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Advances in the biology of JC virus and induction of progressive multifocal leukoencephalopathy

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Since the initial description of progressive multifocal leukoencephalopathy (PML) in 1958, clinical and basic science investigators have demonstrated a growing interest in the area of neurovirology, with a recent focus on polyomaviruses. In this review, the authors present an overview of the biological properties of the human polyomavirus, JC virus (JCV), and its association with PML as the etiologic agent. Additionally, the authors provide a discussion of the current understanding of JCV molecular pathogenesis and therapeutic strategies. *Journal of NeuroVirology* (2003) **9,** 236–246.

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Biology of JC virus

JC virus (JCV) is a double-stranded circular DNA virus belonging to the family Polyomaviridae. Like the related human polyomavirus BKV, JC virus was named after the initials of the patient from whom it was first recovered. JCV virion particles are approximately 45 nm in diameter and display icosahedral symmetry. The prototype JCV genome Mad-1 exists as supercoiled DNA 5130 base pairs in length (Frisque *et al*, 1984). Initially this genome was reported to encode for two nonstructural proteins (large T, small t), three capsid proteins (VP1, VP2, and VP3), and a regulatory protein (agnoprotein) (Okada *et al*, 2001). Counterclockwise transcription from one of the DNA strands initiated near the origin of replication leads to the expression of the "early" large T and small t proteins. In contrast, "late" transcripts made from the opposite strand of DNA in a clockwise direction encode for the agnoprotein, as well as the virion structural proteins, VP1, VP2, and VP3 (Frisque *et al*, 1984). The presence of three additional early proteins, T′135, T′136 and T′165, has more recently been documented in lytically infected cells (Trowbridge and Frisque, 1995). These T′ proteins are reported to be involved in JCV DNA replication and interactions with several cellular proteins that may play roles in interference with certain T-antigen functions (Frisque, 2001). The large T protein also interacts with p53 (Staib *et al*, 1996), a tumor suppressor protein, that influences cell cycle and apoptosis. Studies have shown p53 accumulation and polymorphisms in patients with progressive multifocal leukoencephalopathy (PML) (Power *et al*, 2000; Ariza *et al*, 1996). Even though apoptosis is a factor in many neurodegenerative disorders, its impact on PML remains elusive. To our knowledge, with the exception of the multinuclear astrocytes commonly observed in PML brain tissue, only two studies suggest that apoptosis may contribute to the PML demyelinating process (Yang and Prayson, 2000; Richardson-Burns *et al*, 2002). This laboratory is currently investigating whether apoptotic pathways are associated with JCV pathogenesis and if such pathways contribute to the central nervous system (CNS) demyelination in PML. The role of the small t protein remains unclear and has not been conclusively linked with viral replication or pathogenesis in humans. However, there is evidence for small t involvement in the transformation of infected rodent and nonhuman primate cells (London *et al*, 1978; Walker *et al*, 1973). The virion particle comprises all three viral capsid proteins, although VP1, the largest capsid protein, is capable of self-assembling into icosahedral

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particles, independent of VP2 and VP3 (Chang *et al*, 1997). VP1 also contains the epitopes for antibody induction and recognition. VP1 has been successfully expressed using recombinant baculoviruses and the resulting recombinant VP1 has been demonstrated to form virus-like particles (VLPs) with a typical morphology of empty JCV capsids. VP1 VLPs have been described as an efficient transporter system with potential for gene therapy and PML-specific vaccine development (Goldmann *et al*, 1999, 2000). Agnoprotein, a 71–amino acid polypeptide, is encoded by sequence at the 5′ end of the JCV late gene region. The presence of agnoprotein results in suppression of cell growth and cell proliferation by deregulating cell cycle progression and stalling cells in G2/M phase (Darbinyan *et al*, 2002). Further, it has been shown that agnoprotein-mediated suppression of cell proliferation is independent of T antigen, probably via enhancement of tumor suppressor protein p21 expression and interaction with p53 (Darbinyan *et al*, 2002). The agnoprotein is shown to interact with T protein to down-regulate T protein–modulated viral DNA replication, as well as play a role in capsid assembly (Safak *et al*, 2001; Major, 2001). Furthermore, a newly synthesized polyclonal agnoprotein antibody has been used to demonstrate that agnoprotein is expressed in, and mainly confined to, the cytoplasm of JCV-infected cells, both in culture and brains of PML patients (Okada *et al*, 2002).

