Treatment-related progressive multifocal leukoencephalopathy: current understanding and future steps

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Abstract: Progressive multifocal leukoencephalopathy (PML) is a rare demyelinating disorder of the brain caused by a ubiquitous polyomavirus, JC virus. PML is almost always associated with some underlying immunosuppression and acquired immune deficiency syndrome has been the most common predisposing disorder. Recently, different pharmacological agents have been demonstrated to increase the risk of PML. Therapies that predispose people to PML can be classified into three categories: therapies that uniquely increase the risk for the disorder, such as the monoclonal antibodies natalizumab and efalizumab; therapies that appear to increase the risk in individuals already at risk of PML due to pre-existing conditions, such as rituximab and mycophenolate mofetil; and therapies with a mechanism of action that might suggest a potential for increased PML risk and/or with which rare cases of PML have been observed. Unlike the latter two classes, therapeutic agents uniquely increasing the risk of PML are associated with a much greater prevalence of the disorder and a latent interval from the time of drug initiation to the development of PML. PML development with pharmacological agents has provided new insight into the pathogenesis of this devastating disorder. This review focuses on the risks of PML with multiple pharmacological agents, the proposed pathogenesis with these agents, and potential risk mitigation strategies.

Keywords: alemtuzumab, efalizumab, JC virus, multiple sclerosis, mycophenolate mofetil, natalizumab, progressive multifocal leukoencephalopathy, rituximab

Background

Progressive multifocal leukoencephalopathy (PML) was first described in 1958 by Aström and colleagues [Astrom *et al.* 1958]. They reported unexplained progressive white matter disorder in three patients who had underlying lymphoproliferative diseases. A review of literature by the authors revealed occasional reports of similar demyelinating disorder dating back to 1930. In 1959, a viral etiology was suggested by Cavanaugh and colleagues based on observation of inclusion bodies in oligodendroglial nuclei within PML brain tissue [Cavanaugh *et al.* 1959]. ZuRhein analyzed these inclusion bodies in detail by electron microscopy and suggested papovavirus as the likely viral agent [Zu Rhein, 1965]. This was confirmed by Padgett and colleagues at the University of Wisconsin [Padgett *et al.* 1971]. The virus was labeled JC virus (JCV) after the initials of person from whom it was first isolated (John Cunningham).

From 1958 to 1984, Brooks and Walker identified 230 cases from their own experience and from the extant English language publications [Brooks and Walker, 1984]. Ninety-five percent of patients in this series had a recognized underlying condition that predisposed them to PML. Hematological malignancies, predominantly B-cell disorders, accounted for most of the underlying disorders. Other hematological and solid organ malignancies, autoimmune disorders, immunodeficiency states and granulomatous disorders, like tuberculosis and sarcoidosis, were recognized as predisposing conditions. The incidence rates of PML changed dramatically following the acquired immune deficiency syndrome (AIDS) pandemic in 1981 [Holman *et al.* 1991]. AIDS was the

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underlying predisposing disorder for 87% of PML cases in the USA in 1993[Selik *et al.* 1997], highlighting the unparalleled link between infection with human immunodeficiency virus type 1 (HIV-1) and PML. Since the introduction of highly active antiretroviral therapies, the incidence of AIDS-related PML appears to have declined [Sacktor, 2002].

The contribution of therapeutic agents to the pathogenesis of PML was not widely appreciated until the report of three natalizumab-associated cases in 2005 when PML was described in two patients with multiple sclerosis (MS) and one patient with Crohn's disease [Kleinschmidt-Demasters and Tyler, 2005; Langer-Gould *et al.* 2005; Van Assche *et al.* 2005]. PML had not been previously reported with either MS or inflammatory bowel disorders despite the frequency with which immunosuppressive agents had been used in their treatment. Similarly, the observation of confirmed PML in three patients and suspected PML in a fourth patient who had been treated with efalizumab for psoriasis was similarly astonishing as PML had not been previously reported in psoriasis either [Korman *et al.* 2009; Sobell and Weinberg, 2009]. Recognition of a link between these agents which shared mechanisms of action and PML was obvious and has provided new insights into the pathogenesis of PML. A large number of other immunomodulatory drugs have been considered to increase the risk for development of PML. US Food and Drug Administration (FDA) 'black box' warnings for PML are found not only for natalizumab and efalizumab (since removed from the market), but also rituximab, mycophenolate mofetil, and brentuximab vedotin. However, there are substantial differences between these agents which can be classed into separate categories with respect to risk for PML. Differences include frequency with which PML is observed, the nature of the underlying disorders being treated [Garcia-Suarez *et al.* 2005; Carson *et al.* 2009] and the time from initiation of the agent to the development of PML (Table 1). Nonetheless, the risk for PML must be considered for any therapy that alters immune function.

Acquisition of JC virus

A patient must be infected with JCV to be predisposed to developing PML. Seroprevalence of JCV antibody has varied in different studies, but it has been widely accepted that seroprevalence is more than 50% in the adult population. It causes no recognized clinical symptoms at the time of initial infection, therefore, the timing and the mechanism of infection has remained elusive. Initial serological studies were based on the ability of JCV to hemagglutinate type O erythrocytes [Padgett and Walker, 1983]. Since that time, immunoassays have made serological tests more convenient [Hamilton *et al.* 2000] and more specific. Cross reactivity with other polyomaviruses, such as BK virus, is a potential concern, but is probably not significant [Hamilton *et al.* 2000]. Between the ages of 1 and 5 years, approximately10% of children demonstrate antibody to JCV and by age 10 years, it can be observed in 40–60% of the population [Taguchi *et al.* 1982]. Continued exposure throughout adulthood is suggested by another study that revealed seroprevalence rates of 50% in the 20–29-year-old age group, but 68% in the 68–100-year-old age group [Egli *et al.* 2009]. Annual rates of seroconversion of 2% have been suggested [Gorelik *et al.* 2010]. Studies employing an immunoassay for JCV have resulted in a broad spread of seroprevalence rates ranging from 35% [Knowles *et al.* 2003] to 91% [Matos *et al.* 2010] among adults. Seroconversion rates to JCV have exceeded 90% in some urban areas. In another study, seroprevalence rates in developed countries ranged between 50% and 60% in the adult population with MS [Bozic *et al.* 2011].

