



**A Randomized Double-blind Trial of Fluconazole vs. Voriconazole
for the Prevention of Invasive Fungal Infections in Allogeneic
Blood and Marrow Transplant Patients**

**BMT CTN PROTOCOL 0101
VERSION 7.0**

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PROTOCOL SYNOPSIS – BMT CTN PROTOCOL 0101**A Randomized Double-blind Trial of Fluconazole versus Voriconazole for the Prevention of Invasive Fungal Infections in Allogeneic Blood and Marrow Transplant Recipients**

- Co-Principal Investigators:** John R. Wingard, M.D., Thomas Walsh, M.D.
- Accrual Objective:** Allogeneic blood or marrow transplant recipients and cord blood recipients in children under the age of 12 will be targeted for accrual. Per study arm, approximately 300 recipients (a total of 600 recipients) will be accrued.
- Accrual Period:** The estimated accrual period is three years.
- Study Design:** The study is designed as a Phase III, randomized, double-blind, multicenter, prospective, comparative study of fluconazole versus voriconazole in the prevention of fungal infections in allogeneic transplant recipients. Recipients will be stratified by center and donor type (sibling vs. unrelated) and will be randomized to either the fluconazole or voriconazole arm in a 1:1 ratio.
- Primary Objective:** The primary objective is to compare the fungal-free survival rates between the two study arms through Day 180.
- Secondary Objectives:** The secondary objectives will be to compare the frequency of invasive fungal infection, time to invasive fungal infection, survival rate, duration of amphotericin B or caspofungin therapy for possible invasive fungal infection, time to neutrophil and platelet engraftment, time to and severity of acute and chronic GVHD, and to perform exploratory analyses of quantitative aspects of the galactomannan assay. The relative safety of the two antifungals will also be assessed through the collection of adverse events and routine laboratory monitoring.
- Eligibility Criteria:** Recipients must be diagnosed with leukemia or myelodysplastic syndrome (MDS). Lymphoma patients with chemosensitive disease and a related donor are eligible. Recipients must receive a myeloablative, 5/6 or 6/6 HLA-matched allogeneic blood or marrow transplant, be two years of age or older, have adequate physical function and give signed informed consent prior to enrollment.
- Treatment Description:** Recipients will begin the study drug on Day 0 (day infusion of stem cell product is initiated). The development of any fungal infection during prophylaxis will be classified according to revised EORTC/MSG definitions (see Tables 3.1.1a and 3.1.1b). Study drug will be continued until Day 100 post-transplant or until invasive infection occurs, or the recipient develops a Grade III or IV toxicity attributable to the study drug. For recipients of any type of graft receiving at least 1.0 mg/kg/day of prednisone (or equivalent steroid dose) on Day 90-100 or for recipients of T cell depleted grafts, receiving immunoprophylaxis post-transplant or having CD4+ counts < 200/ μ L on Day 90-100, study drug will be continued until Day 180.

Study Duration:

Recipients will be followed for a minimum of one year post-transplant. Fluconazole or voriconazole will be taken (depending on randomization arm) beginning at Day 0 of transplant for at least 100 days (or longer as specified above).

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CHAPTER 1

1. BACKGROUND AND RATIONALE

1.1. Study Background

Allogeneic blood and marrow transplant patients are highly susceptible to invasive fungal infection prior to engraftment due to neutropenia and mucosal injury. After engraftment, an impairment of cell mediated immunity from GVHD and the use of aggressive immunosuppressive therapies such as corticosteroids [1] leave patients vulnerable to invasive fungal infections. Recipients of alternate donor transplants are especially susceptible due to slow reconstitution of cell mediated immunity.

Fluconazole prophylaxis in prospective randomized trials [2, 3] of both autologous and allogeneic transplant recipients has been demonstrated to reduce invasive fungal infections due to yeasts prior to engraftment. A prolonged course of fluconazole given during the first 75 days (to cover the early post-engraftment period of risk) is highly effective in prevention of early and later yeast infections [4]. This has translated into a survival benefit. A recent analysis of long-term outcomes of these individuals demonstrated a continuing benefit beyond the course of prophylaxis with a further benefit in survival [5]. In another study of various factors associated with survival after matched unrelated donor transplants, fluconazole prophylaxis was an independent predictor for overall survival in a multivariate analyses [6]. Fluconazole prophylaxis has been found to not only be effective but safe with few substantive drug interactions and has been widely adopted by transplant clinicians.

The recently published guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients, a joint effort of the Centers for Disease Control, the Infectious Disease Society of America, and the American Society for Blood and Marrow Transplantation [7], noted that “No regimen has been reported to be clearly effective or superior in preventing aspergillosis, and therefore no recommendation can be made. Further studies are needed to determine the optimal strategy for aspergillosis prevention.” At a “State of the Science” Meeting on Hematopoietic Stem Cell Transplantation held April 1, 2000, jointly sponsored by the American Society for Blood and Marrow Transplantation and the National Institutes of Health, the Infectious Complications Working Group deemed the highest priority study would be testing of new antifungals and development of strategies to control invasive mould infections after BMT [8].

Voriconazole, a triazole with broad-spectrum antifungal activity [9-18 and reviewed in 19], is available in both oral and intravenous formulations. Although there are more drug interactions with voriconazole than fluconazole, it is well tolerated in clinical use [13-15, 18, 20, 21]. As empirical therapy in febrile neutropenic patients, voriconazole proved to be associated with fewer breakthrough infections than liposomal amphotericin B (AmBisome) with less toxicity in a prospective randomized comparison [20]. The availability of an oral formulation of voriconazole makes it appealing for use long-term and its availability as an intravenous formulation allows its use in patients with oral mucositis, nausea, vomiting or other

gastrointestinal toxicities frequently seen in BMT recipients which might impair compliance with an oral agent. Accordingly, prolonged use through 100 days, like fluconazole, may be associated not only with reduced frequency of invasive fungal infection but also with a survival benefit and prolonged protection even beyond cessation of drug.

Two studies have evaluated another azole, itraconazole, as long-term antifungal prophylaxis after allogeneic BMT [22, 23]. Itraconazole has similar broad-spectrum antifungal activity including activity against aspergillus as voriconazole does. However, itraconazole is less reliably and well absorbed by mouth (20%) and has substantial interpatient variability in contrast to excellent, reliable absorbance (96%) of voriconazole. Itraconazole can be difficult to tolerate orally, and a pilot trial including a pharmacokinetic analysis of itraconazole at Stanford was unable to be completed. Itraconazole also has substantial negative inotropic effects that have proven to be deleterious in patients who have received anthracyclines or in those receiving concomitant high dose cyclophosphamide. This cardiotoxicity of itraconazole has resulted in a warning from the FDA; it is not shared by voriconazole. In one of the two itraconazole trials by Winston et al. [22], a reduction in invasive fungal infections was noted (in comparison to fluconazole), but there were inadequate numbers of aspergillus cases to determine how effective it is as prophylaxis against aspergillosis. Of concern was an excess of deaths in the itraconazole group (21 of 71 versus 12 of 67) and an increase in adverse events leading to discontinuation of drug in the itraconazole group (6 versus 1). The second randomized trial comparing itraconazole and fluconazole by Marr et al. [23] used a higher dose of itraconazole to ensure therapeutic blood concentrations. The trial was stopped prematurely after nearly 300 patients were enrolled (substantially more enrollees than in the Winston study) due to excessive toxicity in the itraconazole arm. A significant excess of renal and hepatic toxicity was noted in the patients in the itraconazole arm. The smaller sample size of the Winston study may have missed important toxicities of itraconazole due to too few patients. Thirty-six percent of patients on itraconazole stopped the drug due to toxicity. There was no reduction in the frequency of fungal infections in the itraconazole arm in the Marr trial, although among those who were able to tolerate it in a posthoc subset analysis, there were fewer infections in patients who could take itraconazole. Taking both itraconazole HCT trials together, there currently is insufficient data to state definitively that itraconazole is safe and effective in the allogeneic BMT setting for longterm prophylaxis.

The risk of invasive aspergillosis after allogeneic BMT ranges between 7% and 24% in various series [4, 24-32]. In the center with the lowest rate, 7% (seen in patients transplanted between 1990-1992) [4, 5], a larger analysis over time indicated an increase from 5.7 to 11.2% spanning the earlier report and more recently [31]. A still more recent survey of aspergillosis at that center found an infection rate of 12% [56]. Most centers find rates of invasive aspergillosis in the range of 11-15%. Several of the series included both low-risk patients (autologous transplant recipients) along with high-risk patients (allogeneic transplant recipients). By excluding low-risk transplant recipients (autologous BMT recipients), including other fungal pathogens in addition to aspergillus (those organisms not controlled by fluconazole), adding cases of probable aspergillosis and incorporating the galactomannan assay into the diagnostic criteria, we estimate the rate of invasive fungal infection in patients receiving fluconazole to be at least 15% and are using that as the estimate in this trial.

Because of poor sensitivity and specificity of clinical criteria and difficulty in performing invasive diagnostic procedures in this patient population, more accurate and less invasive diagnostic assays have been sought. Recently, the double-sandwich ELISA assay for galactomannan has been found to be highly sensitive and specific [33-35]; it performs well in the BMT recipient patient population [29, 33]. Maertens et al identified 12 episodes of proven invasive aspergillosis in 99 consecutive allogeneic stem cell transplant recipients plus 3 episodes of probable invasive aspergillosis. Using a sandwich ELISA technique, serum samples were assayed for the presence of galactomannan twice weekly. Two or more consecutive sera tested positive in all episodes documented by clinical and microbiologic criteria. Galactomannan was detected prior to any clinical signs of invasive aspergillosis in 5 of the 12 patients and preceded fever or other symptoms in 9 of the 12. In 93% of culture positive episodes, galactomannan was detected before isolation of aspergillosis. Galactomannan was detected prior to the presence of radiographic abnormalities in 91%. Using classical criteria for initiation of empirical antifungal therapy, the assay was positive a median of 6 days prior to standard criteria for empirical therapy in 87% of cases. Autopsies of patients suspected of having invasive fungal infection but with negative galactomannan assays demonstrated no histologic evidence of invasive aspergillus. In a retrospective survey using banked serum from patients found to have proven invasive aspergillosis when compared with controls, Marr showed that the galactomannan assay has a high degree of sensitivity and specificity [29]. As with the Maertens study, Marr found the presence of galactomannan in the serum prior to the diagnosis in two-thirds of patients using classical diagnostic criteria.

Incorporation of the galactomannan assay along with the classical criteria will improve accuracy and may increase the number of cases documented (by upgrading possible cases not proven by classical criteria to proven or probable cases with the galactomannan assay) in the context of a clinical trial. The assays will be performed at a certified contract laboratory or a certified hospital laboratory associated with the transplant center (see Appendix C). The data will be analyzed in grading aspergillosis cases as either possible, presumptive, probable or proven in accordance with revised EORTC/MSG consensus case definitions (see Section 3.1).

1.2. Human Clinical Pharmacokinetics of Voriconazole

Voriconazole has been administered to over 3000 healthy volunteers or patients with fungal infections in single or multiple dosing regimens, by oral and intravenous routes.

In order to improve its solubility, voriconazole is formulated with sulphobutylether-cyclodextrin (SBECD) for intravenous administration. Volunteer studies show that the cyclodextrin is completely renally cleared at a rate consistent with creatinine clearance. Multiple dosing indicates that there is no accumulation of SBECD and the mean elimination half-life after 10 days dosing is 1.6 hours.

