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Prevention and therapy of JC polyomavirus-mediated progressive multifocal leukoencephalopathy – a realistic possibility?

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Summary

Progressive multifocal leukoencephalopathy (PML) is a serious opportunistic infection of the brain caused by the JC polyomavirus (JCPyV). PML occurs when immune control of persistent infection with JCPyV fails, the virus mutates and changes its cellular tropism, enters the brain and infects astrocytes, oligodendrocytes and, in particular cases, also neurones. Currently, there is no therapy for this often fatal disease. A number of approaches have failed, and only the restoration of protective immunity, if possible, can lead to clearance of the virus once PML has occurred. During the last two decades, investigators have attempted to understand the factors contributing to the development of PML, which immune mechanisms are involved in immune surveillance, and which in clearing JCPyV from the brain once PML has occurred. Recent data suggest that both $CD4^+$ and $CD8^+$ T cells of the cellular immune system, and also JCPyV-specific antibodies, are involved in protection against PML and in resolving the opportunistic infection. Based on the current immunological data, prophylactic and therapeutic vaccination strategies have been proposed, and first treatment attempts in PML patients have provided promising results that indicate therapeutic vaccination may be feasible.

Key words: *JC polyomavirus, progressive multifocal leukoencephalopathy (PML), active and passive vaccination*

Author contributions
Ivan Jelcic, Benoit Combaluzier and Ilijas Jelcic contributed equally to this work.

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Introduction

The clinical disease entity progressive multifocal leukoencephalopathy (PML) was first described in 1959 [1], and in 1971 the polyomavirus JC (JCPyV) was identified as the pathogen that causes it [2]. Characteristics of PML include an underlying hereditary or acquired compromise of immune function, particularly following treatment with immunomodulatory/immunosuppressive agents or human immunodeficiency virus (HIV) infection [3, 4]. A wide range of conditions have been described as potential reasons for the immunocompromise that may lead to PML. These include: immunodeficiencies such as $CD4^+$ lymphopenia and hyper-immunoglobulin E (hyper-IgE) syndrome; broad-spectrum immunosuppressive treatment; highly specific immunomodulatory drugs such as anti-CD20 (rituximab) and anti-VLA4 (natalizumab) specific monoclonal antibodies; infections (HIV); organ transplantation; and autoimmune/inflammatory diseases such as sarcoidosis, rheumatoid arthritis or systemic lupus erythematosus. The breadth of these conditions indicates that immune control of JCPyV probably involves several aspects of adaptive immunity. Infection with JCPyV is widespread, and approximately 60 to 70% of the world's population is infected. In the immunologically healthy individual, the infection remains persistent throughout life in the kidney and urinary tract [5]. Whether JCPyV can also establish persistent infection in other organs and tissues, including the brain, is currently not clear.

Compromised immune control may lead to mutations of the archetypic or wild type (wt) strain of JCPyV to types with rearranged regulatory regions and mutations in the major capsid protein JCPyV VP1. Both are probably relevant for the switch of the cell tropism to glial cells and neurones [6–9]. In the immunocompromised host, PML causes a lytic infection of oligodendrocytes and morphological changes of astrocytes in the absence of an inflammatory response. Once immune function is restored, e.g., following antiretroviral therapy in the case of HIV, adaptive immune mechanisms lead to an inflammation in the area of the PML lesion, which is referred to as immune reconstitution inflammatory syndrome (IRIS) [3, 4, 10–12]. Although the immune mechanisms underlying IRIS mediate the elimination of JCPyV from the brain, the resulting inflammation can cause additional brain damage and may lead to the death of the patient [11, 12]. Nevertheless, restoration of immunity is, to date, the only way to eliminate the virus from the brain and to thereby halt PML. Several potential alternative treatments of PML that looked promising, e.g. with respect to preventing viral entry into glial cells by receptor-blocking drugs, failed [13]. Both cellular and humoral immune components were found to be important players during IRIS in PML lesions [14]. These findings provided the rationale for vaccination approaches against

PML [15]. In parallel to a decline of PML frequency in HIV-infected individuals, there has been an increase associated with therapy with certain biologics, particularly with anti-CD20 treatment in haematopoietic malignancies and autoimmune diseases, and anti-VLA4 treatment in multiple sclerosis (MS) [4, 16].