The mechanism of spread for the JCV remains undetermined. Respiratory or oropharyngeal transmission has been postulated based on the detection of JCV in tonsillar tissue [Monaco *et al.* 1998], however recent studies of these fluids in HIV-infected people and healthy controls indicate that the virus is rarely demonstrated in upper respiratory secretions and, when present, is there in very low titer [Berger *et al.* 2006]. Regardless of the route of infection, JCV in the urological tissues and viral shedding in urine is extraordinarily common. JCV has been demonstrated in 19% [Egli *et al.* 2009] to more than 60% of urine samples from immunologically normal individuals [Berger *et al.* 2006]. Due to its frequent occurrence in urine, perhaps it is not surprising that JCV has been detected worldwide in virtually every sample of sewage that has been examined [Bofill-Mas and Girones, 2001]. Contaminated food and water could be potential sources of infection [Bofill-Mas *et al.* 2001]. Importantly, the virus isolated from the urine, referred to as the archetype virus, is genetically distinct from that

Table 1. Classes of agents predisposing people to progressive multifocal leukoencephalopathy (PML).

Therapeutic agent	Underlying condition previously associated with PML	Latency from initiation of therapeutic agent to the development of PML	Frequency of developing PML
Class 1	*No*	*Long*	*High*
Natalizumab	Multiple sclerosis and Crohn's disease)	>8 months with peak at 24 months	Dependent on JC virus antibody status, duration of administration, and prior immunosuppressant use; range of ~1 in 1000 from 1–24 months with JC virus antibody seropositivity and no prior immunosuppressant use to >1 in 100 after 24 months of use with JC virus antibody positivity and prior immunosuppressant use
Efalizumab	Psoriasis	>3 years for efalizumab	3 of 166 receiving efalizumab for greater than 3 years
Class 2	*Yes*	*No latency to PML development; PML develops stochastically following administration of the agent*	*Infrequent*
Rituximab	Lymphoproliferative disorders; rheumatoid arthritis; systemic lupus erythematosus; AIDS	None	1:30,000
Mycophenolate mofetil	Solid organ transplants; systemic lupus erythematosus and other autoimmune diseases	None	?
Brentuximab vedotin	Hodgkin's disease	None	?

AIDS, acquired immune deficiency syndrome; JC virus, John Cunningham virus.

found in the brain tissue of people with PML. The omnipresent archetype JCV does not replicate effectively in glial tissues and must undergo genetic rearrangement to become neurotropic [Berger and Khalili, 2011].

Pathogenesis of progressive multifocal leukoencephalopathy

Despite the high prevalence rate of JCV, PML is an extremely rare disorder. This suggests that there are significant and multiple barriers to the evolution of the disorder. The following steps have been proposed in the development of PML: initial infection with JCV; establishment of a latent or persistent infection; gene rearrangement in the promoter/enhancer region of the virus to express a neurotropic form of the virus; re-expression of the virus from the sites of latency or persistence; entry of the virus into the brain; establishment of productive infection of glial tissues; failed central nervous system (CNS) immunosurveillance [Berger *et al.* 2009].

There remain many unanswered questions about the pathogenesis of PML. While latent JCV has been detected in tonsils, lung, spleen, bone marrow and kidney [Caldarelli-Stefano *et al.* 1999], controversy has surrounded its potential latency/persistence in the brain of otherwise normal individuals. B lymphocytes have been considered to play a vital role in JCV re-expression and gene rearrangement, leading to a neurotropic strain [Jensen and Major, 1999]. This genetic modification requires insertion of a 98-base-pair tandem repeat in JCV noncoding control region (NCCR). The gene rearrangement in the NCCR of the neurotropic virus allows it to bind to nuclear factor (NF)-1X binding protein, a protein that glial cells share with B cells [Houff *et al.* 1988; Major

Table 2. Proposed steps to progressive multifocal leukoencephalopathy (PML) pathogenesis.

Initial infection with JC virus
Establishment of a latent or persistent infection
Gene rearrangement in the promoter/enhancer region of the virus to express a neurotropic form of the virus
Re-expression of the virus from the sites of latency or persistence
Entry of the virus into the brain
Establishment of productive infection of glial tissues
Failed CNS immunosurveillance.

CNS, central nervous system; JC virus, John Cunningham virus.

et al. 1990]. Either free virus or circulating JCV-infected lymphocytes cross the blood–brain barrier and infect perivascular astrocytes and oligodendrocytes. The association of PML with B-cell malignancies, such as chronic lymphocytic leukemia and Hodgkins's disease, and disorders associated with B-cell activation, such as systemic lupus erythematosis (SLE) and AIDS, increase the conditions which place people at high risk of developing PML. Additionally, the monoclonal antibody natalizumab, which carries the highest risk among biological agents for PML development, causes hematopoietic stem cell mobilization, particularly a release of immature $CD34^+$ B cells from the bone marrow [Berger and Khalili, 2011]. A summary of the proposed events leading to PML is found in Table 2.

Class 1 therapeutic agents with risks of progressive multifocal leukoencephalopathy

Therapeutic agents that result in an unequivocal substantially increased risk of PML have been categorized as class 1. To date, two agents have been identified: natalizumab and efalizumab. These agents are associated with PML in people who have no known disorder that predisposes them to PML. Additionally, they are associated with a latency of many months to years from time of initiation to the onset of PML.

Natalizumab

Natalizumab is a recombinant humanized monoclonal immunoglobulin G4 (IgG4) antibody that binds to the α_4 subunit of the $\alpha_4\beta_1$ subunit [Warnke *et al.* 2010] The $\alpha_4\beta_1$ integrin is one of the four main integrins that are required for the firm arrest of leukocytes following their rolling and adhesion [Luster *et al.* 2005]. Natalizumab interferes with leukocyte α_4-mediated adhesion to its natural ligands of the extracellular matrix and endothelial lining, vascular cell adhesion molecule and fibronectin [Von Andrian and Engelhardt, 2003]. Hence, natalizumab inhibits activated lymphocytes from entering the brain. On 24 November 2004, the FDA approved natalizumab for the treatment of relapsing forms of MS. In 2005, natalizumab was withdrawn from the market after two patients with MS (treated with natalizumab and interferon β-1a) and one patient with Crohn's disease were diagnosed with PML [Kleinschmidt-Demasters and Tyler, 2005; Langer-Gould *et al.* 2005; Van Assche *et al.* 2005]. In summer 2006, natalizumab was reintroduced as a monotherapy for relapsing-remitting MS and moderate to severely active Crohn's disease. After its reapproval, safety and risk control trials were designed, including Tysabri Outreach: Unified Commitment to Health (TOUCH), Tysabri Global Observation Program in Safety (TYGRIS), and Crohn's Disease – Investigating Natalizumab through Further Observational Research and Monitoring (CD INFORM). The initial estimate of PML risk was approximately 1 in 1000 people would develop PML after 17.8 months of treatment [Yousry *et al.* 2006]. Subsequent analyses identified three independent risks for PML development with natalizumab, namely, JCV antibody status, duration of therapy with natalizumab, and the use of immunosuppressant therapy prior to the initiation of natalizumab. The estimated risk in people with JCV antibody who are receiving natalizumab is estimated at up to 1 in 11,000, although no cases of PML have been identified in this population. The estimated incidence of PML stratified by these risk factors can be found in Table 3 [BiogenIdec, 2012].

