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Clinical Trial Report

Designs for clinical trials to test the efficacy of therapeutics in progressive multifocal leukoencephalopathy

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The design of a comparative treatment trial to evaluate the efficacy of therapeutics for progressive multifocal leukoencephalopathy (PML) is outlined. We propose a large simple randomized trial with patient survival as its primary endpoint, completed in a short period of time and involving a cohort with as few enrollment restrictions as possible. We use stratification as the counterweight to the lack of exclusion criteria and suggest that proper stratification will attenuate differences inherent in a heterogeneous subject cohort. Estimation of power, sample size, and study duration, implementation of interim analyses, toxicity management, and validation of secondary clinical measures are also addressed. *Journal of NeuroVirology* (2001) **7,** 369–374.

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Introduction

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease resulting from brain infection with the JC virus. It is rapidly fatal, affecting immunocompromised individuals and up to 5% of human immunodeficiency virus (HIV)-infected patients (Berger *et al*, 1998). No effective therapy has been identified for PML. In HIV infection, increases in survival after PML diagnosis have been noted with the advent of highly active anti-retroviral therapy (HAART) (Clifford *et al*, 1999; Tassie *et al*, 1999; De Luca *et al*, 2000a). Nevertheless, several cases with poor prognosis have been observed in HAART-treated patients (De Luca *et al*, 1998) even among patients otherwise responding to therapy (Weiner *et al*, 2000). PML-specific treatments are urgently needed.

Authoritative assessment of treatment efficacy can only be performed through properly designed and implemented clinical trials. Without such a study, information on the efficacy of a therapeutic agent will be, at best, incomplete and, at worst, contradictory. A salient example in the context of PML therapy is the question of the efficacy of cidofovir, where several contradictory reports have appeared in the literature (Brambilla *et al*, 1999; De Luca *et al*, 2000a; Houston *et al*, 2001; Marra *et al*, 2001).

We describe various aspects of the design of a PML treatment study. The article is organized as a protocol template of a comparative clinical trial. We advocate a simple, large, multicenter randomized study based on a simple outcome: patient survival on a background of optimized anti-retroviral therapy for all subjects (control group). This design can be extended to two or more comparisons with standard treatment taking the place of the no-treatment arm.

Diagnosis of PML

Definitive diagnosis of PML is made through pathological evaluation of stereotactic brain biopsy specimens (Silver *et al*, 1995). A less invasive and adequately accurate diagnosis criterion includes observation of typical clinical and radiological features of PML such as focal signs and symptoms, plus magnetic resonance imaging (MRI) revealing focal demyelination with JC virus DNA detected in the cerebrospinal fluid (CSF) by a polymerase chain

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reaction (PCR) assay. In the proposed trial, the latter criterion will be used. Biopsy will be required if any of the three criteria is negative or not present.

Primary endpoint
Given the rapidly fatal course of PML despite HAART, in approximately 50% of subjects that develop symptoms, survival is a viable endpoint for a PML treatment study. A number of factors have been shown to be predictive of patient outcome, such as JC virus DNA levels in the cerebrospinal fluid (CSF) (Taoufik *et al*, 1998, Koralnik *et al*, 1999, De Luca *et al*, 1999, Yiannoutsos *et al*, 1999), reductions of CD4 counts and HIV-RNA (Berger *et al*, 1998, Clifford *et al*, 1999), proton magnetic resonance spectroscopy (MRS) (Chang *et al*, 1997), Karnofsky score (De Luca *et al*, 2000b), evolution of the neurological (Berger *et al*, 1998, De Luca *et al*, 2000a) and neuroradiological picture (Berger *et al*, 1998), and changes in disability indices (Gasnault *et al*, 2001). However, no endpoint has been validated as a surrogate marker of PML morbidity and mortality. Patient survival is a simpler and unequivocal endpoint that does not require diagnostic technology not universally available (e.g., quantitative JCV-PCR or MRS) and it can be recorded even after subjects have discontinued study participation. Given current knowledge about the disease and its course, patient survival as the primary study endpoint is a viable and easily quantifiable outcome that will form the basis of our design.