Recent studies have shown that treatment of cells with neuraminidase, protease, or phospholipase blocks JCV binding. However, viral infection was specifically abrogated only by neuraminidase, suggesting that receptors for JCV may contain sialic acid. Studies have since confirmed that JCV uses widely expressed alpha 2,6-linked sialic acid residues on glycoproteins for attachment to the cell membrane of several cell types (Liu *et al*, 1998). Virions are transported to the nucleus through a clathrin-dependent endocytosis (Pho *et al*, 2000) where viral replication and assembly occurs. Furthermore, most recent evidence suggests that the oligosaccharides of glycoproteins and glycolipids work as JCV receptor based on the fact that neoglycoproteins and gangliosides with biologically relevant binding to JCV inhibited JCV infection in human glial SVG-A and human neuroblastoma IMR-32 cells (Komagome *et al,* 2002).

The role of DNA binding proteins in cell type–specific regulation of JCV DNA replication and transcription has been emphasized recently (Raj and Khalili, 1995). A number of such proteins has been studied in relation to JCV infection, including Sp1 (Henson *et al*, 1992), c-Jun (Amemiya *et al*, 1992), nuclear factor kappa B (NF-μB) (Ranganathan and Khalili, 1993), Tst-1 (Wegner *et al*, 1993), YB-1 (Kerr *et al*, 1994), GBP-i (Raj and Khalili, 1994), Pur-alpha (Chen and Khalili, 1995), and NF-1 (Amemiya *et al*, 1989). NF-1 encompasses a family of multifunctional proteins that participate in transcription and replication of several genes in an organ- and cell type–specific manner. The NF-1 family of transcription factors is divided into four subclasses, A, B, C, and D or X. NF-1 has several binding sites in the regulatory region of JCV and has been implicated in supporting JCV infection (Raj and Khalili, 1995). The NF-1X class member in particular has stirred significant interest among investigators because it is expressed at higher levels than the other classes in cells that are susceptible to JCV infection (Sumner *et al*, 1996). Human fetal glial cells and the hematopoietic progenitor cell line KG-1 are two recently cited examples with correlation of JCV permissiveness and NF-1X expression. The role of NF-1 in JCV activity has been confirmed by several studies demonstrating that KG-1 cells, which lose JCV susceptibility upon differentiation to macrophage-like cells, exhibit a concomitant down-regulated NF-1X expression. However, transfection with NF-1 class X expression vectors, and subsequent overexpression of NF-1X protein in the same differentiated cells, restored susceptibility to JCV infection (Monaco *et al*, 2001).

The nucleotide sequence arrangement of the JCV regulatory region is believed to be a predominant factor affecting viral host range and cellular tropism. In fact, a classification scheme has been recently proposed to distinguish between JCV variants based on sequence differences in their regulatory regions (Jensen and Major, 2001). Mad-1 sequence includes a 98-bp tandem repeat in the regulatory region. Such arrangements are most frequently identified in brain biopsies from PML patients. In contrast, viral isolates from urine samples generally exhibit regulatory region sequence rearrangements referred to as "archetype," having only a single copy of the 98-bp Mad-1 regulatory region sequence structure with insertions of 23- and 66-bp sequences. The archetype strain has been shown to be biologically inactive *in vitro*, whereas in contrast the Mad-1 and other similarly arranged strains have the most robust viral activity.

Incidence and detection of JCV

JCV has a worldwide distribution, with asymptomatic JCV infection seen in more than 80% of adults, as defined by the presence of JCV antibodies (Major *et al*, 1992; Weber *et al*, 1997a). Serological studies indicate that primary infection occurs in childhood. JCV strains worldwide have been classified into more than 10 main genotypes based on the phylogenetic analyses of viral VP1 gene (Agostini *et al*, 2001). A correlation between JCV genotypes and human populations appears to be evident based on the geographical distribution of JCV. Within populations, a parent-to-child mode of viral transmission has been demonstrated (Kunitake *et al*, 1995; Suzuki *et al*, 2002). JCV was initially designated as strictly neurotropic, and was assumed to replicate and multiply only in human glial cells. However, as recent