It has been recommended that all immunomodulatory agents should be discontinued at least 3 months prior to the initiation of natalizumab [Kappos *et al.* 2007]. No definitive recommendations exist about the discontinuation of platform

Table 3. Estimated incidence of progressive multifocal leukoencephalopathy (PML) stratified by risk factor (BiogenIdec 2012).

	Anti-JC virus positive	
Tysabri (natalizumab) exposure	No prior immunosuppressive use	Prior immunosuppressive use
1–24 months	<1/1000	2/1000
25–48 months	4/1000	11/1000

MS therapies (interferon β and glatiramir acetate) and it is quite likely that there is really no increased risk of PML with the administration of these agents. Longer washout periods may be needed for patients on immunosuppressive agents, but duration of therapy, time from last administration, and the nature of the immunosuppressant agent do not correlate with the subsequent development of PML. Rarely, PML has been reported to occur within the first 12 months of therapy, but it has not been seen prior to 8 months of therapy. The incidence rate in all natalizumab-treated patients was reported to increase after 12 months of therapy to 1.35 per 1000 and this was found to further increase to 1.76 per 1000 after 24 months. Whether longer duration of therapy would result in a further increase, a plateau or a decline in incidence remains unknown, but preliminary evidence suggests either a plateau or a slight decline. Continued surveillance of patients on natalizumab for PML is needed.

The precise explanation for an increased risk of PML in patients on natalizumab is not clear. Due to $\alpha_4\beta_1$ antagonism, treatment with natalizumab results in impaired immune surveillance of the CNS. Reduced numbers of white blood cells including $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, and plasma cells have been seen in the cerebrospinal fluid (CSF) of patients with MS treated with natalizumab compared with those not treated with natalizumab [Stuve *et al.* 2006a]. $CD4^+$ cells express significantly less unbound α_4 integrin before and after natalizumab therapy compared with $CD8^+$ cells, contributing to reduced migration of $CD4^+$ cells across the blood–brain barrier [Stuve *et al.* 2006b]. There is also a reduction in the number of antigen-presenting dendritic cells and major histocompatibility complex II antigens in cerebrovascular spaces [Del Pilar Martin *et al.* 2008]. Natalizumab prevention of the entry of JCV cytotoxic lymphocytes into the CNS and reduction of antigen-presenting dendritic cells may predispose people to PML. Another potential contributor to the development of PML is the release of $CD19^+$ $CD10^+$ pre-B cells that occurs after natalizumab administration [Krumbholz *et al.* 2008]. These cells can be latently infected with JCV. During maturation of these B cells, transcriptional factors are activated that increase transactivation of JCV [Lindberg *et al.* 2008]. Rearrangement of the virus transcriptional control region leads to the evolution of neurotropic JCV. Therefore, natalizumab administration causes multiple effects on the immune system that predispose people to the development of PML.

Efalizumab

Efalizumab is a monoclonal antibody that has been approved for treatment of moderate to severe plaque psoriasis [Gordon *et al.* 2003; Leonardi, 2004]. It is an anti-CD 11a IgG1 antibody that binds to the α chain of CD11a and induces conformational changes in lymphocyte function-associated antigen 1 (LFA-1). LFA is the site that binds to intercellular adhesion molecule [Lub *et al.* 1995]. Hence, efalizumab prevents binding of T cells to endothelial molecules and blocks their passage from circulation to sites of inflammation [Vugmeyster *et al.* 2004]. It also inhibits activation of naïve T cells in lymph nodes and reactivation of memory T cells in response to antigen [Lebwohl *et al.* 2003]. Efalizumab has been shown to result in sustained improvement in psoriasis during 36 months of continuous therapy [Leonardi *et al.* 2008]. In animal models, blockade of CD11a on T cells caused sustained hyporesponsiveness to viral and other pathogens. This decreased responsiveness is fully reversible following efalizumab washout [Guttman-Yassky *et al.* 2008]. Efalizumab also reduces cutaneous dendritic cells [Lowes *et al.* 2005].

More than 6000 patients had been treated with efalizumab before its removal from the European and US markets in 2009. Of these, only 166

patients had received more than 3 years of treatment. There appears to be no increased risk of infection in 2335 patients receiving 12 weeks of therapy, 1115 receiving 24 weeks of therapy and 170 receiving 108 weeks of continuous therapy [Langley *et al.* 2005]. In 2008, three fatal cases of PML treated with efalizumab were reported. A detailed immunological study of one of these cases showed naïve T cells in peripheral blood and lack of clonal expansion in the CSF and periphery [Schwab *et al.* 2012]. Removal of efalizumab by plasmapheresis (PE) resulted in multiple immune changes. Polyclonal expansion of the T cells in CSF was noted with the appearance of $CD8^+$ T cells 3 weeks after PE and $CD4^+$ T cells 5 weeks after PE [Schwab *et al.* 2012]. It has been postulated that restoration of LFA function results in interaction between T-cell LFA-1 and antigen-presenting cells, restoration of memory $CD8^+$ T-cell activation, and accumulation of $CD8^+$ T cells in CSF [Schwab *et al.* 2012].

Efalizumab and natalizumab impair CNS T-cell migration and the subsequent immune responses, but some differences have been proposed in the mechanisms by which natalizumab and efalizumab lead to PML. Natalizumab causes release of progenitor bone marrow cells into the circulation, an effect not seen by efalizumab. Efalizumab predominantly impairs intrathecal antigen-presenting cells and antigen-mediated reactivation of $CD4^+$ and $CD8^+$ T cells, while natalizumab decreases CSF cells counts by 70% by predominantly affecting migration of T cells across the blood–brain barrier [Schwab *et al.* 2012].

Class 2 therapeutic agents with risks of progressive multifocal leukoencephalopathy

Therapeutic agents in class 2 are those that appear to increase the risk of PML but at significantly lower levels than class 1 agents. Most of the patients developing PML with this class of agents have either underlying conditions recognized to predispose them to PML or have been treated with other medications recognized as increasing the risk. Generally, the development of PML following their institution is a stochastic event without the need for a period of latency. The therapeutic agents in this category also carry FDA 'black box' warnings for PML.

