. Clinically, a higher CD4+ T cell count, and contrast enhancement on radiographic imaging and neurological recovery were associated with longer term survival [61, 65-66]. The magnitude of JCV viral load in CSF was inversely correlated to survival (Spearman's rank correlation, −0.83; $p<0.01$) in a pilot study [67]. This was confirmed in a second study where a threshold of 50 to 100 JCV genome copies/μl of CSF was significantly associated with mortality [68]. Prior to the advent of cART only 10% of the HIV-positive patients were alive one year after the PML diagnosis[69]. However, the 1-year survival rate has climbed to 50% after the cART era [70]. Recently two reviews have compared the mortality rate of PML before and after the introduction of cART. First, in a review of PML cases seen during a 20 years period in the Swiss HIV Cohort Study, the 1-year mortality rate of PML decreased from 82.3 cases per 100 person year during the pre cART era, to 37.6 cases per 100 person year after 1996 [71]. Second, the Danish HIV cohort study also showed that after introduction of cART, PML patients' median survival time increased from 0.4 year to 1.8 year [72].

Nevertheless, some PML patients do survive extended periods of time. We followed 24 patients for 60-188 months post PML diagnosis. All but one of these patients were HIV-positive and on cART. During the follow-up period, the majority of the patients (83%) had undetectable HIV virus in the plasma and a mean CD4 count of 389cells/ul, sufficient for protection against most opportunistic infections. Clinically, one third of these patients had no significant disability, whereas the rest were evenly distributed among the slight, moderate, and moderately

severely disabled groups. In addition, 21% of the patients developed seizures. Lastly, survival was linked to the ability to mount a cellular immune response to JCV. Almost all (95%) of the long term survivors in this series displayed detectable JCV-specific CTL in their peripheral blood [73]. Finally, JCV-specific CTL to the VP1 protein is also a major prognostic factor of disease outcome. Indeed, one year survival was 73% among 12 PML patients with detectable CTL in their blood within 3 months of diagnosis, compared to 46% for those without CTL. This trend was more pronounced than the impact of CD4+ counts on survival in HIV-positive patients with PML.

PML-IRIS

Although a cellular immune response directed against JCV is beneficial in classic PML, a rapid global recovery of the immune system may not always be favorable. Indeed it can trigger an immune reconstitution inflammatory syndrome (IRIS). IRIS is an inflammatory response to clinically apparent or subclinical pathogens, associated with recovery of the immune system after a period of immunosuppression. This immune reconstitution is inferred by an increase in T lymphocyte counts, which usually follows the commencement of cART in HIV-positive patients, or with cessation of immunosuppressive therapy in HIV-negative patients. IRIS has been described in context of infections with most pathogens seen in AIDS [74-76]. PML-IRIS can account for up to 23% of PML cases diagnosed in HIV-positive patients[77]. Since PML is a rare disease in HIV-negative patients, the frequency of PML-IRIS in this population has yet to be established.

Clinical presentation—PML-IRIS can be defined as follows: HIV positive patients treated with cART, followed by an increase of $CD4^+$ cell counts and a decrease in HIV plasma RNA level from baseline, present with paradoxical development of inflammatory PML, or developing an inflammatory reaction at a site of previously diagnosed PML lesions. This inflammatory reaction is characterized by contrast enhancement and/or edema of PML lesions on MRI with possible mass effect, and is associated with acute and usually transient clinical worsening not consistent with the expected course of a previously or newly diagnosed PML. A case of PML-IRIS is shown in Fig 3. Cases of PML-IRIS in HIV-positive patients were thoroughly reviewed by Tan et al [78]. Time of onset of PML-IRIS ranged from 1 to 104 weeks after starting cART. Comparing the pathogenesis of IRIS in HIV-positive and HIV-negative patients may further elucidate interplay between different components of the immune system.

Radiological findings—Unlike in classic PML, PML-IRIS lesions may show contrast enhancement on MRI, due to the local inflammation and breakdown of the blood-brain barrier[79]. This inflammation can be associated with brain edema, swelling, mass effect, and in the most extreme cases, causing brain herniation and death [80]. Contrast enhancement in PML lesions can be detected on MRI in both HIV-positive and HIV-negative patients with IRIS [81]. However, contrast enhancement may only be a transient phenomenon, and therefore, not necessarily captured at the time of MRI evaluation.