Voriconazole is widely distributed throughout body fluids and the volume of distribution is about 2 L/kg. Following oral administration of voriconazole, absorption is rapid with a mean time to maximum plasma concentration of 1 to 2 hours post dose. Single and multiple dose administration of voriconazole in the presence of a high-fat meal delays absorption. Multiple dosing in the presence of food reduces systemic exposure by 32% compared to the fasted state.

As a precaution, it is recommended that voriconazole should not be taken within one hour of eating.

Voriconazole in adults has non-linear pharmacokinetics with regard to dose [57, 58]. For example, a two-fold increase in single oral dose resulted in a three to four fold increase in the $AUC_{\tau_{0-last}}$ (the area under the concentration time curve from time 0 to the last observed concentration above the limit of detection). For the intravenous formulation, the single dose pharmacokinetics are linear up to 4 mg/kg, thereafter they become non-linear with similar increase in the AUC seen with a doubling of dose. For oral multiple dosing, comparison across cohorts suggests that for a 1.5 fold increase in dose there is a three to four fold increase in the AUC (the area under the concentration time curve from time 0 to the end of the dosing interval).

There is wide inter-patient variability in the pharmacokinetics of voriconazole with the coefficient of variation of 126% for AUC_{τ} for a 3 mg/kg Q12H oral dose and 76% for intravenous 3 mg/kg Q12H dosing. Multiple dosing at intravenous doses of 3 mg/kg Q12H and oral doses of 200 mg Q12H results in a mean accumulation of approximately three fold and a maximum accumulation of five fold which is not predictable from the single dose data. For both intravenous and oral administration at single and multiple doses, voriconazole has a mean terminal phase half-life of about six hours. Steady state, for both intravenous and oral dosing, is achieved within six days. Use of a loading dose (6 mg/kg) results in steady state being achieved by 24 hours of dosing.

The pharmacokinetic data from phase II studies showed that voriconazole exhibits a high degree of inter-patient variability. In the 175 patients analyzed, the average AUC_{τ} , maximum plasma concentration and time to maximum plasma concentration were consistent with healthy volunteer data at equivalent doses. The average bioavailability was high, 96% of subjects had observed plasma voriconazole concentrations within the range 1-10 mcg/mL.

Salivary levels of voriconazole are approximately 65% of plasma levels (range 51 to 79%). This is consistent with a plasma protein binding of about 58%. Comparison of the oral and intravenous systemic exposure following single doses and at steady state after multiple dosing indicates that the bioavailability of the oral dose lies within the range of 58 to 90%.

Plasma pharmacokinetics of intravenous voriconazole in immunocompromised children aged 2–11 were assessed and compared with those of healthy adults. In contrast to adults where nonlinear kinetics were observed, in children, the elimination of voriconazole was linear after doses of 3 and 4 mg/kg [60]. Comparison of observed and simulated pediatric and adult pharmacokinetic data indicated that plasma concentrations and area under the concentration-time curve τ of 3 and 4 mg/kg in children were lower than the corresponding dosages in adults. Nevertheless, the peak plasma concentrations in pediatrics still substantially exceed the reported mean inhibitory concentrations (MICs) for most species of *Candida*, *aspergillus* and other filamentous fungi. Doses of 4 mg/kg in children appear to achieve AUCs that approximate the AUCs in adults dosed at 3 mg/kg.

1.3. Clinical Efficacy of Voriconazole

The clinical efficacy, safety and tolerance of voriconazole have been tested in three Phase II studies in Europe; oropharyngeal candidiasis in HIV positive patients [36], acute aspergillosis in immunocompromised patients [37] and chronic fungal infections in non-neutropenic patients. In addition, preliminary efficacy data are available in pediatric patients who received voriconazole compassionately.

In a seven day double-blind, dose ranging study in 165 HIV positive patients with oropharyngeal candidiasis (dose levels 50 mg QD, 200 mg QD or 200 mg Q12H), clinical efficacy (cured + improved) in the intention to treat (ITT) analysis was 80% at 50 mg QD, 100% at 200 mg QD and 89% at 200 mg Q12H. Mycological efficacy (reduction in colony counts < 11 colonies/culture) in the ITT analysis increased with dose from 33% at 50 mg QD to 74% at 200 mg Q12H. The drug was generally well tolerated and the only drug-related adverse event of any significance was transient visual disturbance. The visual events had resolved fully in all patients at follow-up.

Based upon the plasma pharmacokinetic profile of voriconazole, there may be a dose-response relationship between higher dosages and improved antifungal efficacy. The geometric-mean inhibitory concentrations (GMIC) of voriconazole vs. *aspergillus* spp. range from 0.19 to 0.58 ug/mL. Although the GMIC cannot be as closely correlated with clinical efficacy as MIC can for an antibiotic, it is generally considered desirable for plasma concentrations of an antifungal agent to cover the GMIC. Plots of C_{min} data showed that a dosage of 4 mg/kg IV BID resulted in trough plasma levels of voriconazole > 0.5 μ g/mL in 6 of 7 subjects. However, a dosage of 3 mg/kg IV BID resulted in trough levels of voriconazole > 0.5 μ g/mL in only 4 of 14 subjects.

A second open, non-comparative study was conducted using 200 mg PO Q12H of voriconazole for up to 24 weeks in chronic aspergillosis or candidiasis of non-neutropenic patients. At the interim analysis of clinical efficacy in 25 patients, favorable clinical response (complete or partial resolution of clinical signs and symptoms, as determined by the investigator) was achieved in 70% (12/17). Mycological response was available in three patients at the end of therapy (EOT) and all three had negative cultures. The compound was generally well tolerated in those patients. Visual disturbance, skin rash and elevated liver function tests were observed and reported as adverse events possibly related to therapy. In two patients, elevation of liver function tests (LFTs) above the protocol-specified maximum resulted in their withdrawal from the study.

The third study was an open, non-comparative study of voriconazole (6 mg/kg IV Q12H for one day then 3 mg/kg IV Q12H for 6-27 days followed by 200 mg PO Q12H for up to a total of 24 weeks) in acute invasive aspergillosis of neutropenic and other immunocompromised patients. Interim analysis of clinical efficacy in 71 patients indicated a favorable clinical response (complete/partial resolution or stable clinical signs and symptoms, as determined by the investigator) was achieved in 83% (54/65). Mycological response at EOT was available in 31 patients; three patients had their infection eradicated, four had persistent infection and 24 were not evaluable. The drug was generally well tolerated by these severely ill patients. LFT

elevations above the protocol-specified maximum resulted in withdrawal of three patients from the study.

The safety and efficacy of voriconazole in children treated within the compassionate release program who were intolerant of, or refractory to, conventional antifungal therapy was recently reported [38]. Seventy-two patients (ages 9 months to 15 years, median 7 years) received voriconazole; 63 had a proven or probable fungal infection. Among these 63 patients, 27 had hematological malignancies and 14 had chronic granulomatous disease as the most frequent underlying conditions. Forty-two patients had aspergillosis, 8 scedosporiosis, 4 invasive candidiasis, and 4 had other invasive fungal infections. The median duration of voriconazole therapy was 93 days. At EOT 27 patients (44%) had a complete or partial response. Four patients (7%) had a stable response, 25 (43%) failed therapy, and 4 (7%) were discontinued from voriconazole because of intolerance. Success rates were highest in patients with CGD (57%) and lowest in patients with hematological malignancies (25%). Two patients experienced treatment-related serious adverse events (ulcerated lips with rash, elevated hepatic transaminases, or bilirubin). Voriconazole-related adverse events included rash (8), increased hepatic transaminases or bilirubin (5), blurred vision (2), and vomiting (1). These data support the use of voriconazole for treatment of invasive fungal infections in pediatric patients who are intolerant of, or refractory to, conventional antifungal therapy.

The results of an open, randomized comparison of voriconazole versus amphotericin B followed by other licensed antifungal therapy for primary treatment of invasive aspergillosis were recently described [39]. Patients with invasive aspergillosis were randomized to receive amphotericin B 1 mg/kg or voriconazole intravenously at 6 mg/kg Q12 hours (2 doses) then 4 mg/kg Q12H. Oral formulation at a dosage of 200 mg Q12H could be given following 4 mg/kg IV dosing. Other licensed antifungal therapy was allowed after initial randomization therapy. Blinded teams assessed eligibility and response, and followed modified EORTC/MSG definition criteria for probable or proven infection. Favorable outcomes were complete response of clinical and radiological findings or partial response, which was significant clinically and > 50% radiological improvement. Unfavorable outcomes were unchanged or worsened condition. The primary outcome was assessed at week 12. Secondary endpoints included outcome of study therapy and survival through week 12. Three hundred ninety-two patients were enrolled over three years in 92 centers in 19 countries. One hundred forty-four voriconazole and 133 amphotericin B patients had confirmed invasive aspergillosis and received ≥ 1 dose of therapy. Underlying diseases represented were allogeneic bone marrow transplant in 26 and 23%, autologous bone marrow transplant or hematologic disease in 56 and 63%, and other in 18 and 14% of voriconazole and amphotericin B patients, respectively. Pulmonary aspergillosis was present in 83% of the voriconazole and 84% of the amphotericin B treated patients. Disseminated disease occurred in 8.3% voriconazole versus 7.5% amphotericin B-treated and CNS disease in 1.4% versus 0.8%, respectively. At week 12, a complete/partial response of 52.8% with voriconazole versus 31.6% with amphotericin B was observed (95% CI for the difference was 32.9% to 10.4%). Survival on the voriconazole arm was 70.8% for voriconazole versus 57.9% on the amphotericin B arm (Hazard ratio 0.59; 95 CI 0.40 to 0.88). Study therapy was continued for a median of 77 days on the voriconazole arm versus 11 days on the amphotericin B arm. End of therapy response was 53.5% on the voriconazole arm versus 21.8% on the amphotericin B arm (95% CI for the difference was 42% to 21%). These data suggest voriconazole was more

effective than amphotericin B followed by other licensed antifungal therapy at week 12. Moreover, voriconazole improved survival in patients with invasive aspergillosis.

Recently reported data on the efficacy of voriconazole against rare or resistant fungal pathogens (*C. neoformans*, *F. solani*, *P. lilacinus*, *Trichosporon* spp, *S. immitis* and *H. capsulatum*) appear promising [40-43]. Patients with documented invasive infections caused by rare or resistant fungal pathogens and enrolled into voriconazole clinical studies or for compassionate use were included in the analysis. Fifty-nine subjects were analyzed; those receiving primary therapy (n=8 success=3, failure=5) and those receiving salvage therapy (n=51 success=21 failure=30). Outcomes were grouped according to underlying condition: AIDS/HIV (n=13 success=3, failure=10), hematologic malignancy (n=15 success=5, failure=8), drug or disease induced immune suppression (n=11 success=4, failure=7), injury/trauma or post surgical (n=5 success=1, failure=4), non-malignant hematological disorders (n=6 success=3, failure=3). Fifteen of the 59 patients had hematologic diseases (n=15 success=7, failure=8). Successful outcomes were seen in 17 of 44 patients without these risk factors. Outcomes based on pathogens were also analyzed: *Cryptococcus* spp was successfully treated in 4 of 13 patients, *Fusarium* spp in 6 of 16 infections, and infections with other rare fungi, 14 of 30. Therefore, voriconazole appears to be a promising antifungal agent in severely immunocompromised patients who have failed or are intolerant to other treatment options, with potent activity against a wide variety of resistant and rare fungal pathogens.

