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Challenges for clinical trials to treat progressive multifocal leukoencephalopathy

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Progressive multifocal leukoencephalopathy is a lethal complication of immunodeficiency for which no direct therapy has been achieved. The issues that have made this disease especially difficult to address are discussed, and an outline for development of future interventions is provided. A controlled trial of therapy utilizing an international group of trial groups is proposed. *Journal of NeuroVirology* (2003) **9(suppl. 1),** 68–72.

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Progressive multifocal leukoencephalopathy (PML) was a rare opportunistic infection seen primarily in association with hematologic malignancies until the human immunodeficiency virus (HIV) epidemic began in the early 1980s. There was a large increase of immunosuppressed individuals resulting from the acquired immunodeficiency syndrome (AIDS) who commonly developed PML, and experienced a rapidly fatal neurological deterioration. This situation provides the impetus and the opportunity to develop treatments for PML. Sadly, two decades into the era of widespread HIV, specific therapy effective against the JC virus that causes PML has yet to be demonstrated in human trials. Issues that must be confronted in developing specific therapy include achieving accurate and timely diagnosis, devising a means of following subjects with varied presentations and courses, defining optimal populations to be studied, and applying appropriate therapy rapidly enough to make a difference while assuring ethical safeguards for providing best current care for such patients. It is hoped that ongoing efforts responding to these challenges will provide the basis for the next generation of treatment trials to address the ongoing challenge of PML.

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Issues in making the diagnosis of PML

Achieving a research diagnosis of PML very rapidly has been one of the most important challenges to treatment trials. Any clinical trial is dependent on isolating a group of patients genuinely suffering from the disease to be studied. Brain biopsy has been the "gold standard" for diagnosis of PML. Biopsy tissue demonstrates that the typical pathological changes associated with JC infection of the brain include demyelination associated with loss of oligodendrocytes, formation of bizarre large astrocytes, and characteristic inclusions. Further evaluation of the tissue by immunohistochemical means, or by polymerase chain reaction (PCR), has further allowed confirmation of this diagnosis. However, brain biopsies are invasive with recognized risk, such that there is reluctance both on the part of patients and families, and of the medical establishment, to perform these procedures. This typically delays or prevents use of biopsy in many centers.

Magnetic resonance (MR) technology has made localization of lesions and observing typical findings of demyelinated areas associated with subacute, progressive, focal neurological disease routine. Diagnosis cannot be made on MR scan, as there are numerous demyelinating diseases. However, the absence of identifiable demyelination on MR brain scans makes PML highly unlikely. Thus, part of establishing this diagnosis is observing lesions on MR scan typical of PML, with characteristics that include bright T2 lesions, which do not contrast with gadolinium, and have little or no mass effect in the brain.

PCR testing has further enhanced the diagnosis of PML with identification of JC DNA in cerebrospinal fluid (CSF). With appropriate sensitivity that is achieved in many diagnostic laboratories, the presence of JC DNA on PCR testing of CSF is highly associated with PML. In the author's experience, the combination of a typical clinical course, including subacute progressive focal neurological disease in an immunosuppressed host, appropriate MR lesions without mass effect or significant contrast enhancement, and positive JC DNA in the spinal fluid, has provided a highly specific diagnostic panel, allowing the diagnosis of more than 80% of PML cases. A remaining smaller percentage still require tissue confirmation when the CSF is negative. Sometimes repeat testing of CSF after short intervals may still avert the requirement for biopsy.

There is developing concern in the modern era of the HIV epidemic that there may be an increasing group of patients with undiagnosed leukoencephalopathy. At present, it is unclear what the appropriate diagnosis is in these cases. In some, the pathology has suggested that there is progressive HIV-associated neurological disease (Langford *et al*, 2002). However, some investigators have been concerned that this could be an alternative presentation for PML modified by the presence of highly active antiretroviral therapy (HAART) (Antinori *et al*, 2001). Enhancement of sensitivity for JC DNA in samples of CSF may also decrease the specificity of the association with active PML and the presence of virus in the CSF. This could force development of quantitative cut-off of JC viral load to suspect PML as the diagnosis for an observed lesion.

At present, it still appears that an adequate sample of subjects may be rapidly identified with sufficient certainty for inclusion in clinical trials by requiring evidence of progressive neurological deterioration, associated with appropriate MR lesion(s), and requiring PCR identification of JC in CSF by assay that is demonstrably negative in a general population. A proposed diagnostic approach would accept biopsy proven **definite** cases of PML and cases where the clinical, MR, and CSF PCR pictures make a **probable PML** diagnosis. Cases in which CSF PCR is negative remain **possible PML** cases but should not be included in studies or series unless repeat studies allow fulfillment of the criteria for probable or definite PML. Undiagnosed leukoencephalopathies, although an issue of substantial interest and merit, should not be included in studies of PML unless better evidence for their relation to JC virus encephalopathy can be produced.