Diagnosis—The CSF PCR for JCV may be negative in PML-IRIS patients. This is due the fact that the recovering immune system is able to partially contain viral replication to a level below the detection threshold of the PCR assay. Specifically, it is likely that the influx of CTL can effectively suppress active JCV replications. The few histological reports of PML-IRIS cases demonstrated, unlike in classic PML cases, an overwhelming infiltrates of CD8+ lymphocytes within demyelinated lesions correlating with contrast enhancement of PML lesions on MRI in both HIV-positive and HIV-negative patients [81]. In some cases, perivascular leukoencephalitis, resembling multiple sclerosis can be found in areas devoid of JCV-infected cells. It has been hypothesized that an exuberant reaction of CD8+ cells, deprived from the control of CD4+ cells, is in part responsible for the pathogenesis of IRIS in the CNS[80].

Treatment—In HIV-positive PML-IRIS patients, treatment with cART allows recovery of functional CD4+ cells while decreasing the plasma HIV viral load. Discontinuation of cART will reduce the immediate stimulus of the immune system. Only two published case-reports and the patient described in Figure 3 reported discontinuation of cART for two to three weeks following detection of PML-IRIS[82-83]. These patients did well during the follow up period. In other case studies, clinicians have chosen to continue cART therapy through PML-IRIS. Indeed, it is not yet clear if resuming cART after the resolution of IRIS will not also reignite the inflammatory reaction. In addition, interruption of cART can increase HIV mutation rate, leading to future drug resistances. In HIV-negative PML-IRIS patients, steroid treatment is usually given in an effort to dampen the inflammatory response. Published cases of steroid use in PML-IRIS showed possible benefits, as recently reviewed in [78]. However the use of steroids in PML-IRIS remains controversial as these medications are immunosuppressants, and may contribute to increase HIV replication in HIV-positive patients, or disrupt treatment plans in HIV-negative patients with cancer or autoimmune diseases. Finally, the inflammatory reaction associated with IRIS does not seem to alter survival of PML. In a recent outcome study including HIV-positive and negative individuals, we observed a similar one year survival rate in PML-IRIS (54%) versus PML without IRIS (49%) [61]. PML-IRIS associated with treatments of monoclonal antibodies is discussed in the next section.

PML associated with monoclonal antibodies: natalizumab, efalizumab, and rituximab

Over the past few years, monoclonal antibodies have been associated with cases of PML in patients with autoimmune diseases, including multiple sclerosis, Crohn's disease, psoriasis or lupus, which are not traditionally included among populations at risk for PML.

A) Natalizumab—Natalizumab is a humanized IgG_4 monoclonal antibody that binds the alpha 4 subunit of the very late antigen-4 integrin present on leukocytes, which prevents the egress of these cells outside of the bloodstream. Treatment indications include multiple sclerosis(MS) and Crohn's disease. The medication was voluntary withdrawn from the market by Biogen Idec in February of 2005 after the initial cases of PML were discovered, and reinstated in the US the summer of 2006 as a monotherapy for MS. A total of 31 PML cases have been reported in MS by 1/12/2010 and one in Crohn's disease patients treated with natalizumab [6-8, 84-86]. The risk for developing PML in natalizumab-treated MS patients which was initially estimated to be 1/1000 at 18 months, has risen to 1.29 (CI 0.82-1.93) cases per 1000 patients with ≥ 24 infusions, suggesting a possible increased risk of developing PML with increased number of natalizumab infusions [87].

B) Efalizumab—Efalizumab is a humanized IgG_1 monoclonal antibody targeting the alpha subunit of the leukocyte function antigen-1 (LFA-1), which binds to intracellular adhesion molecule (ICAM) on antigen-presenting cells. Blockade of these interactions results in prevention of lymphocyte activation, proliferation and migration. It was used for treatment of moderate to severe plaque psoriasis, and was withdrawn from the market in April 2009 after 3 patients were diagnosed with PML [10, 88].

C) Rituximab—Oldest of these three monoclonal antibodies, rituximab, was first approved for use in the US in 1997 and followed with approval by the European Union in 1998. Rituximab is a chimeric IgG_1 monoclonal antibody that targets CD20+ B lymphocytes for lysis and depletion from the peripheral circulation. It is approved for treatment in CD20+ non-Hodgkin lymphoma (NHL). It is also used in conjunction with methotrexate for reduction of symptoms in patients with rheumatoid arthritis who previously failed treatment with tumor necrosis factor-alpha inhibitors, and more recently, in multiple sclerosis and lupus.