The recently completed MSG-42 study of voriconazole versus liposomal amphotericin B (L-AMB) for empirical antifungal therapy in persistently febrile neutropenic patients suggests voriconazole is a safe and effective alternative therapy in this population [44]. Voriconazole was compared to L-AMB in a randomized, multicenter, international trial. Eight hundred forty-nine randomized patients (voriconazole n=421, L-AMB n=428) receiving at least one dose of study drug were evaluable for success. Composite success rates were 26% with voriconazole and 31% with L-AMB (95% CI for difference; -10.6% to 1.6%). These were independent of administration of colony stimulating factors or antifungal prophylaxis. Documented breakthrough fungal infections were fewer among patients treated with voriconazole 8 (1.9%) versus 21 (5.0%). Severe infusion-related reactions (p<0.01) as well as nephrotoxicity (p<0.001) were less in patients receiving voriconazole versus L-AMB. Hepatotoxicity was similar for both drugs. More cases of transient visual changes (22% versus 1%) (p<0.001) and hallucination (4.3% versus 0.5%) occurred on the voriconazole arm than on the L-AMB arm. Ninety-two patients (22%) changed to oral formulation of voriconazole with a reduction of mean duration of hospitalization by one day in all patients and by two days in high-risk patients (p<0.05). The authors concluded that voriconazole is an effective alternative for empirical antifungal therapy in patients at risk.

1.4. Rationale for Study

Given the high mortality rate associated with documented fungal infections in BMT patients despite aggressive therapy with amphotericin B, strategies to prevent infections are warranted [7, 8] and may be more effective than trying to treat a documented established infection. Allogeneic BMT patients are at special risk for infection due to aggressive or prolonged immunosuppressive therapy and are subject to a high case fatality rate. Antifungal agents such as fluconazole and

voriconazole can be administered orally, have good activity against several fungal pathogens and are less toxic than amphotericin B. The availability of intravenous formulations of both agents permit continued compliance with the regimen in the event that the patient cannot tolerate oral medications.

Although voriconazole has a wider antifungal spectrum than fluconazole, there are more drug interactions requiring greater vigilance over the immunoprophylaxis regimen, and there is the potential for greater toxicity. Whether or not, on balance, the potential benefit outweighs these potential risks can only be determined in a prospective, randomized trial.

CHAPTER 2

2. STUDY DESIGN

2.1. Study Overview

This is a randomized, double-blind, multicenter, prospective, comparative study of fluconazole versus voriconazole in the prevention of fungal infections in allogeneic hematopoietic transplant recipients and cord blood recipients in children under the age of 12. Prior to the start of the pre-transplant conditioning regimen, patients will give written informed consent and be screened for eligibility. Patients who meet all entry criteria will be assigned randomly to voriconazole or fluconazole within 72 hours of Day 0. Patients will begin study drug on Day 0 (after completion of the conditioning regimen). Day 0 is defined as the day infusion of the stem cell product is initiated. Study drug will be continued until Day 100 following transplant or until one or more criteria for study drug early withdrawal are met as defined in Section 2.4.6. Continuation of study drug beyond Day 100 is permitted for patients who meet criteria specified in Section 2.4.8. The development of any fungal infection during prophylaxis will be classified according to the definitions listed in Section 3.1.1.

2.2. Hypotheses and Specific Objectives

2.2.1. Primary Hypothesis

Voriconazole prophylaxis will be associated with fewer invasive fungal infections and reduce morbidity and mortality due to fungal infection. This will result in an improvement of fungal-free survival (survival without invasive fungal infection).

2.2.2. Secondary Hypothesis

Reduction in fungal infectious rates will be associated with improvement in post-transplant survival.

2.2.3. Study Objectives

This trial is designed to compare the efficacy of fluconazole 400 mg/day PO (or IV at the same dose if the patient is unable to tolerate it PO) with voriconazole 200 mg PO BID (or IV at the same dose if the patient is unable to tolerate it PO) in prevention of fungal infections in allogeneic hematopoietic transplant recipients during the first 180 days after transplant. Children aged < 12 years will receive age-appropriate dosing, as indicated in Section 2.4.1. The primary objective is to compare the fungal-free survival rates between the two study arms during the first 180 days. Secondary objectives will be to compare invasive fungal infection rates, overall mortality due to fungal infection, engraftment rates, acute and chronic GVHD rates, reasons for failure (e.g., plasma concentrations, tolerance, fungal sensitivity, etc.), and to perform exploratory analyses of quantitative aspects of the galactomannan assay. The relative safety of

the two antifungals also will be assessed through the collection of post-transplant toxicities, serious adverse event reports and routine laboratory monitoring.

2.3. Patient Eligibility

2.3.1. Eligibility Criteria

Patients fulfilling all of the following criteria will be eligible for this study:

1. Patients who receive an allogeneic peripheral blood or marrow transplant from a family or unrelated donor, or for children under the age of 12, a cord blood transplant from either a sibling or other donor.
2. Patients must have a 5 or 6 of 6 HLA-matched donor. The match may be determined at serologic level for HLA-A and HLA-B loci. For sibling donors, matching may be determined at serologic level for HLA-DR; for unrelated donors, matching for HLA-DRB1 must be at the high-resolution molecular level.
3. Patients two years of age or older.
4. Patients and/or legal guardian able to provide informed consent

Patients with one of the following underlying diseases:

- a) AML, with or without a history of myelodysplastic syndrome, in first or second complete remission (see Section 3.4) or in early relapse (< 30% blasts in bone marrow with no circulating blasts in peripheral blood and no extramedullary leukemia); or
- b) ALL, in first or second complete remission (see Section 3.4); or
- c) AUL (acute undifferentiated leukemia) in first or second complete remission (see Section 3.4); or
- d) Acute biphenotypic leukemia in first or second complete remission (see Section 3.4); or
- e) CML in either chronic or accelerated phase; or
- f) One of the following myelodysplastic syndrome(s) defined by the following:
 - 1) Refractory anemia
 - 2) Refractory anemia with ringed sideroblasts
 - 3) Refractory cytopenia with multilineage dysplasia
 - 4) Refractory cytopenia with multilineage dysplasia and ringed sideroblasts
 - 5) Refractory anemia with excess blasts-1 (5-10% blasts)
 - 6) Refractory anemia with excess blasts-2 (10-20% blasts)
 - 7) Myelodysplastic syndrome, unclassified
 - 8) MDS associated with isolated del (5q)

- 9) CMML; or
 - g) Lymphoma (including Hodgkin's) with chemosensitive disease ($\geq 50\%$ response to chemotherapy) and receiving a related donor transplant
6. Patients receiving myeloablative conditioning regimens.
 7. Patients with adequate physical function, within six weeks of initiation of conditioning (preferably within four weeks) unless otherwise specified, as measured by:
 - a) Cardiac: Asymptomatic or, if symptomatic, then left ventricular ejection fraction at rest must be $> 40\%$ and must improve with exercise, or shortening fraction $> 26\%$
 - b) Hepatic: $\leq 5 \times$ ULN ALT (within 72 hours of Day 0)
 ≤ 2.5 mg/dL total serum bilirubin
 - c) Renal: Serum creatinine within normal range for age or if serum creatinine above upper limit of normal range for age then renal function (creatinine clearance) $> 50\%$ LLN for age
 - d) Pulmonary: DLCO, FEV1, FVC (capacity) $> 45\%$ of predicted value (corrected for hemoglobin) or O₂ saturation $> 85\%$ of room air
 8. Patients must have baseline galactomannan blood samples drawn within 30 days prior to randomization with the results available prior to randomization (72 hours prior to transplant)
 9. Patients must have chest CT scans within six weeks prior to randomization if the results of the baseline galactomannan blood sample are not available prior to randomization (72 hours prior to transplant)

2.3.2. Exclusion Criteria

Patients with the following will be ineligible for randomization on this study:

1. Patients with an invasive yeast infection within the eight weeks prior to conditioning regimen initiation. Patients are eligible if colonized or have had superficial infection. Patients with a history of candidemia > 8 weeks prior to conditioning must have a negative blood culture within 14 days of conditioning (within 7 days is recommended), no clinical signs of candidemia, and may not still be requiring antifungal therapy.
2. Patients with a presumptive, proven or probable aspergillus or other mould infection or deep mycoses (including hepatosplenic candidiasis) within 4 months prior to conditioning regimen initiation.
3. Patients with an uncontrolled viral or bacterial infection at the time of study registration.
4. Women who are pregnant (positive β -HCG) or breastfeeding. Women of child-bearing age must avoid becoming pregnant while receiving antifungal agents.
5. Patients with a Karnofsky performance status $< 70\%$ or Lansky $< 50\%$ for patients < 16 years old unless approved by the Medical Monitor or protocol Chair.

6. Patients with a history of allergy or intolerance to azoles (fluconazole, itraconazole, voriconazole, posaconazole, ketoconazole, miconazole, clotrimazole).
7. Patients requiring therapy with rifampin, rifabutin, carbamazepine, cisapride (Propulsid®), terfenadine; (Seldane®), or astemizole (Hismanal®), ergot alkaloids, long-acting barbiturates, or who have received > 3 days treatment with rifampin or carbamazepine within 7 days prior to conditioning regimen initiation. Patients on therapeutic anticoagulation with coumadin (1 mg/day for port prophylaxis is permitted).
8. Patients receiving sirolimus.
9. Patients with prolonged QTc syndrome at study entry.
10. Patients who are HIV positive.
11. Patients receiving another investigational drug unless cleared by the medical monitors.
12. Patients who have received a prior allogeneic or autologous transplant.
13. Patients with active CNS disease.
14. Patients on fungal prophylaxis during conditioning regimen (it is recommended that fungal prophylaxis be suspended once patient is enrolled).
15. Patients with prior malignancies except resected basal cell carcinoma or treated carcinoma in-situ. Cancer treated with curative intent < 5 years previously will not be allowed unless approved by the Medical Monitor or Protocol Chair. Cancer treated with curative intent > 5 years previously will be allowed.

2.4. Treatment Plan

The immediate pre-transplant evaluation will be carried out according to the operating procedures of the participating institutions and should be in keeping with the data reporting requirements of this study. Similarly, special orders and procedures will be those defined by the operations manuals of the Clinical Centers. All patients enrolled on this protocol will be hospitalized in accordance with isolation procedures for recipients of allogeneic blood and marrow transplants as defined by the given institution.

2.4.1. Drug Dosages

The study drugs will be masked by overencapsulation to obscure their identity. Patients randomized to voriconazole will receive one dose in the morning and one dose in the evening. Fluconazole is appropriately efficacious if administered once daily. Those patients randomized to fluconazole will receive their full daily dose in the morning followed by placebo in the evening.

Study drug will begin on Day 0, day of transplant. Patient should be given an AM and PM dose. If necessary, the AM dose may be administered prior to stem cell infusion.

If a patient misses a dose, it should be taken within four hours of the scheduled time. If more than four hours have passed, the patient should not take an additional dose until his next scheduled dose.

In efforts to maintain the blind, both intravenous voriconazole and fluconazole will be administered in equivalent volumes as specified in Appendix F.

Adults: The dose of oral voriconazole is 200 mg twice daily. When voriconazole must be given intravenously, it will be given at a dose of 200 mg every 12 hours for the duration of intravenous therapy. Doses will not be reduced for renal dysfunction.

The dose of fluconazole is 400 mg by mouth or intravenous drip. Doses will be reduced for renal dysfunction in accordance with Section 2.4.3 and Appendix G.

Children (age < 12 years old): Oral doses will be given as follows: In patients weighing ≥ 20 kg, voriconazole will be administered at a dose of 100 mg twice daily. Patients weighing < 20 kg will receive oral voriconazole at a dosage of 50 mg twice daily. If intravenous administration is needed, voriconazole will be at a dose of 4 mg/kg (total body weight) every 12 hours (not to exceed the equivalent oral dose) for the duration of intravenous therapy (see Appendix F).