In this review, we will summarise the types of immunodeficiencies leading to PML and give our outlook on future strategies towards the development of prophylactic and therapeutic approaches for PML.

Development of PML

JCPyV is a small ubiquitous DNA virus with six open reading frames under the control of a regulatory non-coding region [2]. The major capsid protein VP1 can self-assemble into virus-like particles (VLPs) consisting of 72 pentamers of VP1 [17]. The high anti-JCPyV antibody seroprevalence of up to 60% in healthy individuals indicates widespread infection with JCPyV [5]. In the healthy host, JCPyV persists in urothelial cells and is shed into the urine in 50% of individuals. The infection remains clinically invisible throughout life [4]. JCPyV can also establish latency in haematopoietic progenitor cells, which may mobilise under conditions of immunocompromise or immunomodulation from the bone marrow niche into the peripheral circulation [18, 19]. How JCPyV finally enters the central nervous system (CNS) is currently not entirely clear. B cells have been suggested as viral carriers into the CNS [18–20], but the fact that empty VLPs can enter the CNS compartment indicates that free virus may be able to cross the blood brain barrier [21]. JCPyV DNA fragments and proteins have been detected repeatedly in 13 to 50% of non-PML brain biopsies [22–24], which led to speculation that JCPyV may reach the brain and may persist at this site in a latent state without causing PML. In order to avoid potential contamination of brain biopsies with B cells from the peripheral circulation, some research groups used laser capture microdissection and detected fragments of JCPyV DNA in oligodendrocytes, astrocytes and cerebellar granular cell neurones of non-PML patients [25–27]. Because of the paucity of data and lack of definitive proof, the hypothesis that reactivation of latent JCPyV infection of the brain may lead to PML has been intensely debated since.

In PML, JCPyV variants enter the CNS and infect glial cells. This leads to lysis of oligodendrocytes and swelling of astrocytes in PML and of neurones in granule cell neuronopathy. In the vast majority of cases, glio-/neurotropic JCPyV strains isolated from PML patients display both rearrangements in the regulatory noncoding control region and mutations in specific areas of VP1 [7, 8, 28]. Interestingly, VP1 mutations were mainly identified in the three exterior loops of VP1 (fig. 1). In addition to classical PML, which presents with large multifocal, partly confluent white matter lesions, JCPyV infection may in some cases manifest in other regions of the CNS and may thereby cause distinct JCPyV-associated diseases, including GCN, JCPyV encephalopathy and possibly also JCPyV meningitis [6]. In contrast to PML, GCN is characterised by lytic infection of cerebellar granule cell neurones with JCPyV, which results in cerebellar atrophy [29]. Although reports of GCN cases are less frequent, a histopathological survey reported that JCPyV-infected neurones may also be found in classical PML [30]. In GCN, the isolated infection of cerebellar neurones has been linked to mutations in the C-terminal region of VP1, which are probably involved in the altered tropism for neurones [9]. The glycan receptor LSTc (sialylneolacto-N-tetraose c) was identified as functional host receptor of a prototypical JCPyV strain (MAD1) [31]; however, this binding is abrogated in PML-associated JCPyV variants [32]. Therefore, the cellular entry receptor of JCPyV in glial cells and neurones remains unknown.

Protection from viral infections and elimination of virus-infected cells during acute infection involve cellular immunity, which is mediated by virus-specific $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells, as well as virus-specific antibodies from B cells. Their relative importance depends on the respective virus, on its tissue and/or cell tropism, on the structure of its envelope, on the composition of virus-encoded immunogenic epitopes and peptides, on the type of release from the infected cell (i.e. budding from the cell membrane or lytic infection), and other factors. Several lines of evidence suggest an important role of the adaptive immune system to protect the infected host during an asymptomatic persistent infection with archetypic JCPyV, as well as in clinically manifest PML, with infection of the brain with $JCPyV_{PML}$ variants.

