Cryptococcal Infections in Non–HIV Infected Hosts: A Prospective International Study (MSG-11)

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Introduction

Cryptococcus is a basidiomycetous yeast that is acquired from the environment, mostly through inhalation at an early age. Human infections occur worldwide with disease typically involving the lungs and/or the central nervous system. Approximately 1 million cases of cryptococcosis occur annually among HIV-infected persons, with an estimated 650,000 people ultimately dying of the infection (Park, et al. 2009). The majority of infections are caused by Cryptococcus neoformans. Most cases of cryptococcosis are seen among persons with chronic conditions that cause suppression of cellular immunity, such as that which occurs with HIV infection. However, there are several other conditions associated with significant immune suppression, such as treatment for cancer, transplantation, and receipt of a growing list of immunosuppressive drugs and monoclonal antibodies for the treatment of a host of autoimmune disorders. As such, cryptococcosis is commonly regarded as an opportunistic infection (Pappas et al. 2001).

There are few available data which inform us of how the diagnosis, management, outcome, risk factors and epidemiology of cryptococcal infections in non-HIV infected persons differ from those with HIV infections, especially outside of the US. Also, the relative proportion of C neoformans and C gattii infections in persons from these geographic regions is not well understood. Moreover, the impact of species variation (C neoformans vs C gattii) on the clinical spectrum of disease and outcome is incompletely understood.

This project is a prospective, observational, multinational study involving diverse geographic sites with sufficient numbers of non-HIV cryptococcosis proven cases to justify participation. The central purpose of this study is to generate new information pertaining to critical unanswered questions involving the natural history of these infections in selected geographical sites. We will obtain specific data relating to the epidemiology, risk factors, presentation and
diagnosis, antifungal treatment, management and interventions, species distribution, and outcome among non-HIV infected persons with all forms of cryptococcosis. A unique feature of this study is the special emphasis on neurologic outcomes among those patients with CNS cryptococcosis. It is anticipated that these data will provide important insights into the long term morbidity associated with this disorder, with emphasis on the long term neurologic outcomes.

Objectives

1. To define the demographics, risk factors, clinical features, species variation, outcomes, and specifics of antifungal therapy and other treatment interventions among a cohort of prospectively identified non-HIV infected patients with cryptococcal infections from diverse geographic sites.

2. To compare these results according to geographic region, clinical risk factors, antifungal treatment, and cryptococcal species (C neoformans or C gattii).

3. To describe the neurologic complications of CNS cryptococcosis up to 12 months following diagnosis

4. To describe the proportion of Cryptococcus neoformans and Cryptococcus gattii infections observed in these distinct geographic regions

5. To define the antifungal susceptibility of the cryptococcal isolates from this study

Background

The prevalence of cryptococcal infection in people who do not have HIV is not known, but it likely varies according to geographic region. Recent studies have emphasized growing recognition of cryptococcal disease in people who do not have HIV. Evaluation of an administrative database documented 30,840 hospitalizations for cryptococcal meningitis in the U.S. between 1997 and 2007 (Pyrgos et al. 2013). Of these, 79.4% were in HIV-positive individuals, and 21.6% were in people without HIV infection. Rates of hospitalizations in HIV-positive and HIV-negative people clustered according to geography, with the highest rates of hospitalization in HIV-negative people occurring in the southeastern U.S., especially in Georgia,
S. Carolina, and Tennessee. Recent reports also suggest that Alabama is among the highest incidence areas in the United States (Thomas et al. 1998; Brizendine et al. 2013). Common underlying co-morbidities in HIV-negative people include chronic organ failure (e.g., kidney, liver), malignancies, transplantation, and other isolated deficits in immune function (e.g., idiopathic CD4 lymphopenia, hypogammaglobulinemia). In the study by Pyrgos and colleagues, the in-hospital mortality decreased in HIV-positive people, but not in HIV-negative people over the time of observation (Pyrgos et al. 2013). Although this study demonstrates a substantial burden of cryptococcal disease in both HIV-positive and HIV-negative people, use of the administrative database limits conclusions that could be made regarding clinical course, risks and long term outcomes.