advances suggest, JCV is not restricted to the CNS, and can infect diverse cell types within human hosts (Major, 2001). The demonstration of active JCV replication in non-CNS tissues as indicated by polymerase chain reaction (PCR), *in situ* hybridization, Southern blot, and/or restriction enzyme digest patterning has advanced our understanding of JCV pathogenesis and its association with PML. Furthermore, the development of monoclonal and polyclonal antibodies to viral T protein and capsid proteins has helped in following the course of infection *in vitro* and *in vivo* by allowing detection of JCV-infected cells and tissues. Detection of JCV agnoprotein by use of a new specific polyclonal antibody (Ab) has been suggested as an appropriate marker for PML diagnosis and a progressive JCV infection (Okada *et al*, 2002). The presence of host antibodies against JCV has traditionally been determined by hemagglutination inhibition of human type O erythrocytes; however, the recent development of a JCV-specific enzyme-linked immunosorbant assay (ELISA) has facilitated the screening of human serum samples and subsequently larger seroepidemiological studies of JCV infection (Hamilton *et al*, 2000). Collectively, all these methods of detection have been used as markers for PML. The confirmatory diagnosis remains the detection of JCV DNA in demyelinated lesions of brain biopsy tissue or cerebrospinal fluid (CSF) by *in situ* DNA hybridization or PCR amplification, respectively.

Viral pathogenesis and clinical implications of JCV infection

Within the CNS, JCV productively infects oligodendrocytes. Demyelination resulting from this lytic infection and destruction of oligodendrocytes is the pathological cause of the neurological impairments seen in PML. PML was first described as a neuropathologic entity in 1958 (Astrom *et al*, 1958), which was later linked to a viral etiology in 1971, when a unique human polyomavirus was isolated from primary human fetal glial cell cultures inoculated with brain extracts from PML patients (Padgett *et al*, 1971). PML is a chronic, progressive, usually fatal disease in humans, characterized by neuropathological features such as multifocal demyelination, enlarged oligodendrocytes with nuclear inclusions, bizarre looking astrocytes having lobutaled hyperchromatic nuclei, as well as astrocytes containing mitotic bodies (Frisque and White, 1992; ZuRhein, 1969; ZuRhein and Chou, 1965). Pathological alterations of PML are seen in the cerebrum, cerebellum, or brain stem (Richardson, 1961; ZuRhein, 1969; Richardson and Webester, 1983). Although cerebral cortex and deep gray matter appear normal, myelin loss is evident in areas of retraction within subcortical or deep white matter. Histopathologically, demyelinated PML lesions exhibit significant oligodendrocyte death. Remaining intact oligodendrocytes are usually enlarged with nuclear inclusions (Richardson, 1961; ZuRhein GM, 1969). JCV DNA, as well as early and late viral proteins, have been reported in nuclei of infected oligodendrocytes (Greenlee and Keeney, 1986; Aksamit *et al*, 1987). Electron microscopy of PML brain tissue reveals crystalline arrays of viral particles within infected oligodendrocytes. PML can be considered an opportunistic infection that is almost always observed in immunosuppressed patients in clinical settings such as acquired immunodeficiency syndrome (AIDS), certain cases of advanced malignancy, and organ transplantation. Two to five percent of patients with AIDS develop PML, usually leading to rapid mortality due to lack of cellular immunity (Berger and Concha, 1995; Cinque *et al*, 1997). The higher incidence of PML in AIDS patients as compared to other immunocompromised disorders, suggest that the presence of HIV-1 in the CNS may play a direct, or indirect, role in JCV pathogenesis. The fact that the human immunodeficiency virus type 1 (HIV-1)–encoded transregulatory protein, Tat, significantly promotes the rate of transcription from the JCV late promoter supports the hypothesis of this relationship between PML and HIV-1 infection (Chowdhury *et al*, 1993). In immunocompromised individuals, PML involves both a humoral and a T cell–mediated immune response. The impact of the cellular immune response against JCV on the clinical outcome of HIV-positive as well as HIV-negative individuals with PML has been recently examined. These studies show a better clinical outcome for the patients with JCV-specific cellular immune response (Koralnik *et al*, 2001, 2002). In PML cases without AIDS, general impairment of cell-mediated immune response, along with severe and selective impairment of cell-mediated immune response to JCV, has also been shown (Willoughby *et al*, 1980).