Of the 57 reported cases of PML after rituximab therapy, 90% of the patients died [9]. However it is difficult to isolate the role of rituximab in the development of PML in these patients because most of these patients had lymphoproliferative diseases and had previously received several other classes of immunosuppressive agents, including purine analogs, alkylating agents and corticosteroids. Nevertheless, a patient with lupus treated with rituximab was recently reported for developing PML, although no prior TNF inhibitor treatment was administered [89]. Thus, rituximab treated patients usually have both B and T lymphocyte impairment. Indeed, one study documents a median CD4 count of 216 cells/ul in 25 patients who received rituximab[90]. Lastly, the pathogenesis of rituximab in PML can be due to the decrease of B lymphocytes in the cerebral perivascular spaces [91], resulting in decreased antigen presentation to T lymphocytes, and therefore, alteration of the cellular immune response as well. Finally, given the few documented cases of PML occurring in patients with autoimmune diseases treated with rituximab alone, the risk of developing PML in this population has yet to be determined.

Clinical presentation: Since PML is not typically considered in the differential diagnosis of patients with MS, Crohn's disease and autoimmune diseases, the initial diagnosis was often delayed in the early phase of natalizumab post marketing. In the one reported case of patient with Crohn's disease who developed PML after 16 months on natalizumab, the initial symptom of PML was mental confusion without focal neurological deficit. He was initially diagnosed with astrocytoma since the brain biopsy showed a large number of atypical astrocytes. JCV infection was later confirmed after treating clinicians revisited the case when reports of PML in natalizumab-treated MS patients emerged [6]. PML symptoms in MS patients treated with natalizumab are difficult to distinguish initially from those of MS itself. One patient presented with initial mental confusion, another with mild myoclonic jerking of the arm, and a third with difficulties with hand-eye coordination and speech, and the last with attention deficits [7-8, 84-85]. A patient also presented with seizure as the initial diagnosis [84]. With increased awareness of PML occurrence in this population, diagnosis is now made with little delay in most cases (Clifford et al, current issue of Lancet Neurology).

The efalizumab treated patients presented with cognitive difficulties and weaknesses [88]. The initial presenting symptoms in patients treated with rituximab were mental confusion (54.4%), hemiparesis (33.3%), loss of motor coordination (24.6%), speech difficulties (21.1%), and visual changes (17.5%) [9].

Radiological findings: MRI findings are similar to those of classic PML. However, the neutralization of the immune system by the medication may lead to very destructive cavitated lesions which are rarely seen in other settings, including in patients with AIDS [8]. A case of natalizumab-associated PML in an MS patient is shown in Fig 4. In addition, unlike in classic PML where gadolinium enhancement is usually not seen on presentation, 11/28 natalizumab treated patients who developed PML, had gadolinium enhancement at diagnosis, indicating ongoing inflammatory host response to JCV (Clifford, et al, current issue of Lancet Neurology). MRI features may be useful in differentiating PML and MS lesions, as summarized in one study [92], and one recent study attempted to characterize lesions of relapsing-remitting MS from those of PML [93].

Diagnosis: The diagnosis of PML is established by PCR detection of JCV DNA in the CSF. However, this test may be negative, and brain biopsy may become necessary [84]. Unlike HIV-positive PML patients who have significantly altered cellular immune response, where host immune response to JCV is only detected after immune reconstitution with cART, MS patients who develop PML during natalizumab treatment have at baseline cellular immune response to JCV [94]. This may explain why their JC viral load in the CSF is very low. Indeed, 15 of the 28 natalizumab-treated PML patients had a CSF JC viral load of 500copies/ml or less at diagnosis (Clifford, et al, current issue of Lancet Neurology).

Treatment: Clinicians treating natalizumab-associated PML are faced with a conundrum: once discontinued, the drug has a biological activity of 3 months, during which time PML can progress, and the resulting return of lymphocytes in the CNS may lead to IRIS [7]. Natalizumab induces leukocytosis for weeks to months, and alters the CD4/CD8 ratio in the CSF for at least 6 months [95-96]. While plasma exchange/immunoadsorption can reduce the serum concentration of natalizumab, the rapid restoration of immune response may precipitate a severe IRIS reaction up to 3 weeks later [84-85, 97]. One natalizumab-treated patient eventually recovered from the IRIS episode [84], and another was critically ill during IRIS and needed intensive care support [85]. Furthermore, two efalizumab-related PML cases treated with plasma exchange also developed IRIS and had a fatal outcome [88]. Therefore, the restoration of lymphocytes trafficking into the brain parenchyma either by discontinuation of natalizumab or by removal of the drug with plasma exchange has uniformly resulted in IRIS in this population. Aggressive use of corticosteroid in this setting is necessary and helpful in preventing fatal outcome [84-85]. Given the increased risk of developing PML while on a monoclonal antibody therapy overtime, and the risk of developing PML-IRIS after withdrawal of the monoclonal antibody, the characterization of prediagnostic markers of PML would be very valuable. Our recent pilot study showed that asymptomatic reactivation of JCV occurs in the urine and peripheral blood mononuclear cells (PBMC) of natalizumab treated patients over an 18 month period and that decrease in the magnitude of the cellular immune response may trigger this reactivation. Thus, molecular monitoring of JCV in blood and urine may help determine which individuals are at risk of developing PML [94]. However, several recent reports with different study designs did not show similar results [98]. Thus, better understanding of the biology of JCV reactivation with monoclonal antibody treatment and host immune response resulting in IRIS is urgently needed to guide clinicians in the use of these medications and the management of PML in this setting.