Varied presentation and course

PML presents with lesions that are scattered in a variety of white matter regions of the brain, and consequently cause a wide variety of symptoms. Small lesions in exquisitely sensitive regions may cause severe and rapidly progressive clinical pictures, whereas larger lesions in neurologically silent regions may have remarkably little clinical impact. Comparison of clinical courses thus is only a rough guide to the biology of the infectious involvement of brain tissue. Trials must take this into account. Clearly the clinical outcome of preventing further progression, and optimally demonstrating regression of disability, are the most relevant outcomes for treatment, but surrogate markers that can demonstrate arrest of lesion progression on MR, or declining JC DNA in the CSF, provide realistic surrogate markers of clinical disease that are likely relevant. Summary neurological scores constructed from cranial nerve and motor and reflex examinations have been used as a primary outcome for the recent PML trial of cidofovir (Marra *et al*, 2002). This scoring allows identification of disease course in typical areas across presenting localizations.

The variable course of PML also is a challenge to efficient study. Things were easier in the pre-HAART era because a large majority of subjects died within months of the diagnosis, and death was an appropriate end point applicable to most subjects, with the possible exception of a recognized small set of slowly progressing or nonprogressing subjects consisting of about 10% of the population (Berger *et al*, 1998). However, in the HAART era, survival may be prolonged in half or more of subjects due to immune reconstitution. This functional immune reconstitution probably happens at a variable rate, and the separation of the impact of HAART from the impact of an intervention for JC virus becomes very difficult to analyze.

Ideal trial design would require controlled testing of either the JC therapy or HAART alone, a design that might be ethical if neither were known to have impact on this disease at the onset. However, numerous studies have demonstrated that use of HAART has indeed significantly improved treatment for PML (Cinque *et al*, 1998; Clifford *et al*, 1999; Dworkin *et al*, 1999; Giudici *et al*, 2000; Inui *et al*, 1999; Tassie *et al*, 1999). It is assumed that the impact of HAART on PML is the result of immune reconstitution with secondary control of the JC infection. Because PML is lethal, it is ethically necessary to give optimal HAART to all subjects identified with this condition. Coadministration of a therapy to be tested for PML seems required, because much of the progression of disability occurs in the interval between diagnosis and effective immune reconstitution, an interval probably extending at least 3 to 6 months after initiation of HAART. Death intervenes when the early progression is so severe that it is life threatening. A control arm with optimal HIV therapy compared to optimal HIV therapy plus a PML intervention would appear the necessary design for future studies to clearly answer questions of the value of JC-specific interventions.

Patient population for evaluation

Optimal small clinical trials often seek to homogenize the population to be studied as much as is practical to make outcomes clearer. In this light, there would be a number of theoretical options for PML patient studies. One might be to avoid HIV-associated PML entirely and study this disease as encountered in other immune deficient states (Razonable *et al*, 2001). Cases continue to be identified, and often fewer options for intervention in the immune status of these patients are available, simplifying analysis of therapeutic response for the PML. However, there are far fewer cases in this category, and the underlying conditions add a substantial degree of intrinsic variability to this population. These combined features prevented controlled treatment trials in the era before HAART, and remain potent obstacles even now.

Among AIDS patients, one relatively straightforward approach would be to study subjects who present with PML as their AIDS-defining illness, and are naïve to HIV therapy. This too restricts the number of subjects available; but because PML may occur at somewhat higher CD4 counts than many serious opportunistic processes, it is not a rare clinical presentation. It would be possible to learn a great deal about details of the specifics of immune deficiency leading to onset of PML, as well as the details of effective disease-specific immune reconstitution, if this population became the focus of a clinical trial. The problems with this approach include limited numbers of cases, and the fact that the HIV therapy alone is likely to be a very significant part of the clinical response, making it much more difficult to determine the impact of the JC-specific therapy.