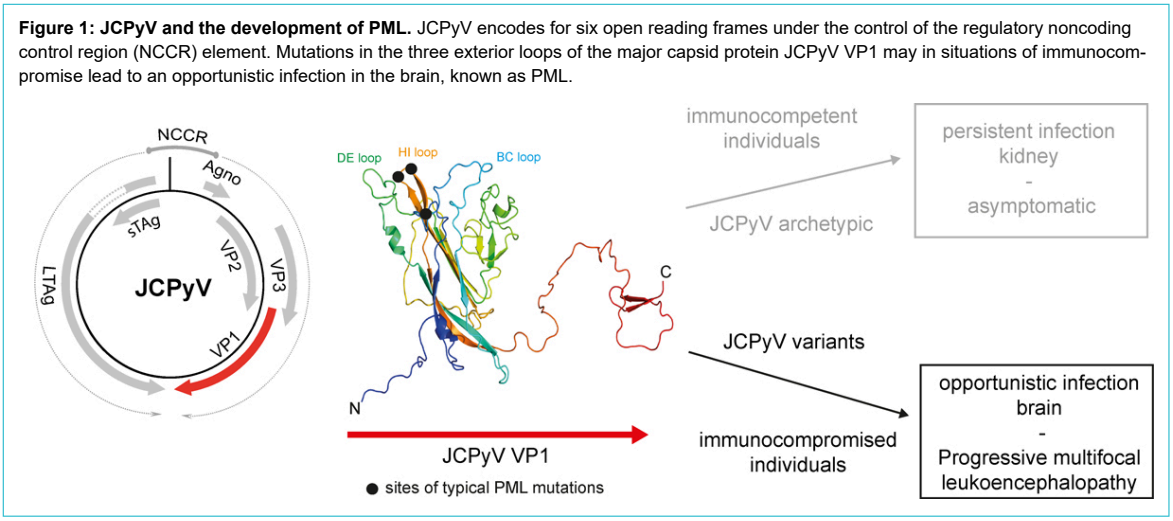


Figure 1: JCPyV and the development of PML. JCPyV encodes for six open reading frames under the control of the regulatory noncoding control region (NCCR) element. Mutations in the three exterior loops of the major capsid protein JCPyV VP1 may in situations of immunocompromise lead to an opportunistic infection in the brain, known as PML.

These pieces of evidence include PML occurrence in states of immunodeficiency of one or several arms of the adaptive immunity, such as hereditary antibody and/or cellular or acquired cellular immunodeficiencies, including HIV infection, or immunodeficiencies induced by chemotherapy, immunomodulatory or -suppressive therapies, allotransplantation or certain autoimmune diseases [14] (table 1). As patients with haematological malignancies are frequently treated with chemotherapy and immunosuppressive therapies, it remains difficult to discern if the primary disorder, the secondary immunosuppression, or both are critical for PML development.

Types of immunodeficiencies leading to PML

After its first description in 1959 [1], PML was considered a rare disease that was mainly diagnosed in immunodeficient patients in the era before the emergence of acquired immunodeficiency syndrome (AIDS) [38]. The number of PML cases drastically increased with the AIDS pandemic, and PML became one of the main opportunistic infections in HIV^+ patients and a major cause of death [39] (table 1). With the introduction of combined highly active antiretroviral therapy (HAART), the incidence of PML decreased, but remains a severe and life-threatening complication for AIDS patients [40–42].

The use of immunomodulatory therapies for various autoimmune diseases has led to an alarming increase of PML cases among patients with conditions that were previously not linked to any substantial PML risk. Immunotherapy-associated PML received broad attention with natalizumab in patients with MS or Crohn's disease [16, 43–45]. Natalizumab had been approved for the treatment of relapsing-remitting MS (RRMS) in 2004, since it effectively reduced CNS inflammation via its inhibition of lymphocyte migration through the blood-brain barrier [46, 47]. It was briefly taken off the market after report of the first PML cases and then reintroduced with a strict safety and risk monitoring programme. Natalizumab is the immunotherapy with the highest number (698 as of December 2016) and highest rate (4.18 per 1000 cases treated) of confirmed PML cases [48]. Efalizumab is a humanised monoclonal antibody that was developed for the treatment of psoriasis by targeting CD11a in order to inhibit lymphocyte activation and migration [49]. It was voluntarily withdrawn from the market by the manufacturer after reports of four efalizumab-associated PML cases [4, 50, 51]. The B cell depleting monoclonal antibody rituximab is widely used to treat haematological cancers and autoimmune diseases, and to avoid graft rejection [52, 53]; although it was approved as long ago as 1997, rituximab has been associated with numerous PML cases only in the last 10 years [54]. The direct role of rituximab in PML development is still debated, since most of the patients suffered from immunosuppressed states either because of their primary haematological disorder or because of additional immunosuppressants, which made them more vulnerable to developing PML [54, 55]. Alemtuzumab, a monoclonal antibody against CD52 used as second-line therapy in MS to deplete B and T cells, was clearly linked to PML in some

Table 1: Hereditary and acquired diseases and immunomodulatory/- treatments associated with a risk of PML or other JCPyV-induced CNS infectious diseases.

