Evaluation of New Anti-Infective Drugs for the Treatment of Toxoplasma Encephalitis

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Toxoplasma gondii is a protozoan that exists in three forms, all of which are potentially infectious for humans. After acute infection, cysts persist in the central nervous system and extra-neural tissue. Human hosts with compromised immunity, particularly those with the acquired immunodeficiency syndrome, are vulnerable to reactivation and dissemination. The most common clinical expression of toxoplasma infection is encephalitis. The diagnosis is established by clinical presentation, computed tomography and/or magnetic resonance imaging, and detection of antibodies to T. gondii in serum of patients positive for human immunodeficiency virus. Brain biopsy may be performed. Protocols may be developed for the evaluation of new regimens for the treatment of acute encephalitis, the suppression of disease after treatment, or the prevention of reactivation before the onset of clinical disease. Assessment of clinical outcome is paramount.

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I. INTRODUCTION

This is one of a series of disease-specific guidelines that have been prepared to assist sponsors and investigators in the
development, conduct, and analysis of studies of new anti-infective drugs. These guidelines deal with the conduct of phase 1 through phase 4 clinical trials and are subsets of the General Guidelines for the Clinical Evaluation of Anti-Infective Drug Products, which should be consulted for the prerequisites to the conduct of studies in humans.

A. Background

Toxoplasma gondii is among the most prevalent causes of latent infection of the CNS throughout the world. T. gondii is a protozoan that exists in three forms: proliferative (tachyzoite), tissue cyst, and oocyst. Felines are the definitive hosts and constitute the reservoir for sporozoite production (oocysts), whereas only tachyzoites and cysts are found in incidental hosts (e.g., mammals). All forms are potentially infectious for humans [1].

After acute infections, cysts of T. gondii persist in the CNS as well as in extraneurial tissues. Although healthy human hosts have immunity sufficient for maintaining infection in a quiescent state, immunocompromised individuals are at risk for reactivation and dissemination [2, 3]. In particular, defective cellular immunity associated with depletion of helper/inducer (CD4) T-cell lymphocytes and macrophage dysfunction in patients with AIDS [4] predisposes to reactivation and dissemination of latent toxoplasma infection. Reactivation leads to clinically apparent disease (toxoplasmosis) that usually presents as life-threatening encephalitis [5, 6]. Thus, patients with AIDS who have been previously infected with Toxoplasma are at considerable risk for the development of CNS toxoplasmosis.

Toxoplasma encephalitis, which was observed early in the AIDS epidemic [7, 8], is recognized as a major cause of opportunistic infection of the CNS [9] and is the most frequent cause of focal intracerebral lesions in patients with AIDS [6, 9–11]. Patients who are infected with the human immunodeficiency virus (HIV) or who have AIDS and who also have antibodies to T. gondii should be considered at significant risk for the development of toxoplasmosis. Seroprevalence varies among geographic locales and within populations in the same locale. In major urban areas in the United States, the prevalence of antibodies to T. gondii among adults at high risk for HIV infection is ~11%–16% (J. S. Remington, et al., unpublished data).

B. Standards of Care

1. Current Standards

Toxoplasma encephalitis is the most common manifestation of the reactivation of T. gondii infection in patients with AIDS [14]. If this entity is left untreated, the mortality rate is virtually 100%. A combination of sulfadiazine and pyrimethamine is synergistic against T. gondii both in vitro and in vivo. Treatment of acute toxoplasma encephalitis is usually successful and is associated with an initial response rate of 65%–90%. However, up to 50% of patients with AIDS manifest drug-associated toxicity sufficiently severe to prompt discontinuation of such treatment [15]. The unique pathogenesis of toxoplasma encephalitis in HIV-infected patients requires that primary therapy be followed by a lifelong suppressive regimen, since the rate of relapse after withdrawal of therapy approaches 100% [16].

Multiple anecdotal reports have described the combination of sulfadiazine and pyrimethamine as effective in controlling but not eradicating the infection. This combination is now considered standard for suppressive therapy, although no single drug or combination of drugs is approved by the U.S. Food and Drug Administration (FDA) for this indication.

There is no general agreement on dosages. The most commonly recommended oral regimen is a loading dose of 75–200 mg of pyrimethamine followed by 25–100 mg of pyrimethamine per day plus 4–6 g of sulfadiazine or trimethoprim per day.