Other JCV associated diseases

JCV Granule Cell Neuronopathy (JCV GCN): Whereas PML results form JCV infection of glial cells in the brain, JCV granule cell neuronopathy(JCV GCN) is caused by JCV infection of granule cell neurons in the cerebellum. Areas of cell loss in the granule cell layer were first described in up to 5% of PML patients prior to the era of HIV [99]. Granule cells with hypochromatic and enlarged nuclei were also seen in AIDS patients with PML [100], and JCV DNA was detected by PCR in the cerebellar biopsy of an AIDS patient with cerebellar atrophy [101]. We initially described a productive infection by JCV in cerebellar granule cell neurons in an HIV-positive patient with cerebellar atrophy, who had PML lesions in the hemispheric white matter, but not in the cerebellum [102-103]. We subsequently found JCV infection in granule cell neurons of an HIV-positive patient with marked cerebellar atrophy, but no radiological or histological lesions of PML, and called this novel entity, distinct from PML, JCV granule cell neuronopathy (JCV GCN) [104]. This syndrome has now been described by several groups in both HIV-positive and HIV-negative patients, including one with sarcoidosis [105-107]. JCV GCN can occur in isolation, or concomitantly to PML [102, 104]. Incidence of JCV GCN may be higher than previously reported since a histological survey of brain samples from 43 known PML patients showed that up to 51% of them also had JCV infected granule cell neurons, regardless whether they also had classic PML lesions in the nearby cerebellar white matter [108].

Since the granule cell neurons are destroyed by JCV, patients with JCV GCN present with subacute or chronic onset of cerebellar dysfunction, including gait ataxia, dysarthria and incoordination. MRI shows cerebellar atrophy. In addition, cerebellar white matter lesions consistent with PML can also be present. Diagnosis is established by cerebellar biopsy, showing a lytic infection of granule cell neurons by JCV. In the proper clinical and radiological setting, JCV GCN can also be diagnosed by detection of JCV DNA by PCR in the CSF. Molecular characterization of JC virus in one case revealed a JCV variant with a 10 base pair

deletion in the C terminus of the VP1 gene. The VP1 gene encodes the VP1 major capsid protein and this deletion caused a change in the deduced last 13 amino acid of this protein [109]. The mechanism by which this deletion leads to a neuronal tropism of this strain is currently under investigation.

JCV encephalopathy (JCVE): Although JCV mainly infects the brain white matter, gray matter infection can occur. We have recently reported a case of JCV induced gray matter disease, secondary to a productive infection of cortical pyramidal neurons in one patient [110]. An HIV-negative woman with history of lung cancer presented with subacute onset of global cognitive decline and aphasia, consistent with encephalopathy. She developed seizures and passed away 4½ months after onset of symptoms. MRI showed non-enhancing lesions initially restricted to the hemispheric gray matter, with subsequent extension into the subcortical areas. The CSF JCV PCR was positive and the diagnosis was confirmed by histological examination which showed fulminant, productive and lytic JCV infection of the cortical pyramidal neurons and astrocytes, associated with laminar necrosis. Since myelinated fibers present in the cortex can be infected by JCV, extension of PML lesions into the gray matter has also been reported by others [111-112].

JCV meningitis: JCV is not routinely checked when patients present with meningitis. However, there are several published cases finding JC virus in the CSF of both immunocompromised and immunocompetent patients who presented with meningeal symptoms only. Although the prevalence of detecting JCV in CSF of patients presenting with meningitis has yet to be determined, investigators screened JCV in CSF from patients with the initial diagnosis of either meningitis or encephalitis and found 2/131 (1.5%) positive for JCV [113]. It is not clear in these cases whether infection was due to JC virus primary infection or reactivation. The presentation of JCV meningitis is consistent with typical meningeal symptoms including headache, nausea, stiff neck, diplopia, with no focal neurological deficits [114-115]. Unlike PML, there is no focal lesion in the white matter of the brain. MRI can detect mild ventricular dilatation. Diagnosis is confirmed with detection of JCV in the CSF along with the exclusion of all other neurotropic viruses.