Before AIDS appeared as a global health threat, PML occurred as an extremely rare complication of malignant diseases such as lymphomas and leukemias, protracted granulomatous disorders such as tuberculosis, sarcoidosis, and immunosuppression for collagen vascular disease or organ transplantation (Greenlee, 1989). However, with the advent of AIDS as a pandemic, the number of PML cases reported worldwide has increased dramatically, and as AIDS patients are now living longer with improved AIDS therapies, the incidence of PML and its complexity in such patients may be expected to rise. JCV-induced PML has hence achieved greater clinical interest. At present, it is unclear whether development of PML is subsequent to the initial entry of JCV into the CNS with infected lymphocytes, or whether it follows reactivation of a persistent latent infection. The underlying mechanism(s) by which PML occurs is an important focus of current research efforts.

For decades, definitive diagnosis of PML required brain biopsy, and detection of JCV DNA or viral antigens by *in situ* nucleic acid hybridization or

immunohistochemistry, respectively. Gene amplification techniques have been successfully applied for the detection of JC viral genome sequences in various tissue types, with remarkable results. For instance, the presence of JC viral DNA has been reported in urine from normal individuals, as well as from PML patients. Extensive PCR studies on peripheral blood mononucleocytes have demonstrated the presence of JCV DNA in more than 75% of PML patients, in a large number of HIV-1–seropositive patients, individuals with renal transplants, and cancer patients (Dorries *et al*, 1994; Tornatore *et al*, 1992). Seroepidemiologic evidence for worldwide distribution of JCV has been confirmed by molecular techniques demonstrating the presence of JCV DNA in the peripheral blood of a majority of healthy individuals. PCR analysis of CSF has shown high sensitivity for viral detection in 80% to 92% of biopsy-confirmed PML patients, some even with very low viral copy numbers (McGuire *et al*, 1995; Weber *et al*, 1994). The diagnostic value of the detection of JCV DNA in CSF and the correlation between the JCV burden in patient CSF samples and PML prognosis has been proposed (Garcia De Viedma *et al*, 2002).

Mechanisms of JCV pathogenesis

In addition to PML, JCV has been implicated in several cancers, including tumors of neural origin (Caldarelli-Stefano *et al*, 2000; Del Valle *et al*, 2001; Khalili *et al*, 1999). Hence, the focus on understanding pathways that lead to viral pathogenesis is important. Recent advances in polyomavirus research have emphasized the involvement of receptors, DNA-binding proteins, as well as variation in the viral regulatory region.

Initial infection

Based on seroepidemiological evidence, introduction of JCV into humans appears to follow a common route of infection. Initially, respiratory inhalation was considered the primary and most likely route of infection, but reports of detection of JCV in lung tissue is uncommon. However, the discovery of the presence of JCV DNA in human tonsillar stromal cells and tonsillar lymphocytes in 1998 has reopened the hypothesis of respiratory tract involvement in JCV infection (Monaco *et al*, 1998). Based on information available from the literature and our present understanding of the virus, we have illustrated different steps and possible sites involved in JCV dissemination throughout the human host in Figure 1. Recent studies have also demonstrated the presence of JCV in epithelial cells of the human colon (Ricciardiello *et al*, 2000). The results provide evidence for a potential gastrointestinal intake, although the virus has previously been shown to be unstable under the conditions of oral ingestion or gastrointestinal infection. However, recent evidence suggest that the virion particle may remain viable at low pH (Bofill-Mas *et al*, 2001). Initial infection of JCV in humans remains a topic of debate; however, the worldwide distribution of this virus calls for research efforts into better understanding of the route of viral infection.