Inclusion criteria
In a comparative PML treatment study, definition of the target population is complicated by several issues. Many patients succumb in the first 12 weeks after diagnosis. Inclusion of these rapidly progressing cases will not allow the efficacy of even beneficial treatments to be observed, resulting in an increased likelihood of a negative study, despite a large number of events (deaths) experienced by this subject population, which will result in a shorter study. With an observed 10% rate of spontaneous remission even in the absence of therapy (Berger and Mucke, 1988), including PML patients with chronic infection will result in a lower number of events and will reduce power, increasing the required sample size, and lengthening study duration. A viable compromise is to include *progressing* cases regardless of the duration of symptoms. Progression can be defined according to a combination of imaging, virological, and neurological markers. Whatever the definition, it must be quantifiable and defined in advance to maintain consistency across subjects and participating medical centers.

The issue of study duration is also important, so we will elaborate briefly here. HIV therapy has been the only factor that has had a beneficial impact on PML survival. Anti-retroviral therapy is a rapidly evolving science, so that in a lengthy study, patient prognosis may be dramatically different among subjects that were enrolled early, versus those that were enrolled later, especially when new anti-retroviral options become available for patients failing their current treatments. It is thus imperative that any trial of PML be completed as soon as possible, to ensure the greatest similarity among potentially confounding factors. Given the difficulties with patient enrollment, disease progression, and study duration—the best option is, in our opinion, conducting a large trial that places the minimum of restrictions on subject enrollment, and uses patient stratification to attenuate the effect of potential confounding or predictive factors of the clinical outcome.

Study design
Until a PML treatment standard has been identified, a comparative treatment trial will entail the experimental treatment along with a "control" arm likely involving anti-retroviral therapy alone. Previous studies have attempted to separate the effect of anti-retroviral and PML-specific therapies by requiring an anti-retroviral therapy lead-in period prior to study entry. We think that this would unnecessarily complicate the study. Furthermore, as PML is a rapidly fatal infection, subjects may elect to be treated by both HIV- and PML-specific therapies, effectively excluding themselves from a study requiring anti-retroviral optimization prior to the initiation of PML treatment. We propose eliminating the requirement of an anti-retroviral lead-in period and thus including all available subjects into the study as soon as they are identified. This may also increase the window of opportunity for the experimental agent to benefit the patient. On the other hand, monitoring anti-retroviral therapy changes during the study as well as HIV viral load in plasma and possibly the CSF will be critical in the effort to distinguish the effect of the anti-retroviral therapy from that of the PML-specific treatment, particularly in a design where no balancing through stratification or other means for anti-retroviral history or treatment at entry has been employed.

Stratification
Stratifying will ensure balancing factor levels across treatment arms. Appropriate subject stratification is a fundamental aspect of our design. It should be clear that we are not attempting to introduce additional subgroup analyses. Most likely, several factor combinations will be sparsely populated and comparisons between these subgroups will be woefully underpowered. Rather, we expect that stratification will eliminate confounding of significant factors with treatment effect (leaving the latter as the most likely cause of any observed differences in clinical outcome). We also expect that the complexity that stratification will confer on the study design will be more than compensated by the increase in sample size resulting from

open enrollment and the attenuation of the effect of differences among subject subgroups. Possible stratification factors may include the following:

- Baseline CSF JCV DNA. We can use detectable versus undetectable DNA levels, or, if quantitative PCR technology is available at all sites, a low JCV DNA threshold could be considered (De Luca *et al*, 1999; Koralnik *et al*, 1999; Yiannoutsos *et al*, 1999).
- CD4 count and plasma HIV viral load at entry. CD4 count above or below 100 cells/μL and detectable versus undetectable viral load—particularly if the subject is on stable anti-retroviral therapy—can be used to determine cases with poor versus better prognosis, although viral-load changes during the study might be more predictive of outcome (Clifford *et al*, 1999).
- Karnofsky score or a related functional scale like the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). A Karnofsky score of 60 (De Luca *et al*, 2000a) and an EDSS cutoff of 6 (Gasnault *et al*, 2001) could be considered.
- Duration of PML symptoms. Time since onset of PML symptoms of less than 8–12 weeks will likely distinguish active cases from slowly progressing ones. Inclusion of symptom duration as a stratification factor may obviate the necessity to stratify subjects according to duration of antiretroviral therapy, as most likely therapy will have been changed or initiated in recently identified cases.

Statistical considerations

We envision a randomized (ideally double-blind) 2-arm trial with patient survival (duration of study enrollment to death) as the primary endpoint. The methods described here are appropriate for multitreatment trials, as long as the duration of a disease-related event from study entry remains the primary endpoint.

Power, sample size, and study duration In a study of the time from entry to the occurrence of a clinical event, its duration, power, and sample depend on the number of observed events rather than the number of subjects. We project the rate of enrollment to be 3 or 4 uniformly enrolled subjects per month. This is 2 times higher than the accrual rate observed in the most recent AIDS Clinical Trials Group (ACTG) trial (protocol 363 [Marra *et al*, 2001]). Such an estimate is not unrealistic, however, if one considers broadening the inclusion criteria to include virtually all progressing PML patients and expanding the study to include additional centers (ACTG 363 was performed in 9 sites). The study will cease once the last enrolled subject has completed a 6-month (26-week) therapy regimen.[1]

[1]Power calculations are not affected substantially for different duration of therapy assumptions. If a 3-month duration of therapy is assumed, power decreases by 1–2%.

Table 1 Power and sample-size calculations

Median survival		*Total sample size*	*Expected number of events*	*Study duration (months)*	*Power %*
Control	*Exp. treatment*				
6	9.65	136	109	40	80
8	16.20	88	53	28	80

Median survival after PML in HAART-treated patients is about 2–4 months for rapidly evolving cases (Clifford *et al*, 1999; Marra *et al*, 2001) but is likely to be much longer in a study enrolling a wider group of subjects (Tassie *et al*, 1999; De Luca *et al*, 2000a). Here, we assume that the median survival of a broad PML subject cohort will be 6–8 months (calculations for 6 and 8 months are given in Table 1).

The minimum clinically significant improvement that one would reasonably expect from an efficacious treatment, given the persistently short survival expected for a large percentage of PML patients, is an increase in survival at a given point in time in subjects randomized to the experimental arm versus controls (Hall *et al*, 1998). We consider a 30% increase in the 6-month survival in the experimental treatment over the control arm. If the median survival is 6-months in the control group (i.e., 50% of the subjects survive at 6 months), a meaningful increase of the 6-month survival rate in the experimental treatment would be 65% (i.e., a 30% increase over 50%). This corresponds to a median survival of 9.7 months, assuming an exponential distribution of the event (death) times. Similarly, if the median survival is assumed to be 8 months in the control arm—corresponding to a 6-month survival rate of 59.5%— the 6-month survival rate in the experimental arm will need to be 77.3%, which corresponds to a 16.2-month survival rate (Table 1). Treatment comparisons were based on a 1-sided log-rank test carried out at the 5% alpha level.

Power estimates are presented in Table 1. With a 4-subject monthly enrollment, median survival of 6 or 8 months, and at least 80% power, the study will involve 136 and 88 subjects, enrolled over 34 and 22 months, respectively. In all cases, patient follow-up ceases 6 months after the enrollment of the last subject. The expected number of events is 109 and 53, respectively.

These estimates do not account for losses to follow-up, as in a study with survival as the primary endpoint, the outcome should be recorded on virtually all subjects, even those that discontinue participation, whether or not they are receiving medication. Nevertheless, a small sample-size increase can be considered. This should not affect power levels appreciably, as all projections are based on number of events rather than the number of subjects.