Category of disease	Diseases
Hereditary immune deficiencies [33]	– Adenosine deaminase deficiency – CD40 ligand deficiency – Combined immune deficiency – Common variable immune deficiency – DOCK8 (dedicator of cytokinesis 8 protein) deficiency – Gamma heavy chain disease – Hyper-IgM syndrome – Immunodeficiency-centromeric instability-facial dysmorphism syndrome syndrome – Purine nucleoside phosphorylase deficiency – Severe combined immune deficiency – Signal transducer and activator of transcription 1 gain-of-function immune deficiency – Wiskott-Aldrich syndrome – X-linked agammaglobulinaemia – Idiopathic $CD4^+$ lymphopenia*
Acquired immune deficiencies [18, 34]	– Human immunodefiency infection or acquired immune deficiency syndrome – Haematopietic stem cell transplantation – Immunosuppressive therapy in organ transplant recipients – Haematological malignancies (e.g. lymphomas and leukaemias) – Immunosuppression during chemotherapy of solid cancers – Systemic lupus erythematosus – Sarcoidosis – Immunosuppressive or -modulatory therapy in autoimmune diseases (rheumatoid arthritis, psoriatic arthritis, psoriasis, juvenile idiopathic arthritis, inflammatory bowel disease, ankylosing spondylitis, multiple sclerosis)
Immunomodulatory treatments [4, 18, 35, 36]	– Natalizumab – Efalizumab – Belimumab – Rituximab – Fingolimod – Dimethylfumarate – Alemtuzumab – Tumour necrosis factor-alpha inhibitors (infliximab, adalimumab, etanercept) – Ofatumumab – Mycophenolate mofetil – Betalacept – Brentuximab – Fludarabine – Ruxolitinib – Leflunomide

* Heterogeneous disease of unknown aetiology, but a genetic aetiology has been described in some cases [37]

other diseases, such as leukaemia. So far, no PML cases have been observed in alemtuzumab-treated MS patients [56–58], and the same holds true for rituximab-treated MS patients, in whom there is so far no reported PML case. Further PML cases were reported with belimumab, a monoclonal antibody targeting the B-cell activating and survival factor BAFF, used for the treatment of systemic lupus erythematosus (SLE) [59]. Furthermore, with several tumour necrosis-alpha (TNFα) inhibitors such as adalimumab, infliximab and etanercept, which are currently used to treat psoriasis, rheumatoid arthritis and other autoimmune diseases, PML was observed in predisposed patients [60, 61].

For other immunomodulatory drugs such as fingolimod and dimethyl fumarate used for the treatment of MS or other autoimmune diseases, only a few PML cases have been reported [62]. In the fingolimod-treated MS patients, it is difficult to clearly link this drug with PML since most of these patients had been treated with natalizumab prior to fingolimod therapy [63, 64]. The first cases of PML under dimethyl fumarate were reported in psoriasis patients, but there are now publications of PML also in the context of MS [65–68]. Multiple PML cases occurred under immunosuppressive treatments to avoid allograft rejection or to treat cancer or autoimmune diseases; however, it is difficult to discern whether the underlying conditions of immunocompromise or the treatment-induced immunosuppression are the main cause for PML [69].

Hereditary immunodeficiencies may exclusively or preferentially compromise one specific immune cell type, e.g., in idiopathic $CD4^+$ lymphopenia (table 1). Similarly, treatments that have been linked to PML may affect specific immune cells more profoundly than others. As an example, natalizumab blocks the entry of $CD4^+$ T cells into the CNS much more efficiently than that of $CD8^+$ T cells [70]. In the case of HIV infection, the virus specifically targets the CD4 receptor and leads to quantitative and qualitative alterations of $CD4^+$ T cells, but each of these conditions also indirectly affects the function of $CD8^+$ T cells and antibody responses. The occurrence of IRIS, such as in AIDS patients starting antiretroviral therapy or in natalizumab-treated MS patients after washout of natalizumab by plasmapheresis, indicates restoration of immune functions that leads to an inflammatory response within the area of the PML lesion [11, 12]. In depth studies of the cellular components of this inflammatory response revealed a central role of $CD4^+$ T cells in orchestrating an efficient $CD8^+$ effector T cell response in order to clear the virus [71–73].