Mycophenolate mofetil

Mycophenolate mofetil is a selective, noncompetitive and reversible inhibitor of inosine-5′-monophosphate (IMP) dehydrogenase. It inhibits the proliferation of T and B lymphocytes by depleting guanosine and dexoyguanosine nucleotides via inhibition of IMP conversion into guanosine monophosphate [Ransom, 1995]. Mycophenolate mofetil is approved for allograft rejection after renal, cardiac and liver transplant. It is also used for treatment of different autoimmune disorders, including SLE, myasthenia gravis and autoimmune glomerular disorders [Villarroel *et al.* 2009].

Cases of PML have been observed in patients taking mycophenolate mofetil but the exact contribution of this agent to the pathogenesis of PML remains unclear. All cases had underlying diseases or were on other immunosuppressive agents that predisposed them to PML. In a retrospective cohort study of nearly 33,000 renal transplant recipients in the USA, the incidence of PML among patients on mycophenolate mofetil was 14.4 cases per 100,000 *versus* 0 in those not receiving mycophenolate mofetil, but this did not meet statistical significance [Neff *et al.* 2008].

The proposed mechanism by which mycophenolate mofetil causes PML may have parallels to natalizumab. By depleting T cells, it impairs immune surveillance for JCV. Additionally, it depletes B cells and as immature B cells are re-expressed, there is a possibility of an upregulation of JCV replication and mutation to the neurotropic strain [Berger, 2010].

Rituximab

Rituximab is a chimeric monoclonal antibody directed against CD20. It depletes B and pre-B cells that express CD20. Like mycophenolate mofetil, rituximab is used for different autoimmune and hematological disorders, including autoimmune pancytopenia, SLE, rheumatoid arthritis (RA) and MS [Hauser *et al.* 2008; Carson *et al.* 2009; Gurcan *et al.* 2009]. It is difficult to determine the exact risk of PML with rituximab as patients identified with rituximab-associated PML had underlying immune abnormalities either related to their primary disorder or secondary to other immunosuppressants [Molloy and Calabrese, 2008]. From 1997 to 2008, 52 patients with lymphoproliferative disorders, 2

with SLE, 1 with RA, 1 each with autoimmune pancytopenia and autoimmune thrombocytopenia were identified with rituximab-related PML [Carson *et al.* 2009]. Data from Genentech indicate that approximately 2 million doses have been given to 1 million patients, among whom 157 cases of PML have been observed (137 with lymphoproliferative disorders, 6 with RA, 8 with SLE and 6 with AIDS). There have been no cases reported with its use in MS or other neurological disorders [Genentech, 2012].

In contrast to natalizumab and efalizumab, PML can occur any time after the administration of rituximab, but averages 5.5 months after the last rituximab dose [Carson *et al.* 2009]. The rate of B-cell reconstitution following rituximab administration varies from 6 months to 24 months depending on other antilymphocyte treatments, especially stem cell transplantation [Leandro *et al.* 2006]. Therefore, immature B cells predominate and this may contribute to transactivation and expression of a neurotropic strain of JCV. Additionally, rituximab also reduces $CD3^+$ T cells in CSF [Cross *et al.* 2006]. One explanation for the short interval from drug administration to PML could be that affected individuals are on the verge of PML due to their underlying disorder. Administration of rituximab tips them over due to failed CNS immunosurveillance caused by the therapeutic agent [Berger and Khalili, 2012]. Similar to natalizumab and efalizumab, rituximab may affect antigen-presenting cells and T-cell regulation, though the exact effect of rituximab on T-cell function is not clear.

The incidence of PML with rituximab is considerably lower than that with natalizumab. However, the case fatality rate with rituximab-associated PML is 90% and 100% among those diagnosed with PML within 3 months of their last dose of rituximab [Carson *et al.* 2009]. This high rate is undoubtedly explained by the nature of their underlying disorder with a possible contribution by the irreversible nature of the immune abnormalities induced by rituximab.

Brentuximab vedotin

Brentuximab vedotin is a recently approved chimeric anti-CD30 monoclonal antibody conjugated with the antimitotic agent monomethyl auristatin E. It is approved for treatment of refractory Hodgkin's lymphoma and relapsed or refractory systemic anaplastic large cell lymphoma. Both of these underlying lymphoproliferative disorders increase the risk of PML and it is difficult to know the degree to which the agent increased the risk of PML in the rare cases observed [Wagner-Johnston *et al.* 2012]. Brentuximab vedotin may decrease CNS immunosurveillance by impairing T-cell function. Alternatively, induction of the NFκβ pathway by the agent may increase JCV transcription [Wollebo *et al.* 2011]. However, whether brentuximab vedotin truly increases the risk of PML remains an open question.

Class 3 therapeutic agents with risks of progressive multifocal leukoencephalopathy

Therapeutic agents in this category have rarely been reported to be associated with PML and the risk of developing the disorder with their use remains uncertain.

Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody against CD52, a glycoprotein that is present on most of the peripheral mononuclear cells except plasma cells. It is an approved therapy for hematological malignancies, post-transplant rejection therapy, and has been studied for MS [Fontoura, 2010]. The use of alemtuzumab causes a profound reduction of B and T lymphocytes following administration. B cells recover within 3 months, but T lymphocytes remain depleted for more than 5 years [Klotz *et al.* 2012]. At least three cases of PML have been described with alemtuzumab, two in patients with CLL and one in a lung transplant recipient [Martin *et al.* 2006; Waggoner *et al.* 2009]. The patient who received a lung transplant was treated for acute rejection with steroids, antithymocyte globulin, and alemtuzumab [Waggoner *et al.* 2009]. As with rituximab, PML has been reported with alemtuzumab in the background of either malignancy or severe immunosuppression due to coadministration of other immunosuppressants. Despite the marked lymphopenia that accompanies alemutuzumab administration, PML and opportunistic infections in general appear to be very rare and no cases of PML have been reported to date in MS cohorts who have been treated with this monoclonal antibody.