Treatment in JCV GCN, JCVE and JCV meningitis is the same as in classic PML, including cART in HIV-positive patients, and removal of medications causing immunosuppression in HIV-negative individuals.

Conclusion—There have been major changes in the epidemiology and the clinical presentation of PML since its initial description in 1958. Novel clinical entities caused by JCV infection of cerebellar and cortical pyramidal neurons have been discovered. These findings expanded the clinical features of JCV infection in the central nervous system (Table 1). Examples of the associated histopathological findings are shown in Fig 5. These new features of JC virus infection provide further challenges to scientist in understanding the biology of this polyomavirus and its pathogenesis and to clinicians in diagnosing CNS infections. Analyzing the course of primary infection, characterizing sites of viral latency and defining mechanisms of reactivation will aid in preventing the onset of these diseases. While a strong cellular immune response is crucial for PML survival, an abrupt restoration of immune response can result in fatal progression of symptoms due to IRIS. Better understanding of the immune control of JC virus infection is needed to devise strategies aiming at preventing iatrogenic complications of PML treatment.

Search strategy and selection criteria

Authors conducted PubMed searches for this paper using terms “progressive multifocal leukoencephalopathy” and “JC virus”. Only papers in English language were reviewed. We

tried to focus on papers published in the last 20 years, when possible, but also included prior papers that made definitive contributions to the field. For the latest updates on the multiple sclerosis patients, we accessed the websites listed in the reference section.

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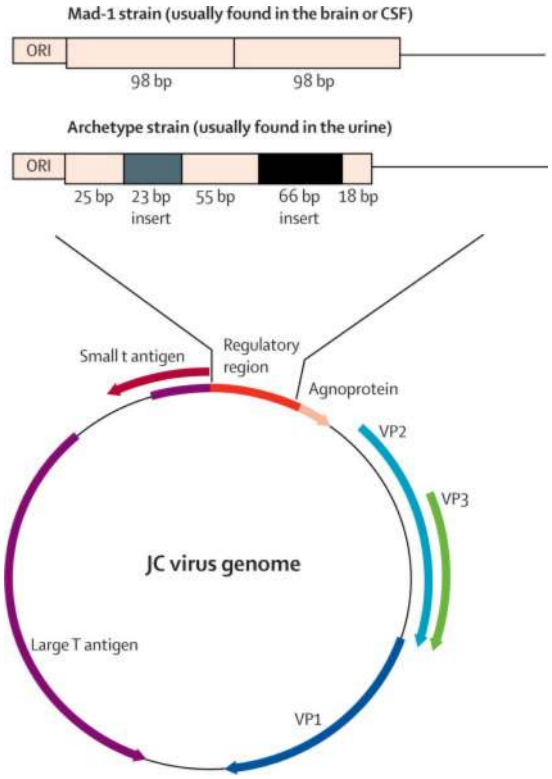

Figure 1. JC virus Genome

JCV genome is composed of a 5kb of double stranded circular DNA. The coding region includes the small t, large T antigens, the capsid proteins VP1, VP2, and VP3, and the agnoprotein (Agno). The non-coding regulatory region (RR) detected in the brain or CSF of PML patients usually consists of tandem repeats of a 98 bp element, as in the Mad-1 strain, and most isolates from urine of healthy and PML patients alike, are similar to the archetype RR. JCV archetype has one 98 bp element with a 23 bp insert (gray box) and a 66 bp insert (black box).