For oral administration, patients weighing ≥ 20 kg will receive fluconazole at a dose of 200 mg once daily. For those weighing < 20 kg, a dose of 100 mg once daily will be used. Doses will be reduced for renal dysfunction in accordance with Section 2.4.3 and Appendix G. When intravenous administration is received, fluconazole will be given at 6 mg/kg/day total body weight (with maximal IV dose not to exceed the equivalent oral dose per weight) once daily (see Appendix F).

Both study drugs will be given orally whenever possible. Both voriconazole and fluconazole are well absorbed after oral administration with bioavailability exceeding 90%. Study drugs should be taken at least one hour after or one hour before a meal. The intravenous formulation will be given only when the oral medication cannot be tolerated. Oral study drug should be resumed as quickly as possible when oral medication can be given.

Total duration of the prophylaxis study drug will be 100 days, or until study drug early withdrawal criteria have been met (Section 2.4.6) including criteria for a presumptive, probable or proven fungal infection (Section 3.1.1). Study drug will be continued beyond Day 100 in patients who meet criteria in Section 2.4.8.

Fungal prophylaxis must not be given after protocol-specified treatment plan is completed on Day 100 (or 180).

2.4.2. Administration Procedures

1. Voriconazole

Voriconazole will be administered orally twice daily. Voriconazole capsules should be taken at least one hour before or one hour after a meal. Taken concomitantly with food,

bioavailability of voriconazole is reduced. If oral drug is not possible, it will be given intravenously at a dosage of 200 mg Q12H over two hours in patients ≥ 12 years. Each voriconazole dose will be diluted to a total volume of 200 mL in patients ≥ 12 years. Volumes of the formulation required to provide 4 mg/kg doses for children (age < 12 years) of various body weights, and the dilution and infusion instructions are in Appendix F. To maintain the blind in children, a standardized volume defined in Appendix F will be used for all doses. Accordingly, dilutions to the specified volume, if necessary, should be made.

2. Fluconazole

Fluconazole will be administered orally once daily. Fluconazole capsules should be taken at least one hour before or one hour after a meal. If oral drug is not possible, it will be given intravenously once daily in a total volume of 200 mL in patients ≥ 12 years. For adults, each 200 mL infusion will be administered over 2 hours. In patients < 12 years, intravenous doses will be prepared in accordance with Appendix F in standardized volumes and administered over two hours.

3. Incompatibilities

Concomitant medications should not be infused in the same line as voriconazole, fluconazole or amphotericin B.

4. Accountability

The investigator (and/or hospital pharmacist) will ensure that all study drug is stored in a secured area under recommended storage conditions and is dispensed by qualified staff members. All study drug will be accounted for on medication inventory sheets.

The BMT CTN clinical monitor will be allowed at intervals, and upon request during the study, to check unused supplies. Accounting for the use of supplies will be by reference to each center's record of supplies received, the dispensing records for the total number of patients enrolled at each center and the unused and returned supplies.

Patients who are discharged from the hospital during the time they are receiving oral voriconazole or fluconazole therapy will be provided with ample supply of study medication between visits. The patient will be requested to return all containers of study drug, including empty containers and containers with unused capsules, at the next visit. Unused drug may be returned to patient if therapy continued.

The investigator is responsible for maintaining drug accountability records. Drug accountability records will be reviewed during monitoring visits. Study drug must be administered only to patients enrolled in this study as per the protocol.

5. Labeling

The investigator or pharmacist will record the patient number on the label of all containers containing voriconazole or fluconazole capsules before dispensing to the patient. The lot number of the bottle should be recorded on the patient's dispensing records at the time they are dispensed.

6. Cardiac Monitoring

In subjects assessed by the treating physician to be at risk for cardiac arrhythmia, the following procedure must be performed: Continuous cardiac monitoring during the first three days of study medication. If the cardiac monitoring reveals a significant arrhythmia, the administration of study medication must be held, and the subject must undergo further assessment by a cardiologist to evaluate the significance of the findings. The cardiologist's assessment and opinion on the significance of any abnormal finding will be summarized in a report and submitted on an Adverse Event Form. If the PI and cardiologist conclude that it is not related to the study drug, study drug can be resumed. Otherwise, the patient will be withdrawn from study drug.

2.4.3. Dosage and Formulation Adjustments

Oral intolerance: Oral study medication will be temporarily discontinued in patients who become unable to take or to tolerate oral medications; the parenteral antifungal formulation should be substituted in the dose according to Section 2.4.1. Patients will be changed back to oral medications as soon as tolerable. If the patient is an outpatient and requires IV study drug for more than 14 days and cannot tolerate oral study drug, the patient must be taken off study drug.

Empirical therapy with Amphotericin B or Caspofungin: Patients who develop a possible invasive fungal infection as defined in Section 3.1.1, but do not meet the criteria for failure of prophylaxis, may be treated empirically with an amphotericin B formulation or caspofungin according to the provisions of Section 2.4.10. These patients will remain on study and continue to be assessed for more definitive evidence of fungal infection. During empirical therapy, the study drug should be continued. Prior to initiation of any empirical trial of amphotericin B or caspofungin, at least two blood samples for the diagnostic galactomannan assay should have been collected during the last seven days (with at least one within 48 hours). No systemic antifungal agent, other than one of the amphotericin B formulations or caspofungin, is permitted while the patient is on study.

Renal dysfunction: In the setting of renal dysfunction, fluconazole doses will be adjusted as follows: estimated CrCl < 50ml/min = 50% dose reduction. For pediatric patients with CrCl < 20ml/min/1.73m², fluconazole dose reduction = 75%. Calculations of creatinine clearance should be made based on the method of Cockcroft and Gault [45] for adult males [CrCl = (140-age in years) X kg weight (ideal) /serum creatinine x72]. For adult females, the equation is multiplied by 0.85. Ideal body weight (IBW) is to be calculated as follows: Male IBW = 50kg + 2.3 kg/inch over 5 feet, Female IBW = 45.5 kg + 2.3 kg/inch over 5 feet. If less than 5 feet, subtract 2.3 kg/inch. For children, the Schwartz calculation will be used (CrCl = k value X height (cm)/plasma creatinine). K values for children and adolescent girls = 0.55, adolescent boys = 0.70. Guidelines for oral dose adjustment are given in Appendix G. No dose adjustments for renal dysfunction will be made in the voriconazole arm. For patients with moderate renal insufficiency (estimated CrCl < 50 mL/min), accumulation of the voriconazole IV vehicle can occur. Oral voriconazole should be administered to these patients unless a benefit/risk assessment justifies the use of IV voriconazole. However, if CrCl declines to 25 mL/min and

persists for more than 14 days and the patient must receive IV drug and is unable to switch to oral drug, then the study drug should be discontinued. Study drug should be discontinued in the setting of hemodialysis (see Section 2.4.6.1.f).

To maintain the blind in patients receiving intravenous study drug, for patients randomized to receive voriconazole who meet the renal impairment criteria, the volume of voriconazole will be adjusted to mimic the volume the patient would receive if randomized to fluconazole. To maintain the blind in patients receiving oral study drug, the number of voriconazole capsules taken in the morning will be adjusted to mimic the number the patient would receive if randomized to fluconazole (see Appendix G).

The physician must order dose adjustments for all patients randomized into the study using an order conforming to type listed in Appendix H. The pharmacist will make the appropriate adjustments based on randomization and the type of renal impairment.

2.4.4. Study Drug Supply

An initial supply of study drug (oral and IV, adult and pediatric doses, if appropriate) will be automatically sent to the investigational pharmacy upon enrollment of the transplant center's first patient onto Segment A (see Section 4.1.1). The transplant center's investigational pharmacist will be responsible for subsequent ordering, receiving, storing, distributing and accounting of the study drug. He/she will be responsible for recording the receipt and administration of all trial drug supplies, for ensuring the supervision of the storage and allocation of these supplies, and for maintaining the drug distribution and accountability logs. These logs must be available for inspection during routine site monitoring visits. The investigator will ensure that all protocol drugs are stored in a secured area under recommended storage conditions and are dispensed by qualified staff members.

2.4.5. Pharmaceutical Information

1. Source

Voriconazole and fluconazole are manufactured by Pfizer, Inc., Groton, CT.

2. Toxicity

Voriconazole: Voriconazole had been administered to over 3000 healthy volunteers or patients with fungal infections in single or multiple dosing regimens, by oral and intravenous routes at the time of FDA review for licensure. The following safety issues have been identified. There were 286 serious adverse events recorded. Of these, a total of eight were reported by the investigators to be potentially causally related to voriconazole. Other events were assessed by the investigators to be related to the disease under study or to other factors. The eight events that were causally related to study drug were: three cases of elevated liver function test (LFT) values, three cases of skin reactions, one case of exacerbated hypoglycemia and one case of atypical pneumonia with hypoglycemia.

Overall, 30% of volunteers and patients have reported adverse events related to vision, regardless of route of administration. The most consistent description offered was of a transient altered/increased perception of light or blurred vision. The events generally lasted less than one hour, with onset occurring between 30 and 60 minutes after an oral dose. Of these, three of four were mild. The remainder were moderate, with 1-2% being severe. All of these events resolved. Abnormal vision is therefore reversible, even during continued therapy, and is not considered to represent a serious risk.

Ophthalmologic tests (visual evoked response), performed in volunteers, did not reveal any abnormalities and provide no basis for a potential mechanism. Also there have been no ophthalmologic or brain/optic nerve histological changes in animals treated for up to six months with high doses of voriconazole. These data suggest that pathological changes to the visual pathways are unlikely.

Analysis of the data from patient studies has shown cases of elevated alkaline phosphatase (ALP) and bilirubin, potentially indicative of cholestasis, which appear to be temporally related to onset of voriconazole therapy. Occasionally, when these levels rose to greater than three times the upper limit of normal (ULN) therapy was discontinued; a total of 16 patients fulfilled this criterion. One of these cases resulted in prolonged hospitalization and therefore was considered to be a serious adverse event.

Also, a number of cases of elevated transaminases (alanine aminotransferase and aspartate aminotransferase, ALT and AST, respectively) have occurred. When levels reached five times the ULN, therapy was discontinued in nine patients from two studies. Two of these cases were hospitalized for observation and therefore were considered serious events. Elevations in liver function tests (LFTs) have only rarely occurred in studies of voriconazole in healthy volunteers.

There have been a small number of skin reactions recorded with the total incidence of rash being 7%. These reactions were largely localized to the sun exposed areas of the skin and may, therefore, indicate a photosensitizing potential with voriconazole. Other azole antifungal agents have been reported to cause skin reactions.

In addition to the visual disturbances, elevated liver function tests, and skin reactions discussed above, the following treatment-related adverse events have occurred at a rate of < 1% in human volunteers and patients that have received voriconazole to date: abdominal pain, headache, nausea, dizziness, asthenia, hypokalemia and injection site reactions.

The following serious adverse events have been reported in patients receiving other azole antifungal agents, and could potentially occur in patients receiving voriconazole: cardiac dysrhythmias (reported in patients receiving itraconazole in combination with terfenadine or astemizole), and anaphylaxis (reported with fluconazole and ketoconazole).

Pediatric patients have received voriconazole in Phase II studies. Patients received between 200-1000 mg per day and tolerated voriconazole without any attributable serious adverse events.

Fluconazole: Sixteen percent of over 4,000 patients treated with fluconazole in clinical trials of seven days or more experienced adverse events. Discontinuation of therapy was required in 1.5% of patients due to adverse clinical events and in 1.3% of patients due to laboratory test abnormalities. Treatment-related clinical adverse events occurring at an incidence of 1% or greater included nausea in 7.7%, headache in 1.9%, skin rash in 1.8%, vomiting in 1.7%, abdominal pain in 1.7%, and diarrhea in 1.5%.