Other observational studies have also emphasized the importance of cryptococcosis among non-HIV infected patients. Most experts recognize 3 major groups among the non-HIV infected patients with cryptococcosis: solid organ transplant recipients, patients with other forms of chronic immunosuppression, and those who are phenotypically ‘normal hosts’. In a recent retrospective review of over 300 cases of cryptococcosis at one institution, over 60% occurred in non-HIV infected patients (Brizendine 2013). Moreover, 90 day and 1 year mortality were higher among those patients who were in the non-HIV, non-transplant category. Among a subgroup of these patients who were phenotypically normal, their 1 year mortality was approximately 10%, the lowest among these groups (Brizendine 2013).

Among patients with no specific host risk factors for infection, some studies suggest that this infection may actually be a ‘sentinel event’ that signals development of a secondary immunologic deficiency that had not yet become clinically apparent, such as with development of auto-antibodies directed against central cytokines such as IFN-γ or GM-CSF (Rosen et al. 2013). Among others, the occurrence of cryptococcosis seems to be a solitary event unassociated with the development of other manifestations of immune dysfunction. Studies examining the genetic risk factors for cryptococcosis in non-HIV infected patients have yielded
some interesting results (Meletiados et al. 2007), but much more work is needed to better
define the immunologic defects associated with disease risk in otherwise normal individuals.

**Microbiology**

The genus Cryptococcus is comprised of basidiomycetes that exist as encapsulated yeasts. The
primary pathogen is C. neoformans, which was historically considered to contain multiple
serotypes (A, B, C, D), characterized by capsular antigens. More recently, the groups B and C
have been raised to a distinct species, designated C. gattii, with C. neoformans being groups A
(C. neoformans var. grubii) and D (C. neoformans var. neoformans). Further taxonomic changes
among the cryptococcal species complex are anticipated (Hagen et al. 2015).

**Global observations of C gattii**

One of the goals of this study is designed to define and compare clinical manifestations and
outcomes of C neoformans and C gattii infection. Experience to date has emphasized several
central themes: 1) Acquisition of disease in a greater proportion of apparently healthy hosts,
with possible observation of subclinical immunologic deficiencies; 2) High rates of severe and
disseminated disease; 3) Poor outcomes, including death and prolonged neurological
impairment; and 4) Focal pulmonary and CNS lesions are frequent and usually delayed in
diagnosis, with evaluation frequently positioned towards malignant disorders.

Historically, the largest published experience describing the epidemiology, immunology, clinical
features and treatment of C gattii infections have come from investigators in Australia (Chen et
al. 2014; Chen et al. 2013; Chen et al. 2009). Two important management questions have been
generated from recent experience in the Pacific Northwest (PNW) outbreak, concerning both
optimal antifungal therapy and the need for anti-inflammatory therapy to manage neurological
immune response syndromes. Development of a typical “immune responses syndrome” has
been described in a small case series from Canada (Refojo et al. 2009), although many appear
to have a simple sterile arachnoiditis. Also, there may be clinically significant differences in
antifungal susceptibility of Cryptococcus species, with more apparent variability in azole in vitro
susceptibilities amongst Cg isolates, especially those of the VGII lineage associated with the recent outbreak in the PNW. Specifically, minimal inhibitory concentrations to fluconazole can be high (≥32 μg/mL) in VGII (a-c) isolates that have been recovered from humans and animals (Khan et al. 2009; Chong et al. 2010; Hagen et al. 2010; Iqbal et al. 2010; Trilles et al. 2012). Whether this causes true microbial ‘resistance’ has not yet been clarified, although our anecdotal observation has been of frequent failure with Cg infection treated with fluconazole alone. These anecdotal experiences suggest that more aggressive (polyene) antifungal therapy for both pulmonary and CNS disease should be considered rather thanazole therapy.

**Immunologic observations among non-HIV infected people**

Underlying diseases that compromise T cell immunity present risks for invasive cryptococcosis caused by both Cn and Cg. Infection can manifest after receipt of biologic agents that suppress the immune system for autoimmune conditions, malignancies, and transplantation, as well as in the setting of severe pulmonary disease. However, some people who develop infection, especially that caused by Cg, have no apparent underlying immunosuppressive condition.