Risk stratification and therapeutic approaches for PML

Natalizumab has been reapproved with the stipulation that factors contributing to PML risk must be identified. Studies of potential risk factors for PML identified prior immunosuppression, duration of natalizumab treatment and presence of anti-JCV antibodies as predisposing factors and, based on these, a risk classification scheme was proposed [74, 75]. The latter stratification involved monitoring of serum and plasma JCPyV VP1-specific antibodies during natalizumab treatment [75]. Interestingly, when the JCPyV antibody indices of natalizumab-treated MS patients were correlated with the incidence rate of PML in the different strata, patients with a higher antibody index had a substantially increased risk for developing PML. The risk stratification protocol ensured a reduction of PML incidence, but also limited the use of this very efficient drug in MS. Moreover, recent data showed that intrathecal production of JCPyV VP1-specific antibodies was enhanced in patients at time of PML [76]. As another potential risk marker, Schwab et al. showed that patients had low percentages of $CD62L^+$ T cells before they developed PML [77, 78]. However, these findings are still debated, since an independent study did not confirm this observation [79]. Furthermore, MS patients with cerebrospinal fluid (CSF) lipid-specific IgM oligoclonal bands have a lower risk of developing natalizumab-associated PML than patients without such bands [80]. It is currently not clear how the above findings translate into increased risk with respect to cellular or humoral immune compromise.

Reconstituting the protective immunity or reversing the immunosuppression is so far the best way to eliminate JCPyV infection from the CNS and to overcome PML. Immune reconstitution in HIV^+ patients can be achieved with HAART, which in turn will lead to the recovery of $CD4^+$ lymphocytes. HAART has greatly reduced the number of HIV^+ PML cases and significantly improved the survival of HIV^+patients developing PML [3, 40, 41, 81–83]. However, immune reconstitution in PML can lead to IRIS in 10 to 30% of HIV^+ patients, resulting in subacute onset of sometimes severe neurological deficits, which often persist after recovery from PML [3, 40, 83, 84]. The same applies to nearly all natalizumab-associated PML patients in whom natalizumab is discontinued and removed by plasma exchange after PML diagnosis [12, 85]. IRIS may be carefully treated with corticosteroids to ameliorate CNS tissue destruction by exuberant JCPyV-specific immune response [86, 87]. Corticosteroid treatment should be tapered slowly to avoid abrogation of the antiviral immune reaction. Alternatively, the C-C motif chemokine receptor (CCR5) antagonist maraviroc approved for the treatment of HIV infection, has been discussed to be useful in preventing overshooting IRIS [88–90]. Since maraviroc was not helpful in other PML cases [91], its therapeutic role for PML-IRIS prevention or treatment has to be defined in larger trials. However, it is not always possible to stop immune suppression, for example in organ transplant patients, in whom it could lead to graft rejection. Furthermore, some PML patients may not develop IRIS at all because of persisting severe immunosuppression related to haematological malignancy, or treatment with rituximab or alemtuzumab. In these cases, other therapies targeting JCPyV have to be considered.

Several antiviral drugs intended to target JCPyV have been investigated *in vitro* and tested in individual medical treatments or in clinical trials. These drugs target the virus at different stages of infection by blocking cell entry or viral replication. JCPyV uses sialylated receptors [31, 92] and the serotonin receptor 2 (5-HT2) [93, 94] to enter the cells. Several serotonin receptor antagonists are currently marketed for the treatment of mental disorders and have been tested as potential therapeutics for PML. Chlorpromazine efficiently blocked the viral infection in cell-based assays [94–96], but has never been tested clinically, probably because of its poor selectivity and the potential side effects.

Mirtazapine was empirically reported to be beneficial in several independent PML case studies, but available data suggest that it is not effective [97–101]. The *in vitro* inhibitory effects of ziprasidone could not be translated into two PML patients [102], and another study [103] reported a PML case who recovered after risperidone treatment despite the fact that this drug could not block the attachment and internalisation of JCPyV into primary human fetal glial cells [104].