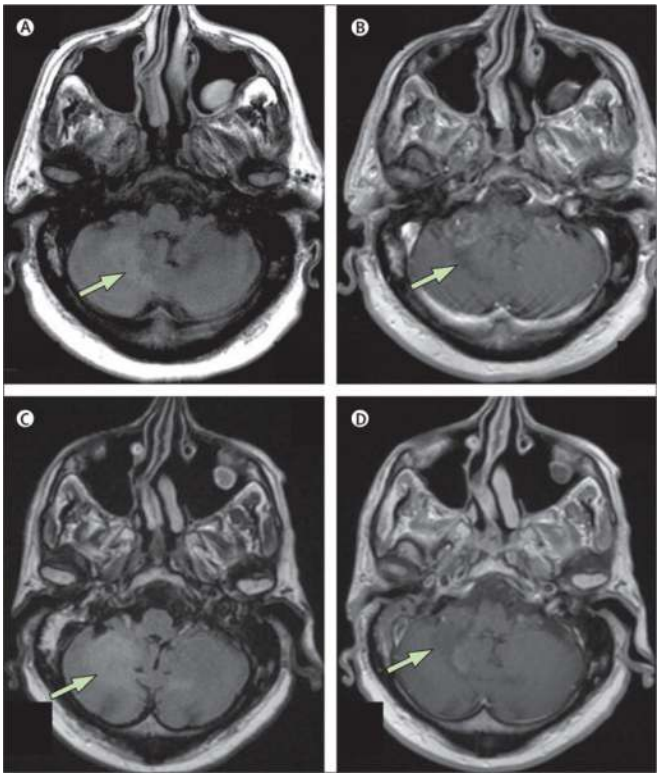

Figure 2. Cerebellar lesions in a patient with classic PML

A 69 years old man with CLL, treated successively with combination chemotherapy and rituximab presented with 6 weeks history of progressive decline in right hand and leg coordination, and new onset of severe vertigo and emesis. MRI showed an area of hyperintense signal in the right cerebellar hemisphere and cerebellar peduncle on FLAIR (A, arrow), which appeared hypointense and was devoid of contrast enhancement on T1-weighted image (B, arrow). JCV PCR was positive in CSF. Rituximab was discontinued and he was started on mirtazapine 15mg per day. His neurological deficit continued to progress, including dysphagia and repeat MRI performed 10 days later showed extension of the lesions to the brain stem and the left cerebellar hemisphere on FLAIR image (C, arrow) and absence of enhancement on T1-weighted image (D, arrow). He passed away 3 months after PML diagnosis.

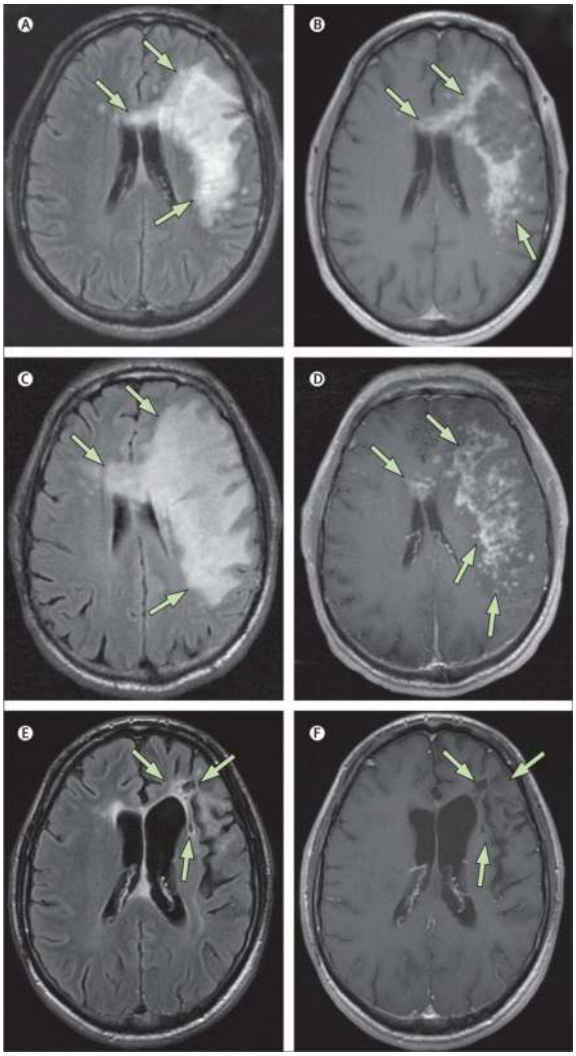

Figure 3. PML-IRIS in an HIV+ patient

A 40 yo man with HIV infection, who presented with progressive onset of word finding difficulties and right hemiparesis followed by seizure, 4 days after starting cART. PCR was positive for JCV in the CSF peripheral CD4 count was 468 cells/ul. MRI performed at another hospital reported a 3 cm focus of abnormal increased signal on FLAIR sequences in the left frontal subcortical white matter, surrounded by linear and punctate foci of enhancement at the margins of the lesion. This lesion extended into the left corona radiata, the corpus callosum and the right frontal white matter. MRI performed at our hospital 3 week after the initial one showed lesions in FLAIR (A, arrows) and contrast enhancement in T1-weighted image post gadolinium injection (B, arrowheads). His aphasia improved progressively with addition of ritonavir to his cART regimen. His CD4 count increased to 558 cells/ul and his HIV plasma viral load was undetectable. He then presented with worsening aphasia. MRI performed 2 and a half month after onset of initial symptoms showed enlargement of the lesions in the left hemispheric white matter and the corpus callosum in FLAIR (C, arrows) which displayed intense contrast enhancement in T1-weighted images (D, arrowheads) as well as mass effect, right to left shift and subfalcine herniation. He was treated with dexamethasone 6 mg three times a day, tapered over 2 weeks, and cART was discontinued for two weeks. All neurological symptoms progressively improved and 2 and a half year later, he has no residual weakness and only minor word finding difficulties. MRI showed leukomalacia and atrophy of the left frontal

lobe with dilatation of the left lateral ventricule in FLAIR (E, arrows) and absence of contrast enhancement in T1-weighted image (F, arrowheads). His CD4 count was 669/ul and HIV plasma viral load continue to be undetectable.