Dose-related bone marrow toxicity is common in response to pyrimethamine and presents as thrombocytopenia, granulocytopenia, and/or megaloblastic anemia. A search for therapeutic alternatives has culminated in one recently completed trial that compared clindamycin plus pyrimethamine with sulfadiazine plus pyrimethamine in 84 patients with presumptive toxoplasma encephalitis treated for 6 weeks [17]. Results were similar with regard to complete or partial clinical response and complete or partial radiological response. The efficacy of the two regimens was similar. Therefore, the combination of pyrimethamine plus clindamycin is an acceptable alternative to that of pyrimethamine plus sulfadiazine. Patients sustaining adverse reactions to one combination may be given the other.

2. Future Trends

Other drugs, including spiramycin, clarithromycin, azithromycin, and roxithromycin, are being developed for therapeutic use either alone or in combination with pyrimethamine. Although some of these drugs have shown activity in a murine model of toxoplasmosis, comparative clinical trials have not been completed [18–20]. In addition, modifiers of biological response, such as interferon gamma, have been shown to act synergistically with antimicrobial agents in experimental infections [19]; again, however, results of clinical trials are not yet available.

C. Scope of the Guideline

The clinical entity included in this guideline is toxoplasma encephalitis. The clinical entities not included are other
forms of toxoplasma infection. The microorganism included is T. gondii.

Definitive diagnosis requires the isolation of T. gondii from brain tissue or blood or the visualization of tachyzoites on histologic evaluation of the brain [16, 21, 22]. Presumptive diagnosis is based on a clinical presentation with the signs and symptoms of toxoplasma encephalitis, serum antibodies to T. gondii, and typical lesions on computed tomography (CT) or magnetic resonance imaging (MRI) of the brain [16, 23].

II. CLINICAL DEFINITIONS OF THE DISEASE

A. General Definitions

Toxoplasma encephalitis is characterized by several signs and symptoms, including headache, seizures, focal neurological signs (e.g., hemiparesis, altered visual acuity), disorientation, and fever. It occurs most commonly among patients with compromised immune function, especially those with AIDS, and among patients who have IgG antibodies to T. gondii in serum.

B. Minimal Diagnostic Criteria for Inclusion

Separate protocols must be developed for evaluating the suppression of disease after the treatment of acute encephalitis and prophylaxis for reactivation disease.

1. Clinical and General Laboratory Criteria

The following findings permit a presumptive diagnosis of toxoplasma encephalitis: IgG antibodies to T. gondii; signs and symptoms of CNS dysfunction; and abnormal findings on CT or MRI that are consistent with toxoplasma CNS infection (single or multiple lesions).

2. Microbiological Criteria

The following findings in a patient with neurological dysfunction and serum IgG antibodies to T. gondii permit a definitive diagnosis of toxoplasma encephalitis: isolation of T. gondii from brain tissue or blood with use of mouse inoculation or visualization of tachyzoites on histologic evaluation of brain tissue.

III. INFORMATION NEEDED BEFORE CONDUCTING CLINICAL TRIALS

A. In Vitro Studies

In vitro tests currently are not sufficiently developed for use in screening.

B. In Vivo Studies

Before the initiation of clinical trials, the activity of the agent or agents to be tested against T. gondii should be confirmed in a systemic model of murine toxoplasmosis with use of drug dosages likely to produce serum concentrations relevant to the situation encountered in humans. The end points should include both survival and resolution of typical toxoplasmic lesions in the brain, as determined by histopathologic examination of cerebral tissue [24].

IV. QUALIFICATIONS OF INVESTIGATORS AND INSTITUTIONS

A. Investigators

See General Guidelines, section VII.

B. Institutions

Institutions should be capable of mouse inoculation studies, determinations of serum antibody to T. gondii, CT and/or MRI, and histologic evaluation of brain biopsy or whole-brain specimens. A neurosurgeon skilled at brain biopsies should be on staff or available to the institution. Alternatively, a reference laboratory may process samples from participating centers. This laboratory should be certified by recognized authorities and should employ personnel skilled in the procedures to be undertaken.

V. DESIGN AND IMPLEMENTATION OF PHASE 1, 2, AND 3 CLINICAL TRIALS

A. Phase 1

See General Guidelines, section III.A.1. Dosages and toxicity should first be studied in a small number of HIV-positive patients who have latent toxoplasmosis (positive results in IgG tests but no evidence of clinical CNS disease). The incremental dose-escalation method should be used.