Early study termination In studies of fatal diseases such as PML, interim data must be reviewed frequently, and allowance must be given to study

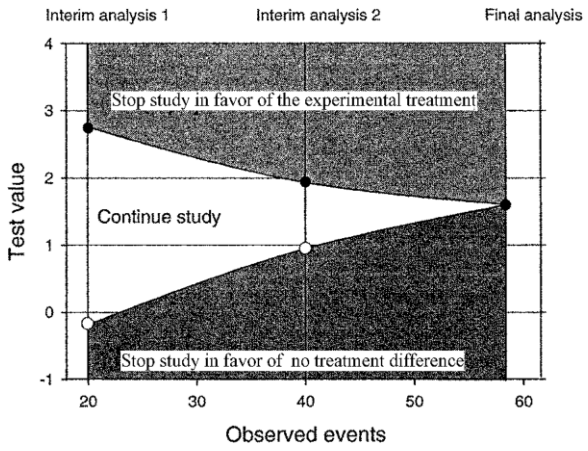

Figure 1 Boundaries for early trial termination.

interruption prior to completion. A study may be interrupted for efficacy considerations either when the benefit of one of the treatments is so unequivocal that to continue treating some of the subjects with the other therapy is unethical, or differences are so insignificant, that the probability that a statistically significant effect will ultimately be detected is small. Detailed calculations are beyond the scope of this short communication. As an illustration, we consider a monitoring plan that encompasses 2 interim analyses, 1 taking place after one third of the total events have been observed and the second after two thirds of the events have occurred. The case of an assumed 8-month median survival and enrollment rate of 4 patients per month is presented in Figure 1. At each analysis, 2 criteria for interruption are specified, based on a statistical test shown as 2 curves. If the value of the statistical test is higher than the upper boundary, the study is interrupted and the experimental treatment is declared superior to the standard treatment. If the value of the statistical test is below the lower boundary, then the study is interrupted, as the probability of ever detecting a treatment difference is very small. The study continues as long as the value of the statistical test falls within the funnel-shaped region. The study is interrupted in favor of the experimental treatment if a highly significant survival advantage is observed ($P = 0.002$ during the first interim analysis and 0.02 at the second), whereas the final analysis is carried out almost at the 5% significance level (Figure 1). On the other hand, nonsignificant P-values ($P = 0.571$ and 0.173) indicate futility with regard to ever detecting significant differences. Boundary curves and P-values were determined by the O'Brien–Fleming criterion (O'Brien and Fleming, 1979). Calculations were carried out by the EaSt 2000 software (Cytel corporation, Cambridge, MA).

Toxicity management We address briefly here incorporation of drug toxicity assessments on study design and monitoring. Drug-related adverse events are of crucial importance when assessing the potential benefit of a therapeutic agent. Toxicity considerations are largely context-specific, in terms of the particular agent and mode of administration. Thus, a study involving cidofovir would involve different toxicity guidelines than one involving IL-2, as would a study of intravenously administered Ara-C versus one involving intrathecal administration of the same drug. To generalize toxicity assessments across trials and events, a consistent grading system must be developed. In the ACTG, toxicity is rated in a 5-step scale, from mild (grade 1) to life threatening (grade 4) and death (grade 5). Existence of a toxicity grading system vastly simplifies the trial design, by allowing comparisons across different adverse events. For example, hemoglobin 6.5–6.9 g/dL is a grade-3 (severe) hematological toxicity according to the Division of AIDS (DAIDS) Table for Grading Severity of Adult Adverse Experiences. It can be straightforwardly compared with a concentration of triglycerides of 400–750 mg/dL, a grade-2 (moderate) toxicity. Comparisons between treatments with regard to toxicity rates are thus distilled to evaluations of proportions of subjects experiencing a specific toxicity grade (e.g., moderate or higher) or the duration of being toxicity-free among patients assigned to the treatments under comparison. Both sample size and power calculations can be produced, and toxicity assessments can be considered systematically during interim reviews of the study.