Viral latency within the host

The presence of JCV in urine samples from healthy, as well as PML patients, suggests a possible site for viral latency and subsequent reactivation under immunocompromised conditions in the kidney (Figure 1). The archetype strain of JCV is the most commonly found strain in kidneys. Interestingly, the archetype regulatory region sequence lacks biological activity *in vitro* and is not as prevalently found in the brain tissue of PML patients as strains containing a prototype or other prototype-like regulatory region. This suggests the possibility of rearrangements and deletions during JC viral replication in the kidney or brain (Daniel *et al*, 1996). Interestingly, there appears to be a correlation between the presence of archetype JCV isolates from plasma and CSF with PML survivability. Comparatively, patients having strains with tandem repeat regulatory region sequence structure are less likely to survive PML (Pfister *et al*, 2001). The inability of the archetype strain to replicate effectively in glial cell cultures has been attributed to the failure of the early viral promoter to produce sufficient mRNA to carry out T protein–mediated viral DNA replication (Daniel *et al*, 1996). JCV has also been reported in the bone marrow and spleen of PML patients, suggesting that lymphoid tissues may be a potential site of viral latency (Dorries *et al*, 1994; Houff *et al*, 1988; Schneider and Dorries, 1993). Further evidence comes from the demonstration of the JCV susceptibility of the CD34+ cell lines KG-1 and KG-1a primary CD34+ hematopoietic progenitor cells, primary B lymphocytes, and stromal tonsillar cells (Monaco *et al*, 1998).

A clear understanding of the site and mechanism of JCV reactivation remains an unanswered question, although the majority of evidence suggests immunosuppression to be a major underlying factor. Several investigators believe that reactivation of JCV may occur following conditions arising from immune suppression that may be conducive for rearrangements and changes in the regulatory region during viral DNA replication. Others, however, believe that loss of specific immune cells that control active infection, or viral replication, results in reactivation. Immunosuppression may hence lead to synthesis or release of biologically active tandem repeat prototype-like strains of JCV from the relatively inactive archetype strain. Evidence that not all JCV-seropositive, immunocompromised individuals develop PML necessitates studies to address the issue of viral latency and reactivation, independent of immune status. The case histories of PML patients studied over the last decades show that, in some patients, PML does not always follow its typical