Some Cn patients have been found to have defects in CD4 numbers (idiopathic CD4 lymphopenia), others have been found to have more subtle defects, as with one patient who had a mild CD8 lymphopenia with a 90% reduction in terminal effector memory cells (TEMRA). Defects in this cell subset have been associated with acquisition of other intracellular pathogens such as tuberculosis after anti-TNFα therapy (Bruns et al. 2009). Infection caused by Cg in the CNS is frequently characterized by an over-exuberant CNS inflammatory response, and neurologic morbidity. This appears out of proportion compared to that observed with Cn infection, although the latter also causes an inflammatory response with immune reconstitution (IRIS). Mechanisms are potentially different, owing to differences in host immunity and differences in Cryptococcus species. While Cn has a capsular polysaccharide that is suppressive to inflammatory Th1 type T cells (Retini et al. 2001), little is known about the microbial differences that dictate IFN-γ mediated inflammatory responses to Cg.
Secondary development of anticytokine autoantibodies has been observed as a cause of immunodeficiency that leads to severe manifestations of infection with “opportunistic” organisms. For example, anti-IFNγ autoantibodies have been associated with infection caused by non-tuberculous mycobacteria, dimorphic fungi, and Salmonella (Browne et al. 2012), and anti-IL-17 or IL-22 autoantibodies were associated with mucocutaneous candidiasis (Kisand et al. 2010; Puel, Doffinger et al. 2010). More recently, infection has been associated with development of anti-GM-CSF autoantibodies, a recognized cause of pulmonary alveolar proteinosis (PAP). A recent study that screened plasma of HIV-negative patients with Cn and Cg infection identified neutralizing anti-GM-CSF autoantibodies in approximately 6% of historical cases, providing a possible mechanism of risk. Anti-GM-CSF autoantibodies, at least in the context of PAP, have broad immunological effects on monocytes and macrophages (Shibata et al. 2001), which appear to be important for host control of Cryptococcus.

These observations have clear therapeutic implications. Studies on monocytes and macrophages and humans with HIV have shown that GM-CSF therapy may augment the effects of triazole therapy and increase cryptococcal killing by monocytes (Tascini et al. 1999; Chiller et al. 2002). Characterization of immune correlates for disease in people without apparent immunodeficiency has the potential of enabling improvement in therapies that employ both specific and non-specific immune enhancing agents and agents that suppress unwanted inflammatory responses.

Treatment of Cryptococcosis

There have been no recent prospective randomized studies to determine the best treatment strategies for patients without HIV infection. The most recent of these trials was conducted almost 3 decades ago (Dismukes et al. 1987). Indeed, current recommendations for the treatment of non-HIV infected persons with cryptococcosis are based in part on these data, but also from data extrapolated from more recent comparative studies in HIV infected patients, anecdotal data, and retrospective series (Day et al 2013, Jarvis et al 2014, Loyse et al. 2012). Thus, treatment approaches to the non-HIV infected patient represent a blend of clinical trial
data, anecdotal experience, and extrapolations from data generated among HIV-infected patients with central nervous system cryptococcosis (Perfect 2010).

All treatment trials for cryptococcosis have been conducted among patients with CNS disease, and most recent studies have been performed in people who have HIV infection. A recent large randomized trial demonstrated that the combination of amphotericin B with 5-fluocytosine yielded the best treatment responses among patients with HIV-associated CNS cryptococcosis, and that aggressive CSF drainage may be needed to avert neurological morbidities (Day et al. 2013). The most recent treatment guidelines produced in 2010 by the Infectious Diseases Society of America (IDSA) state that CNS and disseminated disease caused by C. gattii should be treated the same as that caused by C. neoformans, however, offering the option of fluconazole for isolated pulmonary disease (Perfect, et al. 2010). These recommendations have not been conformed prospectively, and the results of multiple recent studies suggest that there are differences in antifungal susceptibility of Cryptococcus species, with more variability inazole MIC amongst Cg isolates, especially those of the VGII type. As mentioned above, fluconazole MICs can be very high (≥32 μg/mL) in VGIIa-c (Khan et al. 2009; Chong et al. 2010; Hagen et al. 2010; Iqbal et al. 2010; Trilles et al. 2012). Whether this causes true microbial ‘resistance’ has not yet been clarified, although is calls into question the strength of these recommendations.