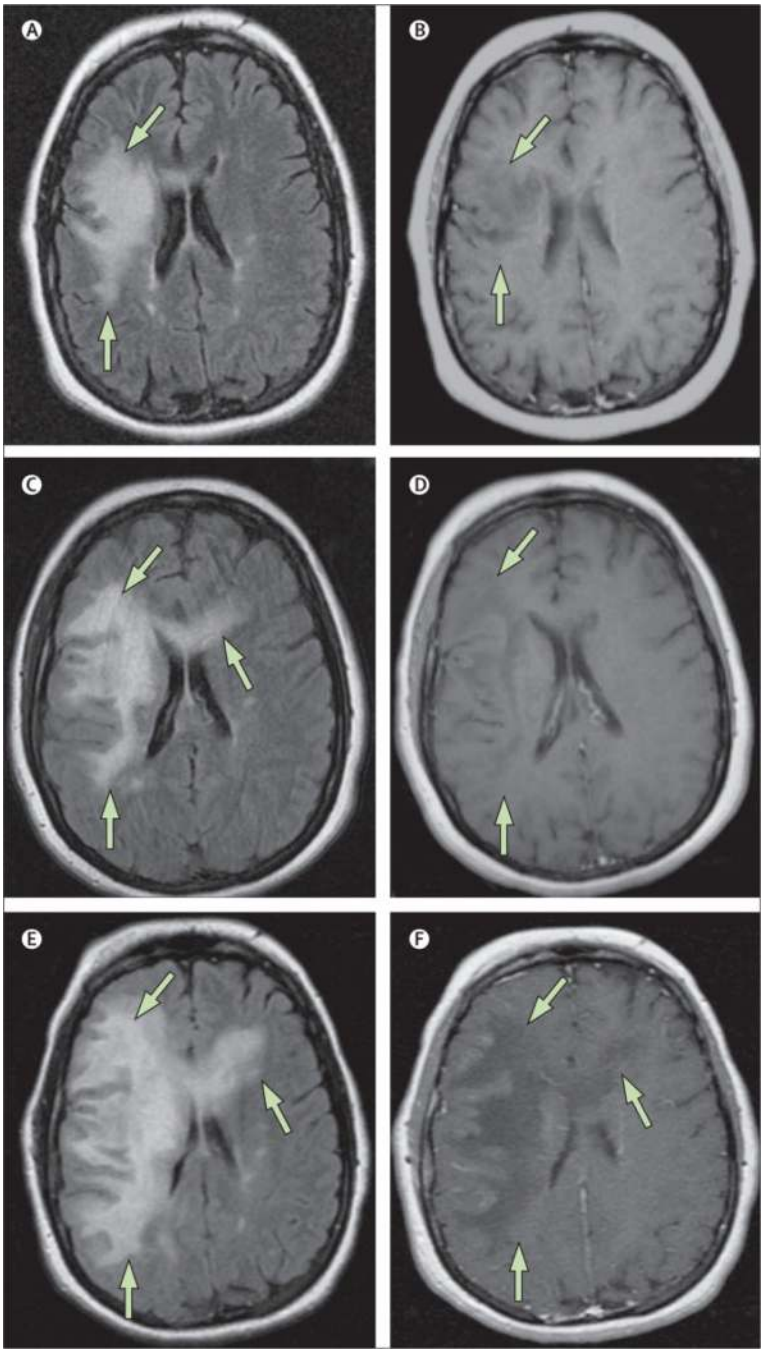

Figure 4. A natalizumab-treated MS patient with worsening PML after plasma exchange

A 35 years old woman with seven years history of relapsing remitting MS was treated with natalizumab monotherapy after failure with other treatments. After almost two years of monthly infusions, she presented with dysarthria, left-sided numbness and weakness. MRI of the brain showed a large right frontal lobe white matter hyperintense lesion on FLAIR, extending through the corpus callosum (A, arrows) which did not enhance on T1-weighted images (B, arrows). The patient was treated with plasma exchange every other day for 5 days. Nevertheless, symptoms worsened and repeat MRI 3 weeks later showed progression of the lesions on FLAIR with slight mass effect on the right lateral ventricle (C) without evidence of enhancement on T1-weighted images (D). The patient's neurological condition worsened and

follow up MRI done 3 ½ weeks later showed extension of the lesions in the right frontal and parietal lobes, as well as in the left frontal lobe, associated with edema and further mass effect on the lateral ventricles in FLAIR (E) which displayed faint areas of linear enhancement on T1 weighted images (F).