The alternative to studying naïve HIV patients with PML is the most practical approach of enrolling all HIV-associated PML patients. A majority of these will have been on some antiviral therapy; in developed countries this will generally be combination HAART therapy. Some cases of PML develop even in the face of HAART therapy (Tantisiriwat *et al*, 1999), but in general the treated patients developing PML have incomplete HIV virologic responses, or are very early in the course of HIV treatment, and thus probably have a changing baseline of immune competence. In this situation, combined attention to HIV therapy and to JC therapy will have varying proportions of impact on the infections depending on the degree of HIV viral resistance, the stage and changing directions of immune competency, and compliance issues in the patients that have often led to incomplete HIV response in the beginning. The combination of these many variables makes this a mixed population, where increased numbers of cases will need to be studied to overcome the significant inhomogeneity of the populations.

Successful clinical studies will optimally define and monitor not only the immediate status of clinical performance, MR lesions, and viral loads for HIV and JC virus, but also will seek to characterize the direction of change in each of these parameters during the course of the study. Given the clinically dynamic nature of PML, characterization at approximately 3-month intervals is probably appropriate for clinical trials of this disease. Change in clinical performance should be scored using batteries of cranial nerve and motor and reflex examination. Blinded reading of MR scans demonstrating increase, decrease, or unchanged MR lesions and quantitative HIV RNA and JC DNA PCR studies on CSF allow characterization of vital directions of change in essential parameters of the diseases in question. Parallel evaluation of cellular and immune response for JC virus and HIV would add further depth to the clinical observations and the impact of therapies introduced.

Selection of therapeutic intervention

Successful development of treatment trials will depend on selection of an optimal treatment strategy. Investigations for PML have been constrained by the lack of animal models for the disease, and the difficulty of *in vitro* models limiting preclinical evaluation of agents. In some cases, serendipitous observations have suggested promising interventions in other diseases. Numerous case reports are available suggesting several treatments for PML, but to date when these observations have been formally tested in trials such as trials of cytosine arabinoside (Hall *et al*, 1998) and cidofovir (Marra *et al*, 2002), results have been disappointing. This is not unexpected in a disease that has a variable course and outcome, and is dependent on changing host immune status. Discussions occurring at an international workshop suggest that advancing understanding of the biology of JC infection may provide promising interventions. Further, it is possible that previously considered therapies can be made more effective by development of more appropriate drug delivery systems. Thus, the possibility that cytosine arabinoside failed in clinical trials because it did not reach affected tissue is certainly possible, and efforts to enhance delivery are currently under investigation in a clinical trial led by Dr. Robert Levy at Northwestern University (Levy *et al*, 2001).

Recognizing the correlation of higher CD4 counts with more frequent arrest of PML (Clifford *et al*, 1998), the possibility of enhancing the CD4 recovery through use of interleukin-2 (IL-2) or other cytokine therapy has been discussed as a plausible intervention. It is recognized that this strategy might not yield augmentation of disease-specific cellular immunity and that cytokines may have either beneficial or detrimental effects on infections. Nevertheless, as an augmentation of our current best mechanism

for arresting disease, this approach appears to warrant consideration. Although current evidence suggests that interferon-α has not had beneficial impact on the disease to date (Geschwind *et al*, 2001), it remains possible that interferons may be interventions worthy of addition evaluation. The possibility of gene therapy, perhaps using agnoprotein, or enhancing immune responses through adoptive transfer therapy, deserves consideration in future years. The very interesting observations of Dr. Walter Atwood and his coinvestigators (Atwood, 2001; Pho *et al*, 2000) noting that JC viral entry may be inhibited by chlorpromazine, a phenothiazine drug, gives an additional rational therapeutic intervention, with immediate toxicity more limited than that of the topoisomerase inhibitors that have also been considered as therapeutic agents.

More extensive *in vitro* modeling of this infection would be highly desirable, screening a broader spectrum of potential therapeutic targets. Limited *in vitro* investigations to date include cytosine arabinoside and several antiretroviral agents (Hou and Major, 1998), but consideration of a much greater number of potential therapies could open up new avenues for trials that might have a greater chance of success. Given the limited number of patients, and potential toxicity of interventions, efficient trial design is clearly crucial when approaching this disease.

When a reasonable level of agreement regarding the next intervention is reached, a multicenter trial with a control arm providing optimal HIV therapy alone seems likely to be the best design. To speed accrual to the study, it would be helpful to include multicenter groups on an international scale. With inclusion of several of the larger international study groups, it should be quite possible to accrue >75 subjects in a controlled trial annually. With clearly diagnosed, progressing patients, substantial activity should be detected with such a sample, as well as generating a toxicity profile. Progress can and should be achieved by ongoing efforts to design studies appropriate to this disease, while refining our understanding of the immunological breakdown resulting in the infection.

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