Another approach to interfering with the life cycle of JCPyV would be to block viral DNA replication, for example, by inhibiting viral DNA polymerases or topoisomerases. Cidofovir, a drug approved for the treatment of human cytomegalovirus (HCMV) infection, and its prodrug brincidofovir suppressed JCPyV replication in a cell-based assay even at a low, non-toxic concentration [105, 106], but neither improved survival nor neurological status of PML patients [107, 108]. Cytarabine reduced JCPyV replication *in vitro* [109], but failed to demonstrate an improvement of patients' prognosis upon treatment [110]. Topotecan, a chemotherapeutic agent for certain ovarian cancers, inhibited JCPyV DNA replication in glial cells [111]. However, the results of a clinical study addressing its efficacy were not convincing, since only a few patients responded to the therapy and several enrolled patients experienced severe adverse events [112]. Mefloquine, a medication used to prevent or treat malaria, was selected as a potential JCPyV inhibitor in a high-throughput screening of approved drugs and was shown to block DNA replication after viral entry [113]. Given the availability and safety of this medicine, mefloquine was tested in a clinical trial that was prematurely terminated after interim data analyses suggested that the study was unlikely to succeed [114]. The lack of signs of efficacy for all these antiviral drugs has been disappointing, in particular after often promising *in vitro* results. The difficult translation into patients could be explained by stability issues, low brain penetration, toxicity issues, lack of specificity for JCPyV or JCPyV variants, and a cell type-dependent effect.

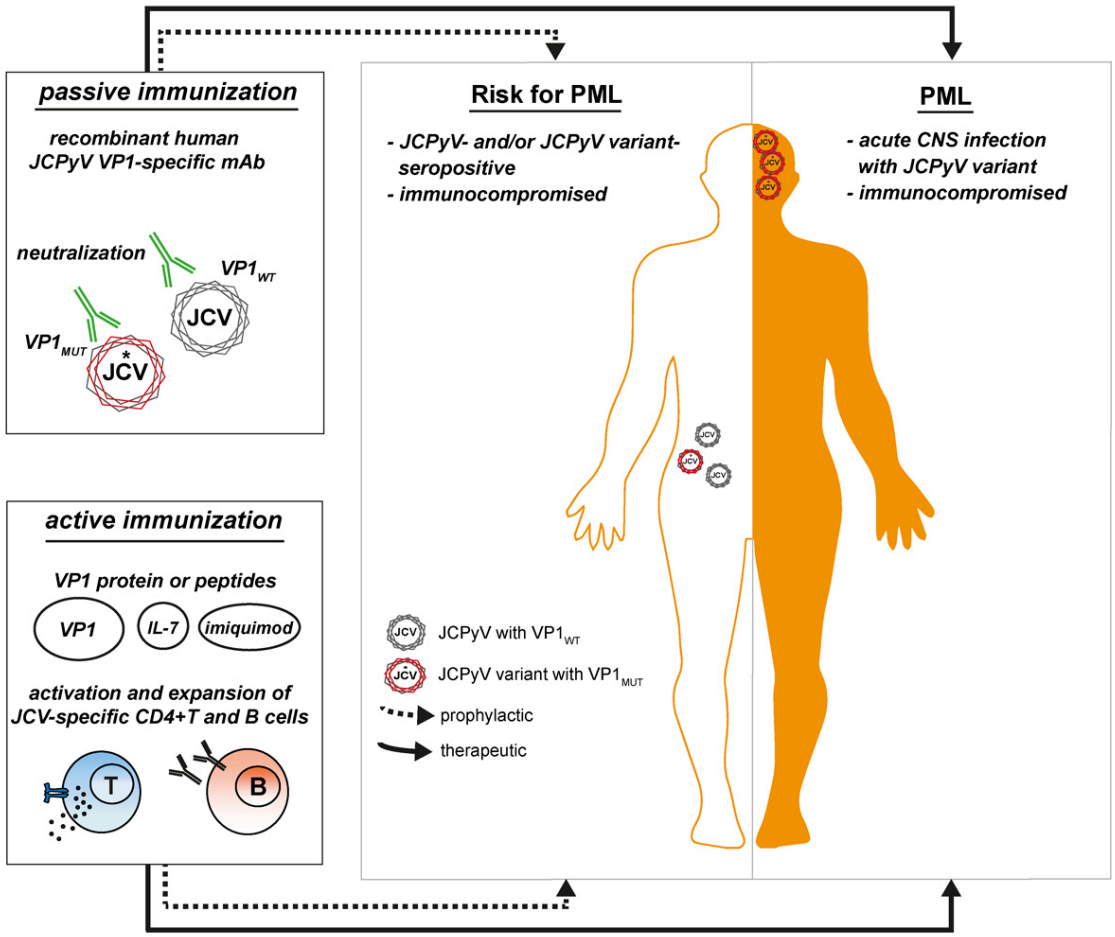
With the recent breakthrough in genome editing using the CRISPR/Cas9 system, two independent groups targeted functional regions in the JCPyV genome to inhibit viral replication in infected cells. The first approach aimed to introduce mutations in the early expressed JCPyV large T antigen in order to suppress viral replication [115], and another study focused on the modification of the regulatory noncoding control region element and the major capsid protein VP1 [116]. In both cases, the infection and spread of JCPyV could be inhibited *in vitro* in the JCPyV-permissive glial SVG-A cell line. However, this technique is still far away from potential clinical use in PML patients, and its efficacy has to be validated for JCPyV variants and different cell types. Furthermore, it is unclear whether those constructs can be delivered successfully to the infected cell types in the CNS and whether such an approach would have safety issues or even be more hazardous in combination of rearranged and mutated JCPyV variants.