Infection outcomes are clearly impacted by therapeutic measures to decrease the sequelae of CNS inflammation. While frequent drainage with serial lumbar puncture has become mainstay for treating people with Cn infection, little is known about how to manage inflammatory responses in people with Cg. Use of intraventricular shunts appears common in persons with Cg CNS infection, and the use of systemic corticosteroid therapy to treat CNS inflammation is reported anecdotally. A recently developed randomized trial comparing initial adjunctive corticosteroids for patients with HIV-associated CNS cryptococcosis in Asia and Africa should provide valuable insights into the role of this intervention in the acute management of patients (Day et al. 2014). More studies are needed to define optimal treatment approaches for disease caused by Cn and Cg in people without HIV as an underlying disease.
Study Methods

This cohort will consist of non-HIV infected patients with cryptococcal disease who have proven or probable disease due to *Cryptococcus neoformans* or *Cryptococcus gattii*. Patients will be identified prospectively and followed for 12 months at 3 month intervals in order to better understand the diagnosis, epidemiology, clinical presentation, treatment, response to therapy, and outcomes of infection due to these organisms. Detailed epidemiologic and clinical data will be obtained for each patient. In addition to collecting the specific isolate from each patient (when possible), investigators will be asked to provide immunologic studies that are routine for care of patients in each location. This may include, but is not limited to, CD4 and CD8 lymphocyte assays and quantitative immunoglobulins.

The present study is designed similar to the ongoing prospective natural history study in the US-(CINCH, MSG 09) with some important caveats:

1. All patients will be followed at baseline and every 3 months for 12 months (rather than 24 months). A study flowsheet is included in Appendix A.
2. We will collect cryptococcal isolates from each case when feasible as determined by local and country standards; available isolates will be shipped and stored at the CDC Mycology Branch.
3. Each investigator will be asked to obtain baseline immunologic studies on study participants according to local standards, and may include CD4/CD8 lymphocyte assays, quantitative serum immunoglobulins, and or other studies that are deemed appropriate by the site investigator.
4. As routine standard of care, patients should have a negative HIV test within 6 months prior to enrollment into this study.
5. Patient DNA will not be collected routinely as part of this study.

For this prospective trial, we will use the electronic CRF RedCap data system which has been modified specifically for this study. The enrollment goal is **150 patients** enrolled from 7 international sites over a 24 month timeframe.
**Participating sites and projected patient enrollment**

Each study site listed below has been queried and asked to provide conservative numbers of patients who might be enrolled into this trial of non-HIV infected patients with any form of cryptococcosis. The sites and principle investigators are listed first.

**Participating Sites**

1. University of Sydney, West Mead campus:  
   PIs - Sharon Chen, MD, PhD and Tania Sorrell, MD
2. Federal University of São Paulo-UNIFESP, Sao Paulo, Brazil:  
   PI - Arnaldo Colombo, MD, PhD
3. Institute Pasteur, Paris, France:  
   PI - Olivier Lortholary, MD, PhD
4. University of Insubria in Varese, Italy.  
   PI - Paolo Grossi, MD, PhD
5. Oxford University, Southeast Asia Research Center, Ho Chi Min City, Vietnam:  
   PI - Jeremy Day, MD, PhD
6. St. Paul’s Hospital and University of British Columbia, Vancouver, British Columbia:  
   PI - Peter Phillips, MD
7. National Taiwan University Hospital and College of Medicine, Taipei, Taiwan:  
   PI - Yee-Chun Chen, MD, PhD

This table provides site specific annual estimates for enrollment:

<table>
<thead>
<tr>
<th>Site</th>
<th>Patients (annually)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sydney, Australia</td>
<td>5-7</td>
</tr>
<tr>
<td>Sao Paolo, Brazil</td>
<td>20-25</td>
</tr>
<tr>
<td>France</td>
<td>15-20</td>
</tr>
<tr>
<td>Ho chi Minh City, Vietnam</td>
<td>20-40</td>
</tr>
<tr>
<td>Vancouver, British Columbia</td>
<td>5-7</td>
</tr>
<tr>
<td>Italy</td>
<td>5-7</td>
</tr>
<tr>
<td>Taiwan</td>
<td>12-20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>82-126</strong></td>
</tr>
</tbody>
</table>