In both combined clinical trials and marketing experience there have been rare cases of serious hepatic reactions thought probably causally associated. The spectrum of these reactions has ranged from mild transient elevations in transaminases to clinical hepatitis, colestasis, and fulminant hepatic failure including fatalities. Generally, liver function returned to baseline on discontinuation of fluconazole.

Other side effects noted with uncertain causal association include seizures, exfoliative skin disorders including Stevens Johnson syndrome and toxic epidermal necroliasis, alopecia, leukopenia, including neutropenia, thrombocytopenia, hypercholesteremia, hypertriglyceridemia, and hypercalcemia.

In children, 13% of 577 patients experienced treatment-related adverse events. These included vomiting in 5%, abdominal pain in 3%, nausea in 2% and diarrhea in 2%. Treatment was stopped in 2.3% of patients due to adverse reactions and in 1.4% of patients due to laboratory test abnormalities. The majority of the laboratory abnormalities were elevations of transaminases or alkaline phosphatase.

3. Formulation and Preparation

a) Voriconazole

Voriconazole for oral use is presented as 50 mg and 200 mg tablets. Tablets will be overencapsulated by the BMT CTN Central Pharmacy to maintain the blind. Capsules must not be opened or altered. Voriconazole for intravenous injection is presented in a 30 mL vial as a lyophilized powder for reconstitution equivalent to 200 mg Voriconazole per vial. The lyophile must be stored at room temperature (15°-30°C). After reconstitution with 19 mL of water for injection USP, each mL contains 10 mg of voriconazole and 160 mg of SBECD. The pH of the reconstituted solution ranges from 5.5 to 7.5. Refer to Appendix F for reconstitution guidelines for IV administration.

b) Fluconazole

Fluconazole for oral use is presented as 100 mg and 200 mg tablets. Tablets will be overencapsulated by the BMT CTN Central Pharmacy to maintain the blind. Capsules must not be opened or altered. Oral fluconazole suspension is not available for this trial. Fluconazole for injection is packaged in Viaflex[®] Plus plastic containers containing 200 mg of fluconazole in a volume of 100 mL in sodium chloride (a

concentration of 2 mg/mL). Refer to Appendix F for reconstitution guidelines for IV administration. Intravenous fluconazole will be stored and prepared according to the manufacturer's instructions provided in the package insert.

c) Placebo

The oral formulations of voriconazole and fluconazole differ in appearance. Accordingly, all oral medications will be masked by overencapsulation to prevent identification. Patients receiving oral fluconazole will receive active masked drug in the morning and an identical appearing placebo in the evening. Similarly, patients receiving IV fluconazole therapy will receive an equivalent volume of normal saline (weight-adjusted as per Appendix F) for the evening infusion.

4. **Stability and Storage**

The safe storage of study drug supplies will be arranged by the investigator until they are dispensed to the patient(s). They will be stored in the hospital's investigational pharmacy under the supervision of the investigational pharmacist.

a) Voriconazole

Voriconazole capsules should be stored at controlled room temperature between 15°-30°C (59°-86°F).

Vials containing unreconstituted lyophilized voriconazole should be stored at controlled room temperature between 15°-30°C (59°-86°F). Vials of reconstituted voriconazole solution must be stored between 2°-8°C (37°-46°F) for no more than 24 hours. They should not be frozen. The reconstituted solution does not contain a preservative, and each vial is intended for single use only.

b) Fluconazole

Fluconazole capsules should be kept below 30°C (86°F).

Fluconazole for injection is packaged in Viaflex[®] Plus plastic containers and should be stored between 5°-25°C (41°-77°F).

2.4.6. Early (Premature) Withdrawal of Study Drug

2.4.6.1. Patient early withdrawal from study treatment

Patients will be prematurely withdrawn from study treatment prior to day 100 (or 180) if:

1. There is evidence at any time during the study of a presumptive, probable or proven invasive fungal infection as defined by criteria (Section 3.1.1).
2. Any of the following toxicities occur. However, if another etiology is judged by the local PI to be the likely cause of the toxicity and an interval of no more than 14 days has lapsed, then the study drug can be resumed at original dose. If the same toxicity recurs, the patient will be permanently withdrawn from the study treatment.

- a) For hepatic toxicity: If ALT exceeds ten times the upper limit of normal and is felt to be at least possibly related to study drug, the study drug should be held until the causality clarifies and until the toxicity resolves to a Grade II or less. If ALT exceeds five times the upper limit of normal and is felt to be at least possibly related to study drug, the study drug may be held at the discretion of the treating physician until the causality clarifies and until the toxicity resolves to less than two and a half times the upper limit of normal.
 - b) For visual toxicity: Photopsia (visual disturbances) is an occasional side effect of voriconazole. It typically is transient and is not associated with organic or enduring sequelae. It does not represent grounds for withdrawal. If a patient experiences loss of vision (blindness), it is unlikely that it is due to the study drug; however, the study drug should be held until etiology is established. Cyclosporine and tacrolimus can cause loss of vision and should be held. Retinal hemorrhage is also a potential cause of visual loss and platelet transfusions and optimization of coagulation parameters should be considered where appropriate. Ophthalmic evaluation should be promptly carried out.
 - c) For cutaneous toxicity: A skin rash can occasionally occur. This may be provoked or exacerbated by sun exposure. Other potential causes of rash should be investigated, including graft-versus-host disease and drug sensitivity. A skin biopsy may be useful in the evaluation of other causes. This ordinarily will not constitute grounds for withdrawal unless there is skin necrosis or ulceration or generalized exfoliative dermatitis.
 - d) For neurologic toxicity: An infrequent observation in patients receiving voriconazole is hallucinations. A preliminary analysis suggests this is due to potentiation of opiate or benzodiazepine effect. If such should occur, attempts should be made to reduce opiate and/or benzodiazepine dosages. If necessary after at least 24 hours after reduction of the dose(s) of concomitant opiates or benzodiazepines, study drug can be held (without withdrawal) up to 14 days as specified above to allow reduction of doses of these concomitant medications.
 - e) For cardiac arrhythmia: If a significant arrhythmia occurs, study medication must be held, and the subject must undergo further assessment by a cardiologist to evaluate the significance of the findings. The cardiologist's assessment and opinion on the significance of any abnormal finding will be summarized in a report and submitted with an Adverse Event Form. If the PI and cardiologist conclude that it is not related to the study drug, study drug can be resumed as specified above. Otherwise, the patient will be withdrawn from study treatment.
 - f) For renal insufficiency: The patient experiences serious renal impairment requiring hemodialysis and IV study drug.
 - g) For any other Grade III or IV toxicity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 which is not typically expected in the course of BMT and may be possibly related to study drug.
3. Systemic amphotericin B (or one of the lipid formulations) or caspofungin is given for more than 14 consecutive days.

If a patient is prematurely withdrawn from study drug prior to Day 100 for reasons other than a presumptive, probable or proven fungal infection, fluconazole will be allowed for fungal prophylaxis until Day 100 with permission of the Medical Monitor or Protocol Chair. No other antifungal agents are allowed.

4. The patient requires terfenadine, astemizole, cisapride or sirolimus, maintenance phenytoin/anticonvulsant therapy, or any of the drugs prohibited at study entry (Section 2.3.2).
5. The patient is an outpatient and requires IV study drug for more than 14 consecutive days and cannot tolerate oral study drug. IV study drug must be prepared by the investigational pharmacist at the transplant center (and not by home health care) in order to maintain the blind.
6. The patient's creatinine clearance declines to < 25 mL/min and persists for more than 14 days and the patient requires IV study drug and cannot tolerate oral study drug.
7. The patient has failed to engraft and requires chemotherapy.
8. The patient has relapsed and requires chemotherapy.
9. The patient becomes pregnant.
10. The patient withdraws consent.

The medical monitor or one of the protocol chairs should be contacted for advice about any of the above considerations for holding or withdrawal of the study drug due to adverse events.

All early study drug withdrawals should be reported to Data Coordinating Center within three business days. Even if withdrawal from study drug occurs for any of the above reasons, all study assessments should continue through the remainder of the one-year period.

If a patient is prematurely withdrawn from study drug prior to Day 100 (or 180) for reasons other than a probable or proven invasive fungal infection, only fluconazole will be allowed as prophylaxis until Day 100 (or 180) with permission of the Medical Monitor or Protocol Chair. No other antifungals are allowed for prophylaxis.

Refer to NCI's Common Terminology Criteria for Adverse Events (CTCAE) as a guideline for toxicity assessment. The CTCAE Document Version 3.0 can be accessed at the following web site: <http://ctep.cancer.gov/reporting/ctc.html>.

2.4.6.2. Breaking the blind

Study drug assignment may be revealed only for reasons relating to the patient's safety or when critical therapeutic decisions are contingent upon knowing the assigned study drug. A decision to break the blind must be discussed with the medical monitor or one of the protocol chairs in advance. Withdrawal of a patient from the study treatment is not a sufficient reason to break the study blind. Suggestions for antifungal therapy for patients withdrawn due to presumptive, probable or proven infection are provided in Section 2.4.9.

2.4.7. Contraindications, Drug Interaction and Monitoring

2.4.7.1 Voriconazole and fluconazole

Voriconazole is metabolized by three separate CYP 450 isoenzymes, including CYP3A4, CYP2C9 and CYP2C19. Fluconazole is also metabolized by the cytochrome P450 3A4 enzyme system and may interact with several medications. Clinically and potentially significant drug interactions that have been observed with fluconazole, as well as voriconazole (i.e., both azoles), include the following:

Co-administration of **terfenadine, cisapride, astemizole, quinidine, pimozide and dofetilide** with voriconazole and fluconazole is contraindicated. Serious cardiovascular adverse events, including death, ventricular tachycardia, torsades de pointes and prolonged QTc have been observed in patients taking other azoles concomitantly with these drugs. Voriconazole causes increased concentrations of terfenadine, cisapride and astemizole.

Other medications with documented drug interactions with fluconazole and voriconazole whose serum concentrations should be closely monitored, or the end effect closely monitored (e.g., blood sugar levels with sulfonylureas), include phenytoin, phenobarbital, digoxin, omeprazole, warfarin and sulfonylureas (can increase plasma levels which may lead to hypoglycemia) [46-51].

1. **Oral Hypoglycemics:** Clinically significant hypoglycemia may be precipitated by the use of fluconazole or voriconazole with oral hypoglycemic agents. One fatality has been reported from hypoglycemia in association with combined fluconazole and glyburide use. Fluconazole reduces the metabolism of tolbutamide, glyburide and glipizide, and increases the plasma concentration of these agents. Blood glucose concentrations should be carefully monitored and the dose of the sulfonylurea should be adjusted as necessary.
2. **Coumadin-Type Anticoagulants:** Prothrombin time may be increased with concomitant fluconazole or voriconazole and coumadin-type anticoagulants. Careful monitoring of prothrombin time is recommended.
3. **Phenytoin:** Fluconazole or voriconazole increase the plasma concentrations of phenytoin. Careful monitoring of phenytoin concentrations is recommended.
4. **Cyclosporine:** Fluconazole or voriconazole may significantly increase cyclosporine levels in renal transplant patients with or without renal impairment. Careful monitoring of cyclosporine concentrations and serum creatinine is recommended [52].
5. **Rifampin:** Rifampin enhances the metabolism of fluconazole. Depending on clinical circumstances, consideration should be given to increasing the dose of fluconazole if administered with rifampin.
6. **Theophylline:** Fluconazole (and probably voriconazole) increases the serum concentrations of theophylline. Careful monitoring of serum theophylline concentrations is recommended.