Immune modulators, such as cytokines, are also used to treat viral diseases because of their potential antiviral capacity or ability to augment the immune response against the pathogen. Type 1 interferons, such as IFN-α, are known for their antiviral capacity, but failed in an open-label study in HIV^+ PML patients [117], despite a retrospective analysis claiming increased survival times [118]. Interestingly, the use of lymphoproliferative cytokines, such as interleukin (IL)-2 of the common gamma-chain cytokine family, revealed beneficial effects on the response and outcome in single case studies with PML patients [119–122]. Another cytokine of the common gamma-chain family, IL-7, was also successfully tested in compassionate use alone or in combination with antiviral drugs [123–125].

On the basis of current immunological data [14], approaches to boost specific immunity against JCPyV have also been explored. Recent studies suggested two novel promising immunotherapeutic approaches for the treatment or prevention of PML that are based on active or passive immunisation specifically targeting JCPyV. The development of prophylactic vaccines based on virus-like particles (VLPs) has been successful in the case of HPV [126], a nonenveloped DNA virus that shares similar structural elements with JCPyV. The use of VLPs formed by the reassembly of JCPyV VP1 proteins appears as a good approach for an effective vaccine since VLPs represent a combination of multiple epitopes and structural arrangement that should be optimal for uptake by antigen-presenting cells, in particular JCPyV-specific B cells. Furthermore, the choice of the right adjuvant is essential in order to efficiently boost the virus-specific immune response. A vaccine consisting of JCPyV VP1 VLPs in combination with IL-7 and imiquimod, a toll-like receptor 7 (TLR7) agonist, has been tested in two PML patients, one suffering from idiopathic $CD4^+$ lymphopenia, the other from breast cancer, polychemotherapy, autologous and allogeneic bone marrow transplantation and subsequent graft-versus-host disease [15]. The treatment was well tolerated and safe, and several lines of evidence suggested that the immunisation caused a "therapeutic" immune response: (1) The CSF JCPyV viral load disappeared, (2) a pronounced proliferation of VP1-specific $CD4^+$ T cells was observed, (3) magnetic resonance imaging studies showed contrast enhancement in the PML lesions as a clear sign of local immune response and IRIS, and (4) an amelioration of the neurological deficits was seen [15]. Although immune reconstitution with IL-7 alone has already shown promise in the management of PML, a combination with VLPs ensured a specific and robust activation and expansion of VP1-specific T cells. However, it is at present not clear whether IL-7 is needed or not in the above vaccination scheme. The same active vaccination approach was also successfully administered to a third PML patient with idiopathic $CD4^+$ lymphopenia [127]. Vaccination substantially reduced the CSF viral load and the progression of PML. The patient also showed a strong rise in neutralising antibodies against her own mutant JCPyV strain, showing that this vaccine broadened the humoral response even against JCPyV variants. The active therapeutic immunisation would be most suitable for patients with residual and restorable immune function. We further assume that active prophylactic immunisation of healthy individuals or patients under treatments with PML risk has the potential to prevent PML or, in seronegative individuals, even the establishment of latent/persistent infection altogether.