Infliximab and other tumor necrosis factor α inhibitors

Tumor necrosis factor α (TNFα) inhibitors have been widely used and the paucity of cases of PML reported with them suggests that any risk, if real, must be very small. A query of the FDA's Adverse Event Reporting System (AERS) database for biological agents commonly employed in the treatment of psoriasis conducted in 2009 [Kothary *et al.* 2011] reported PML associated with adalimumab (1), etanercept (3), and infliximab (3), although these cases were not necessarily confirmed. However, in every instance the use of these agents was for conditions other than psoriasis and was confounded by the use of other immunosuppressive agents. Infliximab is a chimeric monoclonal antibody against soluble, membrane-bound TNF [Maini and Feldmann, 2002]. It is approved for the treatment of RA, psoriasis, psoriatic arthritis, Crohn's disease, ulcerative colitis, and ankylosing spondylitis. One PML case has been reported with infliximab in a patient with RA [Kumar *et al.* 2010]. PML developed after 3 years of intake of infliximab, methotrexate and steroids. Infliximab suppresses TNFα, which decreases interleukin-6 and interleukin-1, thus reducing inflammation. A decrease in cell recruitment occurs through reduced expression of $CD3^+$, $CD68^+$, vascular cell adhesion molecule 1, intercellular adhesion molecule, and E-selectin. Dendritic cell-mediated T-cell activation is also reduced [Gottlieb *et al.* 2005]. This significant immune modulation, particularly T-cell activation, might be implicated in the pathogenesis of PML.

Fludarabine

Fludarabine is a purine nucleoside that has been used as a chemotherapeutic agent for the treatment of hematological malignancies. It is cytotoxic against resting and dividing cells [Keating *et al.* 1989]. In resting cells, it inhibits DNA synthesis; in dividing cells, it prevents the DNA repair process and induces apoptosis. Fludarabine is used for the treatment of chronic lymphocytic leukemia, which is the most common hematological malignancy associated with PML. Hence, association of fludarabine and PML has been questioned in the past. In most reported cases PML developed after fludarabine therapy [Gonzalez *et al.* 1999; Leonard *et al.* 2002; Saumoy *et al.* 2002; Lejniece *et al.* 2011], therefore it is believed that immunosuppression resulted in JCV activation and impaired clearance.

Fumaric acid

Fumaric acid esters (FAEs) are a group of related compounds that have been used for the treatment of psoriasis since 1959. Several lines of evidence suggested immunomodulatory effects for FAEs. FAEs have been shown to reduce the number of T cells, particularly, $CD4^+$ T cells, induce apoptosis of T cells [Treumer *et al.* 2003], and inhibit translocation of NFκB into the nucleus. Disruption of the NFκB pathway leads to decreased expression of NFκB genes that are involved in regulating various inflammatory cytokines, chemokines, and adhesion molecules [Stoof *et al.* 2001]. NFκB has been speculated to inhibit B-cell antiapoptotic protein, resulting in increased apoptosis of B cells, though no definitive studies suggest a direct effect of FAEs on B cells [Moharregh-Khiabani *et al.* 2009]. A phase II clinical trial in patients with relapsing-remitting MS receiving dimethylfumarate (BG-12) showed a significant reduction in the number of gadolinium-enhancing lesions after 24weeks [Kappos *et al.* 2008]. Phase III trials of BG-12 have demonstrated safety and efficacy in relapsing-remitting MS [Moharregh-Khiabani *et al.* 2009].

Recently, a fumaric-acid-related case was reported in Germany in a 74-year-old man who developed pathologically proven PML after 3 years of treatment with fumaric acid for psoriasis. Whether there were other predisposing risks for PML was not precisely stated [Ermis *et al.* 2011]. All other cases of PML reported with fumaric acid from a German database of Fumaderm (Fumedica/Hermal) have had other risk factors for its occurrence, such as, sarcoidosis and treatment with efalizumab.

Risk mitigation strategies

Different strategies have been suggested to mitigate the risk of PML in patients treated with drugs that increase the risk. As JCV exists in at least 50% of the adult population as a latent or persistent infection, serological tests should be done to determine whether the individual has been previously exposed to the virus. Evidence that PML is the consequence of reactivation of a latent infection includes the presence of the IgG antibody to JCV [Weber *et al.* 2001], the rarity of PML in children [Berger *et al.* 1992], the demonstration of genetically identical JCV isolates from the blood and from other tissues months to years before the onset of PML and their isolation from the brain [Fedele *et al.* 2003], and demonstration

of the JCV antibody in all patients to date who have developed natalizumab-associated PML and had stored blood samples available for testing. A commercially available test for JCV antibody is available and should be performed in patients who are being started on natalizumab and other therapies carrying a substantial risk of PML. If a patient is seronegative, repeat determinations at regular intervals (perhaps 6 months) are warranted.

Experience with natalizumab-associated PML has demonstrated that early detection of the disease is an important determinant of survival and outcome. This probably reflects rapid removal of the agent with plasmapheresis and restoration of normal immune function. In radiographically isolated PML, that is, detected by magnetic resonance imaging (MRI) before the recognition of clinical symptoms [Langer-Gould *et al.* 2005; Linda *et al.* 2009], periodic cranial MRIs are warranted. The use of fluid-attenuated inversion recovery images may prove sufficient in detecting suspicious lesions without resorting to a standard set of sequences. The optimal interval for repeat MRI has not been determined, but 6-month intervals once the patient has exceeded a time period of 12 months on natalizumab would appear to be sufficiently prudent.

Other strategies to reduce the risk of PML in patients on these therapeutic agents, in particular natalizumab, such as reducing the dose or frequency of administration, offering drug holidays, or the coadministration of serotonin receptor blockers, remain unsupported by any scientific studies to date. A high index of suspicion for PML needs to be maintained for individuals at risk to identify the disease as early as possible.

Conclusion

PML is a rare demyelinating disorder that is seen in the setting of a disturbed immune system. AIDS, hematological malignancies and many autoimmune conditions predispose people to the development of PML. PML has been observed with the use of new biological agents in the treatment of these disorders. Efalizumab and natalizumab are unique monoclonal antibodies that have caused PML in patients with psoriasis (efalizumab) and MS (natalizumab), conditions that do not increase the risk of PML development. The occurrence of PML with these and other therapeutic agents has provided insight into and spurred the investigation of the pathogenesis of PML. In time, a better understanding of the specific risks associated with these newer therapies and refined strategies for PML risk reduction will undoubtedly be attainable.

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References

Astrom, K., Mancall, E. and Richardson, E. (1958) progressive multifocal leukoencephalopathy; a hitherto unrecognized complication of chronic lymphocytic leukemia and Hodgkin's disease. *Brain* 81: 93–111.

Berger, J. (2010) Progressive multifocal leukoencephalopathy and newer biological agents. *Drug Saf* 33: 969–983.

Berger, J., Houff, S. and Major, E. (2009) Monoclonal antibodies and progressive multifocal leukoencephalopathy. *MAbs* 1: 583–589.