Based on these estimates and assuming a 80% success rate in capturing these patients, it is reasonable to assume that we can identify and enroll 66-101 patients annually leading to the projected enrollment of **132-202** patients during the 2 year patient accrual phase. Our
enrollment goal is 150 patients. The accrual phase of the study will be completed upon enrollment of 150 patients with proven or probable cryptococcosis, or meeting the 24 month patient accrual deadline, whichever comes first. There is a potential increasing the enrollment target, but this will be based on the unanimous consent of the sites and the availability of adequate funding.

Patient informed consent
The requirement for patient informed consent will be determined by individual site IRBs. A consent template in the English language will be made available to each participating site from the UAB MSG Central Unit.

Data Management
Clinical data will be captured using the REDCap system designed for this study. Data will be captured at baseline (BL), 3, 6, 9, and 12 months. REDCap users at each site will have secured, password protected access to the database to enter data. Data will be stored on the UAB Department of Medicine REDCap secured server. Data will be reviewed by the UAB MSG Central Unit personnel and initial queries will be generated at that time. The Biostatistical Unit of the UAB School of Public Health will perform the data cleaning and analysis. Descriptive statistics will be utilized.

These data will be analyzed and presented as an independent body of work. Once analyzed, presented and submitted for publication, these data will be merged with data from MSG 09 (NIH sponsored CINCH) as a combined analysis for publication.

Administration
The study will be coordinated through the central office of the Mycoses Study Group - Central Unit at UAB in the Division of Infectious Diseases. The roles of the coordinating center will be to initiate discussions with each principal investigator, design and provide electronic access to the REDCap database, point-of-contact for each site conducting the study, maintain regulatory
documents, monitor enrollment and site activities, cover the costs of shipping isolates to the Centers for Disease Control and Prevention (CDC), etc. The Central Unit will also be responsible for trouble-shooting as the study progresses, and for performing data quality review on the REDCap electronic case report form (eCRF). Finally, the Central Unit will coordinate review for adjudication of cases (diagnosis and response).

**Study funding and timeline**

Gilead Pharmaceuticals has agreed to provide the financial support for this study. We have proposed the identification and enrollment of 150 patients with proven and probable cryptococcal disease. Patient accrual will occur for up to 24 months after study initiation at each site, and there will be up to 12 months follow-up for each enrolled patient. We anticipate having 7 active international sites once the study is fully underway.

We are seeking separate funding for our proposed Canadian site (UBC, Dr Phillips) through Astellas Canada. The site proposes to identify and enroll up to 14 patients. If successful, then this site will participate as proposed. If attempts to secure funding for the Canadian site though Astellas Canada are not successful, then Gilead must decide whether they can provide funding to the Canadian site to participate in this study.
References


## Appendix A

### Schedule of Procedures

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Baseline</th>
<th>3 mo</th>
<th>6 mo</th>
<th>9 mo</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV negative test results</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(as routine standard of care within 6 months prior to enrollment)</td>
<td></td>
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<tr>
<td>Informed Consent</td>
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<td></td>
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<tr>
<td>Fungal Isolate*</td>
<td>X*</td>
<td>X*</td>
<td>X*</td>
<td>X*</td>
<td>X*</td>
</tr>
<tr>
<td>Immunologic Studies*</td>
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<tr>
<td>Mental Assessments#</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Health Survey (SF-36)</td>
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<td></td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td>Case Report Form</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

* One isolate per fungal organism. If organism is the same throughout the study, only send one isolate.

* Routine standard of care studies that may include, but not limited to CD4 and CD8 lymphocyte assays and quantitative Immunoglobulins. Only enter results if studies performed as routine standard of care.

# Mental assessments include the following: Montreal Cognitive Assessment (MOCA), Glasgow Coma Score (GCS), Patient Disability Questionnaire.