With respect to possible passive vaccination against PML, antibody therapy has been successfully applied for various diseases; broadly neutralising antibodies are promising candidates for the treatment of viral infection and are cur-

Figure 2: Overview of the active and passive immunization approaches for the prophylactic and therapeutic treatment of PML. These novel vaccination approaches are considered for both patients with PML and immunocompromised, JCPyV-positive patients at risk of developing PML. Passive immunisation involves administering broadly neutralising JCPyV VP1-specific recombinant antibodies. The latter are expected to provide protection from and neutralisation of neurotropic pathological JCPyV VP1 variants. The active immunisation strategy implies a direct boost of adaptive immune responses specific for JCPyV variants in patients by vaccinating with whole protein or peptides of the major capsid protein VP1 in combination with an adjuvant (e.g., imiquimod) and supporting cytokines (e.g.. interleukin-7). The latter will lead particularly to the activation and expansion of JCPyV-specific $CD4^+$ effector T cells and antibody-producing B cells, which will confer a broad protection from pathological JCPyV infection to the patients.



rently in development for numerous pathogens, such as influenza, respiratory syncytial, Ebola and Zika viruses [128–132]. The above data indicate that an effective humoral response is important for controlling JCPyV infection and/or eliminating virus from the brain in PML. Evidence includes reports that HIV^+ PML survivors showed higher IgG titres compared with controls [133], that B cells and plasma cells infiltrate the brain during IRIS [71, 134], that intrathecal production of JCPyV-specific antibodies increases with onset of IRIS [76, 135], that hereditary immunodeficiencies affecting B cells or antibodies can cause PML [136, 137] and that immunomodulatory drugs targeting B cells may lead to PML [4, 138]. The level of JCPyV VP1-specific antibodies is usually measured to stratify the patients at risk of developing PML, but this test does not discriminate between archetype JCPyV and PML-associated mutants. Further, there seems to be no correlation between the level of anti-JCPyV antibodies and their neutralising activity in the sera of MS patients [139]. Why higher anti-JCPyV antibody titres in natalizumab-treated MS patients are related to higher risk is at present not clear. We demonstrated that the humoral immune response against JCPyV mutants was compromised in natalizumab-associated PML patients, and that immune reconstitution led to a broadened antibody response against the PML-associated JCPyV variants [135]. We generated highly potent neutralising JCPyV VP1-specific antibodies by recombinant cloning from a PML patient who successfully controlled the viral infection after IRIS and recovered from PML [135]. These human-derived antibodies exhibited exquisite specificity and high affinity towards JCPyV, neutralised the JCPyV infection *in vitro* and showed cross-reactivity against the most common PML-causing JCPyV variants, and represent promising candidates for the development of a passive immunisation of PML patients. Similar work was also successfully carried out in Ebola survivors [128, 129]. Adequate brain exposure to antibodies would be expected, as observed for other human monoclonal antibodies in development for CNS indications [140]. Passive immunisation appears to be a promising approach, in particular for PML related to HIV, transplantation or immunosuppressive drug therapy patients, where immediate immune reconstitution is not possible.

Active and passive immunisation approaches for the prophylactic and therapeutic treatment of PML are summarised in figure 2.

Conclusion

PML remains an untreatable and often fatal opportunistic infection of the brain. Its resurgence with the increasing use of novel potent immunomodulatory treatments for a variety of diseases makes the development of prophylactic and therapeutic strategies for PML a high unmet medical need. Current data suggest that all three arms of the adaptive immune system, – virus-specific $CD4^+$ and $CD8^+$ T cells and virus-specific antibodies – are important for controlling infection with JCPyV and also for resolving PML once it has developed. Active therapeutic vaccination, as well as high affinity, cross-neutralising, anti-JCPyV VP1-specific human monoclonal antibodies are promising approaches to the development of an effective treatment and prophylaxis of PML.

Disclosure statement

B.C., Ivan J., R.M., and J.G. are listed as inventors on a patent of the human monoclonal antibodies against JCPyV VP1 for the treatment of PML. R.M., Ilijas J. and M.S. are listed as coinventors on a patent on vaccination against PML by immunisation with JCPyV VP1 protein. J.G. is employee and shareholder of Neurimmune. B.C. is employee of Neurimmune.

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