7. **Rifabutin:** There have been reports of uveitis in patients to whom fluconazole and rifabutin were co-administered. Patients should be carefully monitored. **The concomitant administration of voriconazole and rifabutin is contraindicated** (see inclusion criteria).
8. **Tacrolimus:** There have been reports of nephrotoxicity in patients in whom fluconazole or voriconazole and tacrolimus were co-administered. Patients should be carefully monitored.

Grapefruit juice should be avoided since it is known to interfere with the cytochrome P450 system. Certain herbal medicines (e.g., St. John's Wort) should be avoided for similar reasons.

2.4.7.2 Voriconazole drug interactions

Co-administration of voriconazole and immunosuppressive agents metabolized by cytochrome P450 3A4 may result in alterations in drug metabolism as follows:

1. **Cyclosporine:** In stable transplant recipients, voriconazole increased cyclosporine C_{max} and AUC (area under the blood concentration time curve to the last quantifiable measurement) by at least 13% and 170%, respectively. Trough cyclosporine concentrations increased by an average of 2.5 fold [52]. These observations have led to the recommendation that when initiating voriconazole in patients already receiving cyclosporine, the cyclosporine dose be halved and blood cyclosporine levels carefully monitored [59]. When voriconazole is discontinued, blood cyclosporine levels must be carefully monitored and the dose increased as necessary.
2. **Tacrolimus:** In healthy volunteers, voriconazole increased tacrolimus (0.1 mg/kg single dose) C_{max} and AUC by 117% and 221%, respectively. These observations have led to the recommendation that when initiating voriconazole in patients already receiving tacrolimus, the tacrolimus dose should be reduced to at least one third of the original dose and blood tacrolimus levels carefully monitored. When voriconazole is discontinued, blood tacrolimus levels must be carefully monitored and the dose increased as necessary.
3. **Sirolimus:** In healthy volunteers, voriconazole increased sirolimus C_{max} and AUC by 6.6 fold and 11.1 fold, respectively. **Voriconazole should not be co-administered with sirolimus.**
4. **Benzodiazepines:** In vitro studies demonstrate the potential for voriconazole to inhibit the metabolism of benzodiazepines (and thereby increasing plasma exposure). Frequent monitoring for adverse events and toxicity (especially prolonged sedation) related to benzodiazepines metabolized by cytochrome P450 3A4 (e.g., midazolam, triazolam, alprazolam) is recommended. Adjustment of benzodiazepine dosage may be needed.
5. **Statins:** In vitro studies demonstrate the potential for voriconazole to inhibit metabolism (thereby increasing plasma exposure). Frequent monitoring for adverse events and toxicity related to statins is advised. Increased statin concentrations in plasma have been associated with rhabdomyolysis. Adjustment of the statin dosage may be needed.
6. **Calcium channel blockers:** Voriconazole may increase the serum concentrations of this class of drugs (e.g., diltiazem, verapamil, felodipine). Frequent monitoring for adverse

events and toxicity related to calcium channel blockers is advised. Adjustment of calcium channel blocker dosage may be required.

2.4.8. Duration of Study Drug

Study drug will be continued without interruption through Day 100 unless the patient has met the early drug withdrawal criteria or has met the criterion for a presumptive, probable or proven fungal infection. In three circumstances, study drug may be continued beyond Day 100 and should be given in those instances until Day 180. An assessment between Day 90 and 100 should be made to determine if either of the following three conditions are met:

- Any patient who is receiving at least 1.0 mg/kg/d of prednisone or other steroid equivalent (based on actual or ideal body weight) during that assessment should continue study drug until Day 180.
- Recipients of T cell depleted transplants receiving post-transplant immunosuppression between Day 90 and 100 should continue to receive study drug until Day 180.
- Recipients of T cell depleted transplants with CD4+ counts < 200/uL should continue study drug until Day 180.

2.4.9. Recommendations for Treatment of Documented Fungal Infections

In the event of the patient demonstrating a presumptive, probable or proven invasive fungal infection as per the criteria of Section 3.1, the patient has reached the primary study endpoint, should stop study drug and the clinician is free to treat the infections according to local institutional guidelines. The following are offered as suggestions.

One option is to use amphotericin B and voriconazole together. This would provide adequate therapy for most fungal pathogens irrespective of which study drug the patient was assigned to and this strategy would ordinarily not require breaking the blind. Voriconazole is to be given at a dose of 6 mg/kg/dose IV twice daily for two days, then at a dose of 4 mg/kg/d thereafter. One can switch to the equivalent dose orally once the patient is stable and taking oral drugs satisfactorily. The dose of amphotericin B is 1.0 mg/kg/d if the conventional formulation is used or is 4.0-5.0 mg/kg/d if a lipid formulation is used. If the pathogen is mucormycoses, an amphotericin B formulation is the therapy of choice; voriconazole is not active against this pathogen and is not needed.

For patients with proven mucormycoses or probable fungal infection with filamentous fungal forms on cytologic or histologic section, which cannot exclude mucormycetes, an amphotericin B formulation (or other investigational therapy) is recommended. Voriconazole is not active against mucormycosis.

2.4.10. Guidelines for Empirical Antifungal Therapy with Amphotericin B or Caspofungin Usage for Patients not “Off Study”

Limited empirical antifungal therapy may be given using amphotericin B (or one of the lipid formulations of amphotericin B) or caspofungin. No other systemic antifungal is permitted without approval of the medical monitor. Empirical antifungal therapy is intended for patients strongly suspected to have invasive fungal infections where exhaustive diagnostic evaluation has excluded other etiologies but has also failed to demonstrate a presumptive, probable or proven invasive fungal infection as defined in Table 3.1.1a and b. It is generally intended for patients in whom the criteria for possible invasive fungal infection in Tables 3.1.1a and b are met. The guidelines for empirical therapy for the most common situations are below. In no case should it be continued beyond 14 consecutive days. During an empirical trial of antifungal therapy, efforts should continue to be undertaken to ascertain the etiology of the fever through cultures and biopsies of pertinent tissue specimens.

2.4.10.1. Required tests prior to use of empirical antifungal therapy

All focal lesions suspicious for fungi must be biopsied if clinically possible. Cultures of urine and blood must be obtained. Isolator culture methods are preferred if available in your institution. At least two blood samples for the diagnostic galactomannan assay should have been obtained during the seven days (with at least one within 48 hours) before institution of empirical antifungal therapy and should continue to be drawn along with blood samples for investigational fungal diagnostic assays during the empirical therapy. In addition, one Pharmacokinetics (PK) blood sample should be obtained prior to the start of empirical antifungal therapy.

2.4.10.2. Pre-engraftment requirements and amphotericin B/caspofungin dose

If the patient is neutropenic, has persistent fever ($\geq 38^{\circ}\text{C}$) of unknown origin refractory to at least 96 hours of broad-spectrum antibiotics or fever that recurs after 96 hours of antibiotics, and a specific etiology cannot be established, an empirical trial of either amphotericin B at a dose of 1.0 mg/kg/d (or one of the lipid formulations at a dose of 4-5 mg/kg/d) or caspofungin (in accordance with manufacturer prescribing guidelines) is permitted. Generally, the loading dose for caspofungin is 70 mg on day one and 50 mg/day on subsequent days for patients over 12 years of age. This may be continued until resolution of neutropenia ($\text{ANC} \geq 500$) but should not be continued beyond 72 hours after resolution of neutropenia and not longer than 14 consecutive days.

2.4.10.3. Post-engraftment requirements and amphotericin B/caspofungin dose

If the patient is post-engraftment and meets the criteria for possible infection in Table 3.1.1b and attempts to document etiology have failed, an empirical trial of an amphotericin B formulation or caspofungin is permitted. Continued radiographic procedures, cultures, biopsies, and other pertinent diagnostic evaluations should be performed in an effort to elucidate the suspected infection. If these measures fail, then the empirical trial should be terminated after no more than 14 days. If the physician feels that additional therapy is warranted this should be discussed with the medical monitor or one of the protocol chairs. The dose of amphotericin B or one of the lipid

formulations of amphotericin B should be 1.0 mg/kg/d (for conventional amphotericin B) or 4-5 mg/kg/d for the lipid formulations. The dose of caspofungin should be in accordance with manufacturing guidelines, generally, 70 mg on the first day followed by 50 mg/day on subsequent days for patients over 12 years of age.

2.4.10.4. Continuation of study drug during empirical antifungal therapy

There are no data to suggest antagonism of voriconazole or fluconazole with amphotericin B or caspofungin. So as to avoid fluctuations in the drug levels of concomitant immunosuppressive agents, it is recommended that the study drug be continued during empirical therapy.

If the clinician does not discontinue empirical amphotericin B or caspofungin within the 14 days, then the study drug will be withdrawn. The patient will continue to be monitored for all study assessments for the remainder of the study duration (one year) and continue to have galactomannan assays performed according to the study schedule.

2.4.11. Other Antifungal Therapy

Topical antifungal therapy is permitted and should be documented on the appropriate case report form. Nebulized or inhaled amphotericin B is not permitted. Systemic amphotericin B (or one of its lipid preparations) and caspofungin are the only systemic antifungal agents permitted on study but are only permitted as specified as empirical therapy for possible infection (or for presumptive, probable or proven invasive infection) as specified above in Section 2.4.10.

If a patient is prematurely withdrawn from study drug prior to Day 100 (or 180) for reasons other than a probable or proven invasive fungal infection, only fluconazole will be allowed as prophylaxis until Day 100 (or 180) with permission of the Medical Monitor or Protocol Chair. No other antifungals are allowed for prophylaxis.

Fungal prophylaxis must not be given after protocol-specified treatment plan is completed on Day 100 (or 180).

If a patient develops a presumptive, proven or probable fungal infection as specified in Table 3.1.1a and b, the clinician can employ other antifungal therapy as appropriate.

2.4.12. Use of the Galactomannan Assay

The galactomannan assay is used with host and clinical factors to determine the likelihood of invasive aspergillus infection. The galactomannan assay detects only aspergillus and penicillium. The sensitivity and specificity of the galactomannan assay are reported as 90% and 98% respectively [32]. The positive and negative predictive values are 88% and 98% respectively [32]. Many invasive fungi other than aspergillus can cause invasive infection without galactomannan positivity. Thus, a negative test does not exclude the possibility of infection by fungi.

A positive galactomannan assay satisfies only the microbiologic criterion for invasive fungal infection as defined in Tables 3.1.1a and b. In the event that a positive result is obtained, additional radiographic, cultural, and other diagnostic tests should be performed, and a repeat specimen for the galactomannan assay should be sent. The study drug should be continued while these investigations are underway.

There have been reports of false positive results using the galactomannan assay in patients receiving concomitant piperacillin-tazobactam (Zosyn). In addition, certain lots of Zosyn have tested positive. However, piperacillin alone does not test positive. As a result, positive galactomannan results in patients receiving piperacillin/tazobactam (Zosyn) should NOT be considered a microbiological criterion to support documentation of an invasive fungal infection. Other diagnostic methods should be pursued. Subsequent positive galactomannan results should not be considered as microbiological criteria until the patient has been off of Zosyn for 5 days.

One other secondary endpoint to be evaluated is the utility of the galactomannan assay to determine response to therapy. Accordingly, if the patient develops a presumptive, probable or proven invasive fungal infection, twice weekly monitoring of treatment galactomannan samples should be collected at onset and after initiation of treatment for the infection for four weeks, then once every two weeks for eight weeks for a total of 12 samples. These samples will be frozen and batch shipped to the Repository. The assays will be performed after completion of study enrollment at Dr. Kieren Marr's laboratory at FHCRC. The investigators will be blinded to the results.