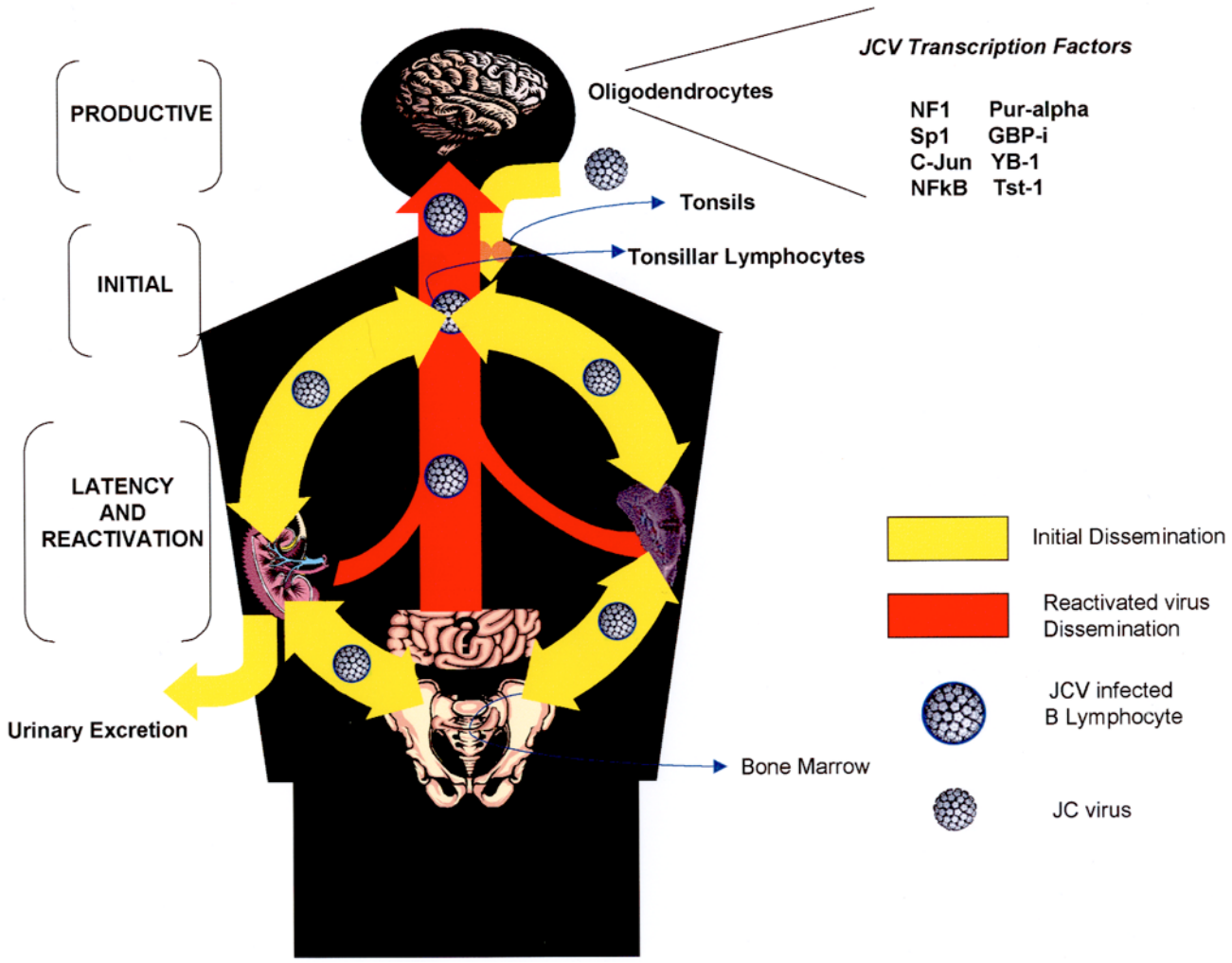

Figure 1 Initial JCV infection may occur through respiratory inhalation followed by tonsillar B lymphocyte dissemination to one or more organs and tissues, including other B lymphocytes, kidneys (one shown), spleen, and bone marrow, where JCV is believed to persist in a latent state. Data are still inconclusive whether colon epithelial cells play a role in the JCV infectious cycle. Following immunosuppression, JC virus productively replicates and could make its way into the brain via B-lymphocyte trafficking. Once in the brain, productive infection is triggered and/or maintained by a number of cellular transcription factors, leading to demyelination and PML. Mainly archetype strains have been found in the urine of healthy and infected patients. Other organs mentioned in the text are not shown for simplicity.

features of being progressive, multifocal, and restricted to white matter. Currently, clinical and basic science investigators in the field of PML are initiating a consensus for redefining the terminology and definition of PML (Cinque *et al*, 2003; *Journal of NeuroVirology* 9(s1), report of a conference).

Dissemination of JCV to brain

Absence of animal models for JCV-induced demyelination leading to PML has hampered our understanding of the trafficking of JCV to the brain and the initiation and development of CNS infection. Unsuccessful attempts to develop relevant transgenic mice, or produce acute infection in nonhuman primates, have made the mechanism(s) of JCV entry into the human brain more elusive. The presence of JCV in different organs such as kidney, brain, heart, spleen, bone marrow, lung, liver, and colon (Major *et al*, 1992; Weber and Major, 1997b), as well as positive *in situ* DNA hybridization for JCV in cells surrounding blood vessels in brain tissue, suggest a hematogenous route for progression of infection within the host (Houff *et al*, 1988). JCV has been shown to be present in prostate tissue as well (Zambrano *et al,* 2002). Efforts have been directed toward studying different lymphocyte populations and their involvement in trafficking virus to the brain. For example, the presence of JCV infected B lymphocytes in PML brain lesions (Major *et al*, 1990) could be partially explained by the enriched blood supply circulating these cells through the brain. JCV has been shown to bind to several different cell types, both susceptible and nonsusceptible. Virus internalization occurs quite rapidly

via clathrin-dependent receptor-mediated endocytosis (Pho *et al*, 2000). However, highly susceptible cells, like glial cells, positively regulate and promote JCV transcription and DNA replication due to the presence of specific NF-1 DNA-binding proteins. NF-1 class X, in conjunction with other transcription factors, regulates JCV activity in glial cells, resulting in a productive infection, that spreads virus to adjacent cells. Infected oligodendrocytes undergo lytic infection, resulting in CNS demyelination. The loss of the myelin sheath surrounding neuronal axons impairs signal transmission, causing compromised neurological function and appearance of clinical symptoms consistent with affected brain regions. The condition worsens as demyelinated lesions grow. Prognosis of such PML patients is usually poor, succumbing to the disease within months of clinical diagnosis, with some exceptions (Major *et al*, 1992; Kepes *et al*, 1975).