Berger, J. and Khalili, K. (2011) The pathogenesis of progressive multifocal leukoencephalopathy. *Discov Med* 12: 495–503.

Berger, J. and Khalili, K. (2012) The pathogenesis of progressive multifocal leukoencephalopathy. *Discov Med* 12: 495–503.

Berger, J., Miller, C., Mootoor, Y., Avdiushko, S., Kryscio, R. and Zhu, H. (2006) JC virus detection in bodily fluids: clues to transmission. *Clin Infect Dis* 43: e9–e12.

Berger, J., Scott, G., Albrecht, J., Belman, A., Tornatore, C. and Major, E. (1992) Progressive multifocal leukoencephalopathy in HIV-1-infected children. *AIDS* 6: 837–841.

BiogenIdec (2012) PML incidence in patients receiving Tysabri. Cambridge, MA. Available at: https://medinfo.biogenidec.com/medinfo/secure/pmlresource.do?resource=TYSABRIPMLSafetyUpdate (accessed 25 March 2012).

Bofill-Mas, S. and Girones, R. (2001) Excretion and transmission of JCV in human populations. *J Neurovirol* 7: 345–349.

Bozic, C., Richman, S., Plavina, T., Natarajan, A., Scanlon, J., Subramanyam, M. *et al.* (2011) Anti-John Cunningham virus antibody prevalence in multiple

sclerosis patients: baseline results of STRATIFY-1. *Ann Neurol* 70: 742–750.

Brooks, B. and Walker, D. (1984) Progressive multifocal leukoencephalopathy. *Neurol Clin* 2: 299–313.

Caldarelli-Stefano, R., Vago, L., Omodeo-Zorini, E., Mediati, M., Losciale, L., Nebuloni, M. *et al.* (1999) Detection and typing of JC virus in autopsy brains and extraneural organs of AIDS patients and non-immunocompromised individuals. *J Neurovirol* 5: 125–133.

Carson, K., Evens, A., Richey, E., Habermann, T., Focosi, D., Seymour, J. *et al.* (2009) Progressive multifocal leukoencephalopathy after rituximab therapy in HIV-negative patients: a report of 57 cases from the Research on Adverse Drug Events and Reports project. *Blood* 113: 4834–4840.

Cavanaugh, J., Greenbaum, D., Marshall, A. and Rubenstein, L. (1959) Cerebral demyelination associated with disorders of the reticuloendothelial system. *Lancet* 2: 524–529.

Cross, A., Stark, J., Lauber, J., Ramsbottom, M. and Lyons, J. (2006) Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients. *J Neuroimmunol* 180: 63–70.

Del Pilar Martin, M., Cravens, P., Winger, R., Frohman, E., Racke, M., Eagar, T. *et al.* (2008) Decrease in the numbers of dendritic cells and CD4+ T cells in cerebral perivascular spaces due to natalizumab. *Arch Neurol* 65: 1596–1603.

Egli, A., Infanti, L., Dumoulin, A., Buser, A., Samaridis, J., Stebler, C. *et al.* (2009) Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. *J Infect Dis* 199: 837–846.

Ermis, U., Wiesmann, M., Nolte, K., Chan, A., Adams, O., Weis, J. *et al.* (2011) Fumaric acid-associated progressive multifocal leukencephalopathy (PML), treatment and survival in a patient with psoriasis (abstract #84). At the Kongress der Deutschen Gesellschaft fur Neurologie mit Fortbidlungsakademie. Nurnberg, Germany, September 30, 2011.

Fedele, C., Ciardi, M., Delia, S., Contreras, G., Perez, J., De Ona, M. *et al.* (2003) Identical rearranged forms of JC polyomavirus transcriptional control region in plasma and cerebrospinal fluid of acquired immunodeficiency syndrome patients with progressive multifocal leukoencephalopathy. *J Neurovirol* 9: 551–558.

Fontoura, P. (2010) Monoclonal antibody therapy in multiple sclerosis: paradigm shifts and emerging challenges. *MAbs* 2: 670–681.

Garcia-Suarez, J., De Miguel, D., Krsnik, I., Banas, H., Arribas, I. and Burgaleta, C. (2005) Changes in the natural history of progressive multifocal leukoencephalopathy in HIV-negative lymphoproliferative disorders: impact of novel therapies. *Am J Hematol* 80: 271–281.

Genentech (2012) *PML with Rituximab*. Joseph R., Berger (ed). San Francisco.

Gonzalez, H., Bolgert, F., Camporo, P. and Leblond, V. (1999) Progressive multifocal leukoencephalitis (PML) in three patients treated with standard-dose fludarabine (FAMP). *Hematol Cell Ther* 41: 183–186.

Gordon, K., Papp, K., Hamilton, T., Walicke, P., Dummer, W., Li, N. *et al.* (2003) Efalizumab for patients with moderate to severe plaque psoriasis: a randomized controlled trial. *JAMA* 290: 3073–3080.

Gorelik, L., Lerner, M., Bixler, S., Crossman, M., Schlain, B., Simon, K. *et al.* (2010) Anti-JC virus antibodies: implications for PML risk stratification. *Ann Neurol* 68: 295–303.

Gottlieb, A., Chamian, F., Masud, S., Cardinale, I., Abello, M., Lowes, M. *et al.* (2005) TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. *J Immunol* 175: 2721–2729.

Gurcan, H., Keskin, D., Stern, J., Nitzberg, M., Shekhani, H. and Ahmed, A. (2009) A review of the current use of rituximab in autoimmune diseases. *Int Immunopharmacol* 9: 10–25.

Guttman-Yassky, E., Vugmeyster, Y., Lowes, M., Chamian, F., Kikuchi, T., Kagen, M. *et al.* (2008) Blockade of CD11a by efalizumab in psoriasis patients induces a unique state of T-cell hyporesponsiveness. *J Invest Dermatol* 128: 1182–1191.

Hamilton, R., Gravell, M. and Major, E. (2000) Comparison of antibody titers determined by hemagglutination inhibition and enzyme immunoassay for JC virus and BK virus. *J Clin Microbiol* 38: 105–109.

Hauser, S., Waubant, E., Arnold, D., Vollmer, T., Antel, J., Fox, R. *et al.* (2008) B-Cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med* 358: 676–688.

Holman, R., Janssen, R., Buehler, J., Zelasky, M. and Hooper, W. (1991) Epidemiology of progressive multifocal leukoencephalopathy in the United States: analysis of national mortality and AIDS surveillance data. *Neurology* 41: 1733–1736.