For the galactomannan assays, all specimens are to be collected, processed, and transported in accordance with the procedures in Appendices C and D.

2.4.13. Supportive Care

Blood products must be irradiated.

Antibiotics and intravenous immunoglobulin should be given according to institutional practice.

Growth factors should be given according to institutional practice.

Granulocyte transfusions are not permitted as part of routine care.

2.4.13.1. Prophylaxis against infections

Patient should be given prophylaxis for:

1. **Pneumocystis carinii:** According to institutional practice.
2. **Herpes viruses (HSV, VZV):** According to institutional practice until neutrophil engraftment for HSV+ recipients.
3. **Encapsulated bacteria:** According to institutional practice.

The Manual of Procedures for Infection should be followed for patients with cytomegalovirus (CMV) infections. In general, the guidelines for CMV prevention jointly developed by the CDC/IDSA/ASBMT should be used. These can be accessed from the CDC website: <http://www.cdc.gov/mmwr/PDF/RR/RR4910.pdf>.

2.4.13.2. Identification of opportunistic infections

In the event that a patient develops fever, sinusitis, interstitial pneumonia, diarrhea, or hepatitis, all efforts will be made to identify the responsible organism. Cultures will include routine bacterial, mycobacterial, fungal, and viral cultures. Bronchial lavages and open lung biopsies will also be evaluated for pneumocystis carinii. If possible, these samples will also be evaluated for RSV, other respiratory viral pathogens, and legionella. Stool samples will also be evaluated for C. difficile toxin, cryptosporidium, and rotavirus. Samples will not be routinely sent for EM studies. If a GI biopsy is performed, evaluation for CMV with immunofluorescence and PCR should be considered.

When a fungal infection is suspected, radiographic studies and invasive diagnostic procedures will be obtained as deemed necessary by the investigator. CT scans of the chest are recommended in persistently febrile patients. CT scans of the sinuses are recommended in patients with symptoms of sinusitis. Biopsies should be obtained whenever possible of tissue from sites suspected to be infected. If biopsy is not feasible, BAL or a percutaneous needle biopsy should be performed for evaluation of pulmonary infiltrates. Assays for bacteria, CMV, fungi, PCP and respiratory viruses should be performed. If visual symptoms occur, an ophthalmologic consult should be obtained if signs or symptoms are persistent. Blood and urine fungal cultures should be obtained in all febrile patients and fungal cultures and stains obtained of all specimens of suspected infection.

For non-fungal infections, a Post-Transplant Infection Report Form should be completed for each infectious episode with a severity grade assigned for each known agent contributing to the episode. A Fungal Infection Report Form incorporating the fungal infection-specific definitions will be used for fungal infections.

2.4.14. Diagnosis of GVHD

2.4.14.1. Acute GVHD

Acute GVHD generally develops within the first three months after transplantation and appears as a characteristic dermatitis often accompanied by hepatic cholestasis and enteritis. The clinical appearance of skin GVHD can be mimicked by toxicity of the transplant conditioning regimen and by drug reactions. Therefore, documentation of the diagnosis by skin biopsy is recommended. Severity of liver GVHD is usually described according to the serum bilirubin level. Hepatic GVHD cannot be assessed solely on clinical grounds in patients who have concurrent drug toxicity, viral hepatitis, or toxicity caused by the pre-transplant chemotherapy and irradiation. Liver biopsy can be helpful but often cannot be done because of clinical contraindications such as thrombocytopenia. Gastrointestinal GVHD is characterized by watery diarrhea with anorexia, nausea and vomiting accompanied in more severe cases by abdominal cramps, gastrointestinal hemorrhage, and ileus. Eating often exacerbates symptoms. The volume of diarrhea has been used as an indicator of the severity of gut GVHD, but this can be inaccurate and highly variable from day to day. In many cases, it can be difficult to distinguish GVHD from infectious enteritis, and endoscopic biopsy is often helpful and should be done wherever possible. Prospective data related to symptoms of acute GVHD will be collected.

2.4.14.2. Chronic GVHD

Manifestations of chronic GVHD typically do not occur until three to twelve months after transplantation. Initial symptoms frequently include nausea, anorexia and weight loss, ocular and oral sicca, and skin changes. Rash characteristically appears with pigmentary changes, vitiligo, mottling, erythema, plaques, papules, nodules, poikiloderma, or exfoliation progressing to sclerosis and contractures. Hair loss and onychodystrophy may also indicate chronic GVHD. Cough and dyspnea with wheezing, rales, and abnormal PFTS may indicate pulmonary involvement. Diarrhea and abdominal pain may occur but are relatively infrequent. Liver involvement may be indicated by increased bilirubin and alkaline phosphatase and less frequently by increased transaminase levels.

2.4.15. Risks and Toxicities

Recipients of allogeneic blood or marrow transplants incur risks from pre-transplant conditioning and post-transplant therapy, which must be weighed against the risk of the disease for which the transplant is prescribed. Major risks following transplantation include: 1) Infection which can be of a fungal, bacterial, viral or parasitic nature. Often, these infections are life-threatening, particularly when caused by viral or fungal agents, and are associated with a high mortality rate in the transplant population; 2) GVHD, either acute or chronic in nature, may occur following allogeneic blood or marrow transplants. The degree of GVHD varies from mild cutaneous reactions to extensive widespread and systemic involvement of skin, liver, and gastrointestinal tract. Probably due to a direct association, the incidence of fatal infection is greater in patients developing GVHD; 3) Graft Failure can occur and is associated with a high risk of mortality; 4) End Organ Damage of all or any of the major organs may occur as a result of reactions to drugs (e.g., antibiotics, antifungal medications, etc.), and as a result of destructive processes (e.g.,

infection, GVHD, etc.) or radiotherapy, and may have a fatal outcome; 5) for patients transplanted for malignant disorders, Relapse of the underlying disease may occur, especially in patients with advanced disease status at time of transplant; 6) Unknown Toxicities may occur in any individual patient due to multiple events and cumulative effects which may involve any and all organs, including the brain. Brain damage can result in severe loss of cognitive or neurologic function; and 7) Death.

Damage to major body organs may include the brain, eyes, heart, lung, liver, and kidneys. Possible late effects may include growth retardation deformities, cataracts, changes in endocrine function, sterility, learning disabilities or brain damage, bone and joint abnormalities, and secondary malignancy.

For specific study drug risks/interactions refer to Sections 2.4.5 and 2.4.7.

For toxicity assessment, refer to NCI's Common Terminology Criteria for Adverse Events (CTCAE) as a guideline for toxicity assessment. The CTCAE Document Version 3.0 can be accessed at the web site: <http://ctep.cancer.gov/reporting/ctc.html>.

2.4.16. Genetic Variation Study Sampling: Single Nucleotide Polymorphisms from Donor

Subtle differences in different molecules of innate immunity may contribute to either the predisposition or clinical course of infection with filamentous fungi. The allelic frequencies of 24 different genes [MBL2 (mannose-binding lectin 2), CCR5 (chemokine receptor 5), IL1RN (interleukin-1 receptor antagonist), IL1A (interleukin-1 alpha), IL1B (interleukin-1 beta), IL6 (interleukin-6), IL8 (interleukin-8), IL8RA (interleukin-8 receptor type A), IL8RB (interleukin-8 receptor type B), IL10 (interleukin-10), TNFA (tumor necrosis factor alpha), TNFB (tumor necrosis factor beta), MPO (myeloperoxidase), NRAMP1 (natural resistance-associated macrophage protein 1), CHIT1 (chitotriosidase 1), FCGR2A (Fc-gamma receptor 2a), FCGR3A (Fc-gamma receptor 3a), FCGR3B (Fc-gamma receptor 3b), MICA (MHC, major histocompatibility complex, class I chain-related gene A), MICB (MHC class I chain-related gene B), TLR4 (toll-like receptor 4), CD14, HBD-1 (beta defensin), IL-18 (interleukin 18) and their intragenic polymorphic forms will be analyzed and this data will be compared to the incidence and severity of fungal infection. The purpose of this study is to identify a group of molecules of innate immunity, which may influence the risk and severity of invasive fungal infection. Single Nucleotide Polymorphisms (SNPs) of the genes encoding the following two cytochrome P450 proteins (3A4 and 2C19) will also be studied for a total of 26 genes.

CHAPTER 3

3. STUDY ENDPOINTS

3.1. Primary Endpoint

The primary endpoint for this study of prevention of invasive fungal infections in allogeneic blood and marrow transplants is fungal-free survival (proportion of patients alive and free from proven, probable or presumptive invasive fungal infection) at 180 days post-transplant.

Fungal infections graded as colonization, superficial infection or possible invasive infection (defined below) will not be counted as events for this endpoint. A fungal infection will be defined in accordance with the following consensus EORTC/MSG¹ criteria [53]:

Colonization: Defined as fungus or mould present in surveillance cultures in the absence of clinical findings of fungal infection.

Superficial Infection: Includes cutaneous fungal infections, oropharyngeal candidiasis and vulvovaginal candidiasis defined as:

1. Typical signs of mucocutaneous fungal infection
2. Positive fungal culture of scrapings, swab or exudate
3. Hyphae or pseudohyphae noted on Gram or other stain, or biopsy demonstrating invasive fungal elements
4. Patients who develop superficial infections will be treated with topical antifungals and continued on study medication

Invasive Infection: Defined as per Table 3.1.1a and 3.1.1b.

¹ European Organization for Research and Treatment of Cancer-Invasive Fungal Infection Cooperative Group/National Institutes of Allergy and Infectious Diseases-Mycoses Study Group.

Table 3.1.1a
Definitions of Invasive Fungal Infections in Patients with Cancer and Recipients of Hematopoietic Stem Cell Transplants

Category, Type of Infection	Description
Proven invasive fungal infections	
Deep tissue infections	
Moulds ^a	Histopathologic or cytopathologic examination showing hyphae from needle aspiration or biopsy specimen with evidence of associated tissue damage (either microscopically or unequivocally by imaging); or positive culture result for a sample obtained by sterile procedure from normally sterile and clinically or radiologically abnormal site consistent with infection, excluding urine and mucous membranes
Yeasts ^a	Histopathologic or cytopathologic examination showing yeast cells (<i>Candida</i> species may also show pseudohyphae or true hyphae) from specimens of needle aspiration or biopsy excluding mucous membranes; or positive culture result on sample obtained by sterile procedure from normally sterile and clinically or radiologically abnormal site consistent with infection, excluding urine, sinuses, and mucous membranes; or microscopy (India ink, mucicarmine stain) or antigen positivity ^b for <i>Cryptococcus</i> species in CSF
Fungemia	
Moulds ^a	Blood culture that yields fungi, excluding aspergillus species and <i>Penicillium</i> species other than <i>Penicillium marneffe</i> , accompanied by temporally related clinical signs and symptoms compatible with relevant organism
Yeasts ^a	Blood culture that yields <i>Candida</i> species and other yeasts in patients with temporally related clinical signs and symptoms compatible with relevant organism
Endemic fungal infections ^c	
Systemic or confined to lungs	Must be proven by culture from site affected, in host with symptoms attributed to fungal infection; if culture results are negative or unattainable, histopathologic or direct microscopic demonstration of appropriate morphological forms is considered adequate for dimorphic fungi (<i>Blastomyces</i> , <i>Coccidioides</i> and <i>Paracoccidioides</i> species) having truly distinctive appearance; <i>Histoplasma capsulatum</i> variant capsulatum may resemble <i>Candida glabrata</i>
Disseminated	May be established by positive blood culture result or positive for urine or serum antigen by means of RIA [17]
Probable invasive fungal infections	At least 1 host factor criterion (see Table 3.1.1b); and 1 microbiological criterion; and either a) 1 major (or 2 minor) clinical criteria from abnormal site consistent with infection for sites other than lower respiratory tract, or b) one of the clinical criteria for lower respiratory tract site