Current therapies for PML

Because AIDS currently accounts for most of PML cases, it is not surprising that antiretroviral therapies have been extensively used to treat AIDS-associated PML. Studies have shown prolonged survival and improved or stabilized neurological conditions in AIDS-associated PML patients undergoing highly active antiretroviral therapy (HAART) (Clifford *et al*, 1999; De Luca *et al*, 2000b). Cidofovir, a nucleotide analog used to treat cytomegalovirus (CMV) retinitis, proved to have considerable *in vitro* activity against polyomaviruses (Andrei *et al*, 1997) and has therefore been administered in combination with HAART to treat PML. Although some case studies did not find improvement following cidofovir treatment (Marra *et al*, 2002; Houston *et al*, 2001), anecdotal reports and small studies have shown prolonged survival and improved radiological and neurological conditions in AIDS-associated PML (Portilla *et al*, 2000; De Luca *et al*, 1999). However, identifying cidofovir as the agent responsible for the clinical effects is difficult in most of these studies. Some studies have reported cidofovir to be effective only after nonresponsive HAART treatment (De Luca *et al*, 1999; Razonable *et al*, 2001). This temporal relation between the start of cidofovir treatment and clinical improvement fails to eliminate the long-term effects of HAART. Also, cidofovir has proven ineffective when used as the sole treatment (Houston *et al*, 2001). Although undetectable levels of JCV DNA after therapy is associated with longer survival (De Luca *et al*, 2001; Giudici *et al*, 2000; Miralles *et al*, 1998), cidofovir's ability to decrease JCV DNA in CSF more rapidly is inconclusive (Gasnault *et al*, 2001; De Luca *et al*, 2000a). Also, cidofovir did not show any effect in JCV multiplication as well as replication *in vitro* (Hou and Major, 1998). Despite some promising results, a recent controlled pilot study showed no benefits following cidofovir treatment in HIV-associated PML patients, even in conjunction with potent antiretroviral therapy (Marra *et al*, 2002). These results suggest cidofovir, even as a HAART adjunct, is not as effective as previously thought.

Although the outcome of some clinical studies seem promising, they fall short of presenting the clinician and patient with a reliable therapy for PML. Patients with high JC viral load and/or low CD4+ cell counts do not respond to HAART treatment or HAART-cidofovir combinations (De Luca *et al*, 2001; Taoufik *et al*, 2000). Moreover, prolonged survival without neurological improvement after potent combined antiretroviral treatment including cidofovir has been reported (Gasnault *et al*, 1999). The current belief is that immune reconstitution is a requisite for clinical improvement. However, some patients develop PML shortly after HAART treatment. Miralles *et al* (2001) reported clinical and radiological deterioration following HAART, accompanied by reductions in HIV viral load and increased CD4+ cell counts in 3 out of 28 patients, as well as inflammatory changes in half of the PML patients treated with HAART. These findings raise the possibility that rapid immune reconstitution might worsen the course of PML in some patients. However, it is unclear whether initial deterioration precedes improvement as has been previously reported (Miralles *et al*, 1998).

Additional therapies that have been considered include topoisomerase inhibitors, alpha-interferon, or heparin sulfate. But in general, no clear benefit from these treatments has been shown (Mamidi *et al*, 2002). A pre-HAART open study suggested some benefit from alpha-interferon therapy (Huang *et al*, 1998), but a more recent study suggested no additional benefit when administered in combination with HAART (Geschwind *et al*, 2001). Recently, nontoxic doses of chlorpromazine, an antipsychotic, were shown to be effective at inhibiting JCV multiplication and dissemination in an *in vitro* model (Atwood, 2001), but further studies are warranted before it can be considered for use in PML patients. Another area of research that shows some promise is antisense oligonucleotides. These have been used to hinder viral DNA replication and transcription (Ma and Doan, 1994) and techniques for widespread and targeted delivery into the brain are being explored (Levy *et al*, 1997). Cytosine arabinoside or Ara-c inhibits JCV replication and multiplication *in vitro* (Hou and Major, 1998), but a controlled clinical study failed to show any benefits (Hall *et al*, 1998). As the intrathecal drug delivery was inefficient in targeting demyelinating areas, a new clinical trial is being conducted using convection enhanced delivery (Levy *et al*, 1997).