Houff, S., Major, E., Katz, D., Kufta, C., Sever, J., Pittaluga, S. *et al.* (1988) Involvement of JC virus-infected mononuclear cells from the bone marrow and spleen in the pathogenesis of progressive multifocal leukoencephalopathy. *N Engl J Med* 318: 301–305.

Jensen, P. and Major, E. (1999) Viral variant nucleotide sequences help expose leukocytic positioning in the JC virus pathway to the CNS. *J Leukoc Biol* 65: 428–438.

Kappos, L., Bates, D., Hartung, H., Havrdova, E., Miller, D., Polman, C. *et al.* (2007) Natalizumab treatment for multiple sclerosis: recommendations for patient selection and monitoring. *Lancet Neurol* 6: 431–441.

Kappos, L., Gold, R., Miller, D., Macmanus, D., Havrdova, E., Limmroth, V. *et al.* (2008) Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study. *Lancet* 372: 1463–1472.

Keating, M., Kantarjian, H., Talpaz, M., Redman, J., Koller, C., Barlogie, B. *et al.* (1989) Fludarabine: a new agent with major activity against chronic lymphocytic leukemia. *Blood* 74: 19–25.

Kleinschmidt-Demasters, B. and Tyler, K. (2005) Progressive multifocal leukoencephalopathy complicating treatment with natalizumab and interferon beta-1a for multiple sclerosis. *N Engl J Med* 353: 369–374.

Klotz, L., Meuth, S. and Wiendl, H. (2012) Immune mechanisms of new therapeutic strategies in multiple sclerosis – a focus on alemtuzumab. *Clin Immunol* 142: 25–30.

Knowles, W., Pipkin, P., Andrews, N., Vyse, A., Minor, P., Brown, D. *et al.* (2003) Population-based study of antibody to the human polyomaviruses BKV and JCV and the simian polyomavirus SV40. *J Med Virol* 71: 115–123.

Korman, B., Tyler, K. and Korman, N. (2009) Progressive multifocal leukoencephalopathy, efalizumab, and immunosuppression: a cautionary tale for dermatologists. *Arch Dermatol* 145: 937–942.

Kothary, N., Diak, I., Brinker, A., Bezabeh, S., Avigan, M. and Pan, G. (2011) Progressive multifocal leukoencephalopathy associated with efalizumab use in psoriasis patients. *J Am Acad Dermatol* 65: 546–551.

Krumbholz, M., Meinl, I., Kumpfel, T., Hohlfeld, R. and Meinl, E. (2008) Natalizumab disproportionately increases circulating pre-B and B cells in multiple sclerosis. *Neurology* 71: 1350–1354.

Kumar, D., Bouldin, T. and Berger, R. (2010) A case of progressive multifocal leukoencephalopathy in a patient treated with infliximab. *Arthritis Rheum* 62: 3191–3195.

Langer-Gould, A., Atlas, S., Green, A., Bollen, A. and Pelletier, D. (2005) Progressive multifocal leukoencephalopathy in a patient treated with natalizumab. *N Engl J Med* 353: 375–381.

Langley, R., Carey, W., Rafal, E., Tyring, S., Caro, I., Wang, X. *et al.* (2005) Incidence of infection during efalizumab therapy for psoriasis: analysis of the clinical trial experience. *Clin Ther* 27: 1317–1328.

Leandro, M., Cambridge, G., Ehrenstein, M. and Edwards, J. (2006) Reconstitution of peripheral blood B cells after depletion with rituximab in patients with rheumatoid arthritis. *Arthritis Rheum* 54: 613–620.

Lebwohl, M., Tyring, S., Hamilton, T., Toth, D., Glazer, S., Tawfik, N. *et al.* (2003) A novel targeted T-cell modulator, efalizumab, for plaque psoriasis. *N Engl J Med* 349: 2004–2013.

Lejniece, S., Murovska, M., Chapenko, S., Breiksa, B., Jaunmuktane, Z., Feldmane, L. *et al.* (2011) Progressive multifocal leukoencephalopathy following fludarabine treatment in a chronic lymphocytic leukemia patient. *Exp Oncol* 33: 239–241.

Leonard, S., Hulin, C., Anxionnat, R., Grignon, Y., Taillandier, L. and Vespignani, H. (2002) [Multifocal progressive leukoencephalitis in a patient given fludarabine for chronic lymphoid leukemia]. *Rev Neurol (Paris)* 158: 1121–1123.

Leonardi, C. (2004) Efalizumab in the treatment of psoriasis. *Dermatol Ther* 17: 393–400.

Leonardi, C., Menter, A., Hamilton, T., Caro, I., Xing, B. and Gottlieb, A. (2008) Efalizumab: results of a 3-year continuous dosing study for the long-term control of psoriasis. *Br J Dermatol* 158: 1107–1116.

Linda, H., Von Heijne, A., Major, E., Ryschkewitsch, C., Berg, J., Olsson, T. *et al.* (2009) Progressive multifocal leukoencephalopathy after natalizumab monotherapy. *N Engl J Med* 361: 1081–1087.

Lindberg, R., Achtnichts, L., Hoffmann, F., Kuhle, J. and Kappos, L. (2008) Natalizumab alters transcriptional expression profiles of blood cell subpopulations of multiple sclerosis patients. *J Neuroimmunol* 194: 153–164.

Lowes, M., Chamian, F., Abello, M., Fuentes-Duculan, J., Lin, S., Nussbaum, R. *et al.* (2005) Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc Natl Acad Sci U S A* 102: 19057–19062.

Lub, M., Van Kooyk, Y. and Figdor, C. (1995) Ins and outs of LFA-1. *Immunol Today* 16: 479–483.

Luster, A., Alon, R. and Von Andrian, U. (2005) Immune cell migration in inflammation: present and future therapeutic targets. *Nat Immunol* 6: 1182–1190.

Maini, R. and Feldmann, M. (2002) How does infliximab work in rheumatoid arthritis? *Arthritis Res* 4(Suppl. 2): S22–S28.

Major, E., Amemiya, K., Elder, G. and Houff, S. (1990) Glial cells of the human developing brain and B cells of the immune system share a common DNA binding factor for recognition of the regulatory sequences of the human polyomavirus, JCV. *J Neurosci Res* 27: 461–471.

Martin, S., Marty, F., Fiumara, K., Treon, S., Gribben, J. and Baden, L. (2006) Infectious complications associated with alemtuzumab use for lymphoproliferative disorders. *Clin Infect Dis* 43: 16–24.