As an illustration, suppose that in a placebo-controlled study we want to ensure that the rate of grade 3 (severe) or higher toxicity in the experimental arm is no more than 20% higher compared to the arm comprised of anti-retroviral therapy alone. A sample of 88 subjects equally assigned to the 2 arms (e.g., assuming 6 months median survival and accrual rate of 4 patients per month) will produce power of 83% if the baseline (control-arm) toxicity rate is 10%.

Secondary analyses

Comparative trials, such as the one proposed here, offer an unparalleled opportunity to validate markers that are associated with the disease. With decreasing patient mortality resulting from successful therapies, future studies of PML therapies that focus on survival as their primary outcome will be too long and thus difficult to implement and interpret. On the other hand, with increasing survival, the significance of other aspects of the disease, such as neurological functioning, will continue to grow. Validation of several measures can be performed in subgroups of subjects enrolled on all participating centers, or within a subset of centers with the requisite expertise. Examples of potential diagnostic tests (and possible endpoints) are indices of humoral or cellular immunological response such as the JC virus–specific antibody index (Sindic *et al*, 1997; Weber *et al*, 1997; Giudici *et al*, 2000; Koralnik *et al*, 2001) and qualitative

or quantitative PCR (Taoufik *et al*, 1998; Garcia de Viedma *et al*, 1999; Koralnik *et al*, 1999; Yiannoutsos *et al*, 1999). In addition, measures of neurological status such as the EDSS commonly used in multiple sclerosis, or, alternatively a combination of a neurological evaluation with a disability index (e.g., the Barthel index (Colin *et al*, 1988, 105)) and imaging methods such as MRS (Chang *et al*, 1997) can be considered.

Discussion

Authoritative decisions on treatment efficacy can only be made through randomized comparative clinical trials. We provide an outline of such a study in the context of therapeutic agents of PML. We advocate a relatively large simple study, with patient survival as its primary endpoint. Subjects are to be randomized equally to two treatment arms. In the current state of PML treatment research, this probably means one experimental agent compared to maximized anti-retroviral therapy alone but this design can be extended straightforwardly to comparisons of two or more active treatments, or different doses of the same treatment. The resulting heterogeneity of the studied subjects will be balanced across treatments by stratifying on several important factors. Examples of these include baseline JCV CSF DNA levels, CD4 count, and plasma HIV RNA levels, functional status and duration of symptoms of PML, with possibly special attention paid to cases where PML is the AIDS-defining event, as these have better prognosis.

We recognize that some of our proposals may be controversial, such as our suggestion to enroll virtually "all identified cases of PML." We feel, however, that as long as progressing cases are sought, the simplicity in the design and speed of accrual more than compensate. A similar controversy may arise from our intention not to require an anti-retroviral lead-in period that may confound the response to a certain extent. However, we expect that stratification with regard to duration of PML symptoms will attenuate this effect, and the monitoring of plasma and possibly CSF HIV viral load throughout the study, the simplicity and convenience introduced in the design and the resulting sample-size increase will adequately compensate for possible confounding. In addition, there is recent evidence that anti-retroviral therapy initiation at the onset of PML symptoms may be detrimental for some patients (Miralles *et al*, 2001). Ultimately, we are convinced that a subject cohort selected with minimum restrictions will be more representative of the target patient population, that is, subjects with progressing PML-related symptoms.

A final note concerns ancillary causes of death in a trial based on survival. We considered a sample-size increase to account for non-PML causes of death but decided against recommending it because deciphering which competing reason primarily accounts for each event would be speculative if not impossible. We thus, in essence, equate PML with non-PML causes of death and expect that subtle differences across treatment arms will ultimately balance out. This is a reasonable expectation given stratification on HIV- and PML-specific factors that we expect will make the two treatment groups as similar as possible in terms of prognosis.

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