It is difficult to compare many of the results reported because of the different parameters used for both diagnosis and, subsequently, as measures of improvement. Cinque *et al* (2003) proposed a classification scheme for PML in order to develop consistency among researchers both for diagnosis and

Table 1 Current therapies and their clinical outcomes for PML patients

Therapy	*CD4+ cell count*	*HIV load (plasma)*	*JCV load (CSF)*	*Summary of results*	*References*
HAART	Increase	Decrease	NR	Neurological deterioration despite clinical and virologic response to HAART	Tantisiriwat *et al*, 1999
	Increase	Decrease	NR	Clinical and radiological deterioration attributable to inflammatory reactions	Miralles *et al*, 2001
	NR	Decrease	NR	Longer survival was accompanies by HIV RNA reduction	Clifford *et al*, 1999
	Increase	Decrease	Undetectable in 6 of 7 patients	Increased survival and improved or stabilized clinical status and radiological features. Initial deterioration in some patients	Miralles *et al*, 1998
	Increase	NR	Undetectable in most survivors	Increased survival on patients with low JCV loads at diagnosis and higher CD4+ counts	Taoufik *et al*, 2000
	Increase	Decrease[a]	Undetectable	JCV VP1 antibody production; MRI worsening after 3 months. Clinical and radiological improvement or stabilization after 1 year. Longer survival associated with JCV clearance	Giudici *et al*, 2000
	Increase	Undetectable[b]	Undetectable (57%)	26% showed neurological improvement or stability after 2 months; longer survival associated with CSF JCV clearance and higher CD4+ cells at baseline. CSF and plasma HIV not associated with survival	De Luca *et al*, 2000b
CART-P	Increase	Decrease or undetectable	NR	Longer survival without neurological improvement (EDSS)	Gasnault *et al*, 1999
HAART–μ interferon	NR	NR	NR	HAART but not alpha-interferon increased survival. Higher CD4+ cells associated with decreased risk of death	Geschwind *et al*, 2001
Cidofovir-HAART	Increase	Decrease	NR	No neurological improvement on week 8. Two subjects with 25% improvement had low HIV RNA levels	Marra *et al*, 2002
	Increase	Undetectable (70%)	Undetectable (33%)	No neurological benefit (EDSS); longer survival associated with cidofovir	Gasnault *et al*, 2001
Cidofovir	NR	NR	NR	Clinical and radiological progression[c]	Houston *et al*, 2001
Ara-c (i.v.)	NR	NR	NR	Some radiological improvement; 36% chance for stabilization; bone marrow toxicity[c]	Aksamit, 2001
ART with Ara-c (i.v., i.t.)	NR	NR	NR	No benefit; inefficient intrathecal drug delivery into demyelinated areas	Hall *et al*, 1998

ART: antiretroviral therapy; CART-P: combined antiretroviral therapy with protease inhibitor; Ara-c: cytosine arabinoside; NR: not reported; i.t: intrathecal; i.v: intravenous. [a]CSF HIV RNA decreased at same time JCV was cleared from CSF; [b]76% of patients in plasma, 35% CSF; [c]Non-HIV associated PML.

therapy outcome. Such a classification is important especially because a growing number of PML cases deviate from the more typical progressive and lethal types, common in earlier years (Cinque *et al*, 2003). Table 1 summarizes some of the most recent results.

Of the treatments discussed, HAART is the most used treatment for AIDS-associated PML to date. HAART is somewhat effective in 50% of patients, but low JC viral loads, early treatment administration, and high CD4+ cell counts are usually prerequisites for positive prognosis. Although some might argue that the benefits of cidofovir as an effective adjunct to HAART are inconclusive, the recent study conducted by Marra *et al* (2002) shows that it offers no additional benefit, leaving the patient with only one effective treatment, HAART. The appearance of PML during HAART in some cases calls for further studies on immunoregulation during the progression and remission of PML in order to develop new treatments. The need of an effective therapy for this usually fatal disease continues and is further highlighted by the slow advances in treatment of PML when compared to other AIDS-associated disorders during the HAART era (Antinori *et al*, 2001). It is hoped that improved understanding of the underlying mechanisms of JC infection in humans will drive the emergence of new therapeutics and strategies for ameliorating the sequelae of neuroinfection.

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