Matos, A., Duque, V., Beato, S., Da Silva, J., Major, E. and Melico-Silvestre, A. (2010) Characterization of JC human polyomavirus infection in a Portuguese population. *J Med Virol* 82: 494–504.

Moharregh-Khiabani, D., Linker, R., Gold, R. and Stangel, M. (2009) Fumaric acid and its esters: an emerging treatment for multiple sclerosis. *Curr Neuropharmacol* 7: 60–64.

Molloy, E. and Calabrese, L. (2008) Progressive multifocal leukoencephalopathy in patients with rheumatic diseases: are patients with systemic lupus erythematosus at particular risk? *Autoimmun Rev* 8: 144–146.

Monaco, M., Jensen, P., Hou, J., Durham, L. and Major, E. (1998) Detection of JC virus DNA in human tonsil tissue: evidence for site of initial viral infection. *J Virol* 72: 9918–9923.

Neff, R., Hurst, F., Falta, E., Bohen, E., Lentine, K., Dharnidharka, V. *et al.* (2008) Progressive multifocal leukoencephalopathy and use of mycophenolate mofetil after kidney transplantation. *Transplantation* 86: 1474–1478.

Padgett, B. and Walker, D. (1983) Virologic and serologic studies of progressive multifocal leukoencephalopathy. *Prog Clin Biol Res* 105: 107–117.

Padgett, B., Walker, D., ZuRhein, G., Eckorade, R. and Dessel, B. (1971) Cultivation of papova like virus from human brain with progressive multifocal leukoencephalopathy. *Lancet* 1: 1257–1260.

Ransom, J. (1995) Mechanism of action of mycophenolate mofetil. *Ther Drug Monit* 17: 681–684.

Sacktor, N. (2002) The epidemiology of human immunodeficiency virus-associated neurological disease in the era of highly active antiretroviral therapy. *J Neurovirol* 8(Suppl. 2): 115–121.

Saumoy, M., Castells, G., Escoda, L., Mares, R., Richart, C. and Ugarriza, A. (2002) Progressive multifocal leukoencephalopathy in chronic lymphocytic leukemia after treatment with fludarabine. *Leuk Lymphoma* 43: 433–436.

Schwab, N., Ulzheimer, J., Fox, R., Schneider-Hohendorf, T., Kieseier, B., Monoranu, C. *et al.* (2012) Fatal PML associated with efalizumab therapy: insights into integrin alphalbeta2 in JC virus control. *Neurology* 78: 458–467.

Selik, R., Karon, J. and Ward, J. (1997) Effect of the human immunodeficiency virus epidemic on mortality from opportunistic infections in the United States in 1993. *J Infect Dis* 176: 632–636.

Sobell, J. and Weinberg, J. (2009) Patient fatalities potentially associated with efalizumab use. *J Drugs Dermatol* 8: 215.

Stoof, T., Flier, J., Sampat, S., Nieboer, C., Tensen, C. and Boorsma, D. (2001) The antipsoriatic drug dimethylfumarate strongly suppresses chemokine production in human keratinocytes and peripheral blood mononuclear cells. *Br J Dermatol* 144: 1114–1120.

Stuve, O., Marra, C., Bar-or, A., Niino, M., Cravens, P., Cepok, S. *et al.* (2006a) Altered CD4+/CD8+ T-cell ratios in cerebrospinal fluid of natalizumab-treated patients with multiple sclerosis. *Arch Neurol* 63: 1383–1387.

Stuve, O., Marra, C., Jerome, K., Cook, L., Cravens, P., Cepok, S. *et al.* (2006b) Immune surveillance in multiple sclerosis patients treated with natalizumab. *Ann Neurol* 59: 743–747.

Taguchi, F., Kajioka, J. and Miyamura, T. (1982) Prevalence rate and age of acquisition of antibodies against JC virus and BK virus in human sera. *Microbiol Immunol* 26: 1057–1064.

Treumer, F., Zhu, K., Glaser, R. and Mrowietz, U. (2003) Dimethylfumarate is a potent inducer of apoptosis in human T cells. *J Invest Dermatol* 121: 1383–1388.

Van Assche, G., Van Ranst, M., Sciot, R., Dubois, B., Vermeire, S., Noman, M. *et al.* (2005) Progressive multifocal leukoencephalopathy after natalizumab therapy for Crohn's disease. *N Engl J Med* 353: 362–368.

Villarroel, M., Hidalgo, M. and Jimeno, A. (2009) Mycophenolate mofetil: an update. *Drugs Today (Barc)* 45: 521–532.

Von Andrian, U. and Engelhardt, B. (2003) Alpha4 integrins as therapeutic targets in autoimmune disease. *N Engl J Med* 348: 68–72.

Vugmeyster, Y., Kikuchi, T., Lowes, M., Chamian, F., Kagen, M., Gilleaudeau, P. *et al.* (2004) Efalizumab (Anti-CD11a)-induced increase in peripheral blood leukocytes in psoriasis patients is preferentially mediated by altered trafficking of memory CD8+ T cells into lesional skin. *Clin Immunol* 113: 38–46.

Waggoner, J., Martinu, T. and Palmer, S. (2009) Progressive multifocal leukoencephalopathy following heightened immunosuppression after lung transplant. *J Heart Lung Transplant* 28: 395–398.

Wagner-Johnston, N., Bartlett, N., Cashen, A., Grove, L. and Berger, J. (2012) Progressive multifocal leukoencephalopathy (PML) in a patient with Hodgkin's lymphoma treated with brentuximab

vedotin. *Leuk Lymphoma* 23 April (epub ahead of print).

Warnke, C., Menge, T., Hartung, H., Racke, M., Cravens, P., Bennett, J. *et al.* (2010) Natalizumab and progressive multifocal leukoencephalopathy: what are the causal factors and can it be avoided? *Arch Neurol* 67: 923–930.

Weber, T., Weber, F., Petry, H. and Luke, W. (2001) Immune response in progressive multifocal leukoencephalopathy: an overview. *J Neurovirol* 7: 311–317.

Wollebo, H., Safak, M., Del Valle, L., Khalili, K. and White, M. (2011) Role for tumor necrosis factor-alpha in JC virus reactivation and progressive multifocal leukoencephalopathy. *J Neuroimmunol* 233: 46–53.

Yousry, T., Major, E., Ryschkewitsch, C., Fahle, G., Fischer, S., Hou, J. *et al.* (2006) Evaluation of patients treated with natalizumab for progressive multifocal leukoencephalopathy. *N Engl J Med* 354: 924–933.

ZuRhein, G. (1965) Particles resembling papovavirus in human cerebral demyelinating disease. *Science* 148: 1477–1479.