B. Phase 2

See General Guidelines, section III.A.2. In phase 2 studies a small number of selected patients (at least 10–20) with toxoplasma encephalitis may be treated with the study drug according to the dosage schedule determined to be promising in phase 1 trials. It is recommended that phase 2 studies be performed at centers where clinically suspected toxoplasma encephalitis is confirmed by brain biopsy. Activity of the study drug can be assessed by the evaluation of clinical status and the results of imaging studies. If the clinical and radiologic responses are adequate, treatment should be continued for a predefined period (usually 6 weeks). Patients should then be given a standard maintenance regimen.
C. Phase 3

See General Guidelines, section III.A.3. A prospective, randomized trial in which the study regimen is compared with standard treatment (i.e., sulfadiazine in combination with pyrimethamine) is recommended. The goal of the study should be a degree of efficacy equal (within 15% limits) to that of standard treatment. A multicenter study is required. End points must be clearly defined and should include neurological improvement and reduction in mass lesions, as assessed by imaging criteria. A separate objective may be to determine whether the new regimen results in microbiological eradication. The study design should be reviewed with the FDA before initiation of the clinical trial.

VI. ELEMENTS TO BE CONSIDERED IN DESIGNING PHASE 2 AND 3 CLINICAL TRIALS

A. Presumptive Diagnosis

Empirical therapy for toxoplasma encephalitis has become an accepted standard of care in patients with AIDS who have a positive serological IgG test for toxoplasma antibodies, typical clinical features, and focal CNS lesions demonstrated by CT or MRI. More than 90% of patients respond clinically to treatment, with a detectable improvement in presenting symptoms within 10–14 days. When a patient does not respond, other causes of encephalitis (e.g., CNS lymphoma, tuberculoma, fungal or bacterial abscess) should be sought. Brain biopsy, the only procedure that permits a definitive diagnosis, should not be a requirement for participation in clinical trials. Findings of follow-up CT or MRI generally correlate with the clinical course, thus providing strong inferential support for the diagnosis of toxoplasma encephalitis. In patients with persistent focal neurological signs (e.g., hemiparesis), persistent focal lesions are usually found on imaging studies.

B. Early Death

Up to 15% of patients die during the first 10–14 days of therapy for acute toxoplasma encephalitis. Most of those who die present with advanced disease and coma. Therefore, comatose patients should be excluded from trials of new therapies until preliminary evidence of the efficacy of these therapies in less advanced disease has been obtained, since the diagnosis is usually presumptive and the appraisal of outcome is dependent on clinical response. Unless proven otherwise by biopsy, death could be due to advanced toxoplasma encephalitis, inappropriate treatment because of an incorrect initial diagnosis (e.g., lymphoma), or failure of the new therapy—possibilities that make the evaluation of efficacy very difficult.

C. Adjunctive Therapy

Use of adjunctive therapy, such as that with corticosteroids for cerebral edema, may alter the therapeutic efficacy of the study drug. If the trial is conducted as an open study, patients should be stratified on the basis of the use of such therapy. If the trial is comparative and employs an active control, either all or no patients should receive adjunctive therapy.

D. End Points for Evaluating New Therapies

The relative response to therapy should be determined by careful measurement of the rate of return of neurological function, serial recording of results of tests of cognitive and motor function, and resolution of lesions as determined by CT and/or MRI. Scans should be read in a blinded fashion for reducing bias. Since the rate of response to conventional therapy is rapid (often with complete resolution in 7–10 days), significant differences in therapies may become apparent after the enrollment of only a few patients. Interim analysis of early data by an independent data review board is recommended. Since toxoplasma encephalitis in patients with AIDS is currently an incurable disease, the end points classically used in evaluating experimental therapies for infectious diseases (e.g., clinical cure or microbiological eradication of the infecting organism) are not applicable.

E. Stratification

Comparative clinical trials will require careful randomization and stratification of patients by the Glasgow Coma Score. Other neurological abnormalities and grading of CNS lesions by imaging techniques may be evaluated by post hoc stratification.

F. Need for Drugs of Equal Efficacy but Lesser Toxicity

New drugs associated with convincing evidence of therapeutic efficacy equal to that of existing drug combinations but with greater safety would be extremely valuable.

G. Demographic Characteristics of the Study Population

In general, patients enrolled in clinical trials of treatment for toxoplasma encephalitis should be adults. However, because of the uniform fatality of the untreated disease, infants, children, and adolescents should be enrolled in clinical trials as soon as possible. These groups should be evaluated for safety and efficacy by post hoc stratification on the basis of age.
H. Inclusion and Exclusion Criteria

Inclusion criteria for a presumptive diagnosis are based on clinical presentation, CT and/or MRI, and positive serology; a definitive diagnosis is based on brain biopsy; and positive serology for HIV antibody should generally be documented. Patients who have negative serology for HIV antibody may be enrolled but should be stratified either before randomization or during post hoc statistical analysis.