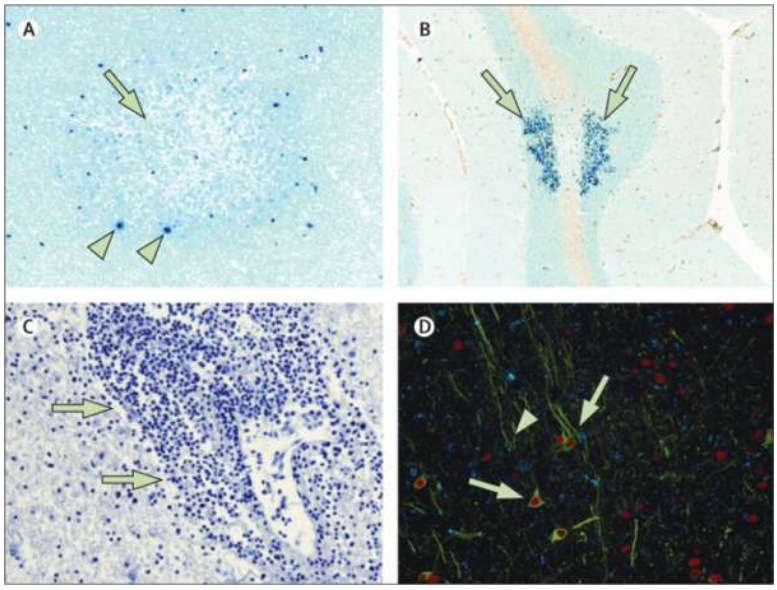

Figure 5. Histological spectrum of JCV infection of the central nervous system

A) Classic PML: demyelinating lesion of the white matter (arrow) surrounded by multiple JCV-infected glial cells (arrowheads). B) JCV GCN: JCV infection of granule cell neurons (arrows). C) PML-IRIS: marked lymphocytes penetrating the perivascular region (arrows). D) JCV encephalopathy: JCV infected (arrow) hemispheric cortical neurons (arrowhead). Panels A, B, and D courtesy of Dr. Christian Wurthrich. Pannel C courtesy of Dr Francoise Gray)

Table 1

Clinical presentations of JC virus infection.

	Classic PML	PML-IRIS	JC virus granule Cell neuronopathy	JC virus encephalopathy	JC virus meningitis
Onset	Subacute	Immune recovery	Chronic	Subacute	Acute
Radiological findings (MRI)	Asymmetric, well demarcated, non-enhancing subcortical white matter lesions, hyperintense in T2 and FLAIR, hypointense in T1	Contrast enhancement and mass effect	Cerebellar atrophy	Cortical lesions	No defined brain lesions, ventricular dilatation
Neurological symptoms	Based on location	Based on location and inflammation	Cerebellar syndrome	Encephalopathy	Headache, stiff neck, fever
Diagnosis	JC virus detection in the CSF, brain biopsy, radiographical findings and symptoms	JC virus in the CSF, brain biopsy, radiographical findings and symptoms	Cerebellar biopsy, JC virus in the CSF, radiographical findings and symptoms	Brain biopsy, JC virus PCR in the CSF, radiographical findings and symptoms	JC virus in the CSF and exclusion of other viruses
Histology	Demyelinating lesions often at grey/ white junction, JC virus detected in enlarged oligodendrocytes, bizarre astrocytes	Demyelination similar to classic PML, with addition of inflammatory infiltrates	Lytic infection of granule cell neurons in the cerebellum by JC virus	Lytic infection of cortical pyramidal neurons and cortical astrocytes by JC virus	
Treatment	cART for HIV-positive patients, discontinue or decrease immunosuppression for HIV-negative patients, plasma exchange for natalizumab-treated patients	Similar to PML, consider steriods in cases with notable neurological worsening or signs of impending brain hemiation	Similar to classic PML	Similar to classic PML	Similar to classic PML

PML: progressive multifocal leukoencephalopathy; IRIS: immune reconstitution inflammatory syndrome; GCN: granule cell neuronopathy; E: encephalopathy; MRI: magnetic resonance imaging; FLAIR: fluid-attenuated inversion recovery, cART: combined antiretroviral therapy.

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