Patients who are receiving concurrent therapy with a non-protocol drug that demonstrates in vivo activity against *T. gondii* should be excluded from clinical trials of therapy for toxoplasma encephalitis. Pregnant women may be included only if sufficient evidence assures the safety of the mother and the fetus. Lactating women may be enrolled only if they agree not to continue to breast-feed their infants. See General Guidelines, sections VI.B. and VI.C.

I. Selection of the Comparison Drug

It is considered unethical to use a placebo-controlled trial for the treatment of toxoplasma encephalitis. Instead, a concurrent active control should be used. Currently, pyrimethamine plus sulfadiazine is the accepted standard.

For the suppression of disease after therapy for acute encephalitis, a concurrent active-control regimen should be used. The choice of the control regimen should be based on tolerability as well as efficacy.

In primary prophylaxis for reactivation disease among vulnerable patients with IgG antibodies to *T. gondii*, a placebo-controlled design is preferred until a regimen has been proven safe and effective.

J. Administration of the Study Drug

Therapy for acute encephalitis is currently provided for 6 weeks. The duration of hospitalization should be minimized. Whenever possible, protocols should allow for outpatient parenteral therapy or for conversion from parenteral to oral therapy.

K. Modification During the Study

Because patients are commonly intolerant of currently available drugs for the treatment of toxoplasma encephalitis, a crossover study design is encouraged. Patients intolerant of one or more drugs but tolerant of a different regimen may be evaluable for safety and partially evaluable for efficacy by Kaplan-Meier estimates of the probability of survival or by other methods. A Kaplan-Meier analysis of the probability of freedom from serious toxicity should be performed.

L. Definitions of Response to Therapy

Since most study participants will receive chronic suppressive therapy, outcomes must be defined at the end of treatment of acute encephalitis.

1. Clinical Response

Clinical response can be categorized as follows: improvement (resolution of signs and symptoms, diminution or disappearance of CT and/or MRI abnormalities); improvement with major neurological residua; improvement with minor neurological residua; no change; progression of disease; and death. Evaluations should be conducted 5–7 weeks after the initiation of therapy. Criteria used in judging the severity of neurological residua must be defined in the protocol.

2. Microbiological Response

In the event that the patient dies, every effort should be made to perform a postmortem examination. The brain tissue should be evaluated by an isolation technique and by histopathology for verifying the accuracy of the original diagnosis (if presumptive) and for determining the microbiological or histopathologic efficacy of therapy. Follow-up serological studies are not of proven value. For patients whose conditions improve clinically and who receive chronic suppressive therapy, the microbiological response should be classified as "presumed persistence."

3. Final Evaluation

In patients with a presumptive or definitive diagnosis of toxoplasma encephalitis, clinical outcome is paramount. Since microbiological or histopathologic data are unlikely to be available for the majority of study participants, these data are merely supportive.

M. Methods of Assessing Safety

See General Guidelines, section XIV.

N. Methods of Presenting and Analyzing Data

See General Guidelines, section XVI and appendix.

O. Methods of Assuring Compliance or Ethical Conduct

See General Guidelines, sections IV and XI.E.

VII. INFORMED CONSENT

See General Guidelines, section IV.D.

VIII. SUMMARY OF THE GUIDELINE

A. Baseline Assessment

Baseline assessment should include the following steps: medical history and physical examination; evaluation by
means of the Glasgow Coma Score or an acceptable alternative; CT and/or MRI; assay for serum IgG antibodies to *T. gondii*; assay for serum antibodies to HIV (unless the patient's HIV status is previously established as positive); brain biopsy (optional); and routine laboratory tests for monitoring toxicity (complete and differential blood cell count, liver function tests, serum electrolyte determinations, renal function tests, and urinalysis).

### B. Assessment During Therapy

Assessment during therapy should include daily neurological examination, routine laboratory tests every 3–5 days, and repeated CT and/or MRI once during therapy.

### C. Assessment at Completion of Therapy and During Follow-Up

Physical examination, routine laboratory tests, and CT and/or MRI should be performed upon completion of therapy. Follow-up evaluation 17–24 days and 38–45 days after completion of therapy should include the same procedures.

### D. Overall Assessment

The outcomes of treatment may be categorized as clinical, microbiological, and final. Assessment of clinical outcome (neurological status and CT/MRI findings) is paramount. Microbiological outcome can be determined only in those instances in which the patient dies. Death is considered indicative of clinical treatment failure unless postmortem examination identifies the resolution of toxoplasma infection and establishes an alternative cause of death. Final outcome should be determined at the follow-up visit 38–45 days after therapy.

### References