Category, Type of Infection	Description
Presumptive invasive fungal infection	<ul style="list-style-type: none"> • At least 1 host factor criterion (see Table 3.1.1b) and 1 clinical criterion for lower respiratory tract infection as listed below. A microbiological criterion is NOT required. The clinical criterion for lower respiratory tract infection must be consistent with the microbiological findings, if any, temporally related to current episode and other potential causes must have been eliminated, along with: <ul style="list-style-type: none"> - The presence of one of the following “specific” imaging signs on CT: (1) Halo sign, (2) wedge-shaped infiltrate, (3) air crescent sign, OR - The presence of a new non-specific focal infiltrate, PLUS at least one of the following: pleural rub, pleural pain, or haemoptysis, AND no evidence of other etiology demonstrated by bronchoscopic examination • Or if lacking a host criterion, but otherwise meets the microbiological and clinical criteria for a presumptive, probable or proven invasive fungal infection. The clinical criterion for lower respiratory tract infection must be consistent with the microbiological findings, if any, temporally related to current episode and other potential causes must have been eliminated, along with: <ul style="list-style-type: none"> - The presence of one of the following “specific” imaging signs on CT: (1) well-defined nodule with or without a halo sign, (2) halo sign, (3) wedge-shaped infiltrate, (4) air crescent sign, (5) cavity within area of consolidation OR - The presence of a new non-specific focal infiltrate, PLUS at least one of the following: pleural rub, pleural pain, or haemoptysis AND no evidence of other etiology demonstrated by bronchoscopic examination

Table 3.1.1a cont'd

Category, Type of Infection	Description
Possible ^d invasive fungal infections	At least 1 host factor criterion; and 1 microbiological criterion; or either a) 1 major (or 2 minor) clinical criteria from abnormal site consistent with infection for sites other than lower respiratory tract, or b) one of the clinical criteria for lower respiratory tract site

Notes for Table 3.1.1a

^a Append identification at genus or species level from culture, if available.^b False-positive cryptococcal antigen reactions due to infection with *Trichosporan beigelli* (1), infection with *Stomatococcus mucilaginosus* (2), circulating rheumatoid factor (3), and concomitant malignancy (4) may occur and should be eliminated if positive antigen test is only positive result in this category.^c Histoplasmosis, blastomycosis, coccidioidomycosis, and paracoccidioidomycosis.^d This category is not recommended for use in clinical trials of antifungal agents but might be considered for studies of empirical treatment, epidemiological studies, and studies of health economics**Table 3.1.1b****Host Factor, Microbiological, and Clinical Criteria for Invasive Fungal Infections in Patients with Cancer and Recipients of Hematopoietic Stem Cell Transplants**

Type of Criteria	Criteria
Host factors	Neutropenia (< 500 neutrophils/mm ³ for > 10 days) Persistent fever (≥ 38°C) for > 96 h refractory to appropriate broad-spectrum antibacterial treatment in high-risk patients Body temperature either > 38°C or < 36°C and any of the following predisposing conditions: prolonged neutropenia (> 10 days) in previous 60 days, recent or current use of significant immunosuppressive agents in previous 30 days, proven or probable invasive fungal infection during previous episode of neutropenia, or coexistence of symptomatic AIDS Signs and symptoms indicating graft-versus-host disease, particularly severe (Grade ≥ 2) or extensive chronic disease Prolonged (> 3 weeks) use of corticosteroids in previous 60 days
Microbiological	Positive result of culture for mould (including aspergillus, <i>Fusarium</i> , or <i>Scedosporium</i> species or Zygomycetes) or <i>Cryptococcus neoformans</i> or an endemic fungal pathogen ^a from sputum or bronchoalveolar lavage fluid samples Positive result of culture or findings of cytologic/direct microscopic evaluation for mould from sinus aspirate specimen Positive findings of cytologic/direct microscopic evaluation for mould or <i>Cryptococcus</i> species from sputum or bronchoalveolar lavage fluid samples Positive result for aspergillus antigen in blood samples as defined in Section 3.2 Positive result for aspergillus antigen in specimens of bronchoalveolar lavage fluid or CSF Positive result for cryptococcal antigen in blood sample ^b Positive findings of cytologic or direct microscopic examination for fungal elements in sterile body fluid samples (e.g., <i>Cryptococcus</i> species in CSF)

Table 3.1.1b cont'd

Type of Criteria	Criteria
Microbiological (cont'd)	Positive result for <i>Histoplasma capsulatum</i> antigen in blood, urine, or CSF specimens [17] Two positive results of culture of urine samples for yeasts in absence of urinary catheter <i>Candida</i> casts in urine in absence of urinary catheter Positive result of blood culture for <i>Candida</i> species
Clinical	Must be related to site of microbiological criteria and temporally related to current episode
Lower respiratory tract infection	<ul style="list-style-type: none"> The presence of one of the following “specific” imaging signs on CT: (1) well defined nodule(s) of at least 1 cm in diameter with or without a halo sign, (2) wedge-shaped infiltrate, (3) air crescent sign, or (4) cavity within area of consolidation^c, OR The presence of a new non-specific focal infiltrate, PLUS at least one of the following: pleural rub, pleural pain or hemoptysis
Sinonasal infection	
Major	Suggestive radiological evidence of invasive infection in sinuses (i.e., erosion of sinus walls or extension of infection to neighboring structures, extensive skull base destruction)
Minor	Upper respiratory symptoms (e.g., nasal discharge, stuffiness); nose ulceration or eschar of nasal mucosa or epistaxis; periorbital swelling; maxillary tenderness; black necrotic lesions or perforation of hard palate
CNS infection	
Major	Radiological evidence suggesting CNS infection (e.g., mastoiditis or other parameningeal foci, extradural empyema, intraparenchymal brain or spinal cord mass lesion)
Minor	Focal neurological symptoms and signs (including focal seizures, hemiparesis, and cranial nerve palsies); mental changes; meningeal irritation findings; abnormalities in CSF biochemistry and cell count (provided that CSF is negative for other pathogens by culture or microscopy and negative for malignant cells)
Disseminated fungal infection	Papular or nodular skin lesions without any other explanation; intraocular findings suggestive of hematogenous fungal chorioretinitis or endophthalmitis
Chronic disseminated candidiasis	Small, peripheral, target-like abscesses (bull’s-eye lesions) in liver and/or spleen demonstrated by CT, MRI, or ultrasound, as well as elevated serum alkaline phosphatase level; supporting microbiological criteria are not required for probable category
Candidemia	Clinical criteria are not required for probable candidemia; there is no definition for possible candidemia

Notes for Table 3.1.1.b

a *H. capsulatum* variant *capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, or *Paracoccidioides brasiliensis*.

b See Table 3.1.1a footnote b for causes of false-positive reactions that must be considered and eliminated from consideration.

c In absence of infection by organisms that may lead to similar radiological findings including cavitation, such as *Mycobacterium*, *Legionella*, and *Nocardia* species.

3.2. Secondary Endpoints

The secondary endpoints are:

1. Frequency of invasive fungal infections
2. Time to invasive fungal infection
3. Six-month and one-year patient survival
4. Empirical use of amphotericin B or caspofungin, frequency and duration of use
5. Time to and severity of acute and chronic graft-versus-host disease (GVHD)
6. Utility of galactomannan assay in diagnosis of aspergillus and response to therapy
7. Time to neutrophil engraftment
8. Time to platelet engraftment
9. Freedom from possible, presumptive, probable, or proven invasive fungal infection, death, or withdrawal of study drug due to toxicity, intolerance, or an empirical trial of amphotericin B or caspofungin > 14 consecutive days.

Other complications that are ordinarily seen during the course of BMT will be captured in BMT CTN and IBMTR follow-up forms. Planned data to be collected, tabulated and analyses to be performed include: association of CD4 counts with infection risk, rates of hepatotoxicity and hepatic GVHD in the two cohorts, drug levels on Day 14 and 28 as they relate to subsequent infection and toxicities, in vitro fungal susceptibility and occurrence of breakthrough infection and response to therapy, and rates of possible fungal infections, fever of uncertain etiology, and pulmonary infiltrates of uncertain etiology. Galactomannan levels will be collected, tabulated and analyses of these data with various endpoints will be performed.

Neutrophil Engraftment: Neutrophil engraftment is defined as achieving ANC $\geq 500/\text{mm}^3$ for three consecutive measurements over three or more days by Day 42. The first of the three measurements may occur on Day 42. A patient receiving a second stem cell infusion prior to Day 42 will be considered a graft failure.

Platelet Engraftment: Platelet engraftment will be defined as the first day of a minimum of three consecutive measurements over three or more days such that the patient:

1. Has achieved a platelet count $> 50,000/\text{mm}^3$, and
2. Is platelet transfusion independent for a minimum of seven days.

Acute and Chronic GVHD: Acute GVHD usually develops within the first three months after transplantation and appears as a characteristic dermatitis often accompanied by cholestasis and enteritis. Initial symptoms of chronic GVHD frequently include nausea and anorexia with ocular and oral sicca. Rash characteristically appears with pigmentary changes progressing to sclerosis and contractures. Other organs may be involved. Symptoms may mimic those seen in patients with scleroderma and other autoimmune disorders.

The staging of acute GVHD will follow NMDP guidelines but will include weekly capture of symptoms and characterization of alternative causes. The highest level of organ abnormalities, the etiologies contributing to the abnormalities and biopsy results pertaining to GVHD will be identified. Staging and grading of acute GVHD are listed in the BMT CTN Manual of Procedures (MOP).

Chronic GVHD typically does not occur until three or more months after transplantation. Details regarding the definition and diagnosis are listed in the BMT CTN MOP.

Infection Grading: Definitions for fungal infections are provided in Section 3.1.1. Non-fungal infections will be graded according to the severity scale defined in the BMT CTN Data Management Handbook.

Galactomannan Positivity: A positive galactomannan assay is defined as two positives on the same specimen or two consecutive positives on different specimens, i.e., a repeat test on the first specimen was not performed. Sera with an index less than 0.5 are considered to be negative. Sera with an index greater than or equal to 0.5 are considered to be initially positive.

Whenever a sample has an index ≥ 0.5 , positivity must be confirmed by re-testing the sample, including repeating the heat treatment on a new aliquot, and by testing another sample obtained from the patient. This confirmation is necessary in order to eliminate any false-positive results due to contamination of the sample after collection.

3.3. Safety Monitoring Endpoints

The rates of neural, cardiac, hepatic, and renal toxicities will be monitored up to 100 days post-transplant, separately in each treatment arm. The definitions of these toxicities are described below:

1. Neural toxicity is defined as convulsions.
2. Cardiac toxicity, defined as grade III toxicity on the Bearman scale, is severe EKG abnormalities with no or partial response to medical intervention; or, heart failure with no or minor response to medical intervention; or, decrease in voltage by more than 50%.
3. Hepatic toxicity, defined as grade III toxicity on the Bearman scale, is severe hepatic dysfunction with bilirubin > 20 mg/dL; or, hepatic encephalopathy; or, ascites compromising respiratory function.
4. Renal toxicity is defined as use of dialysis.

Monitoring will be performed monthly until enrollment to that treatment arm is closed. Each month, the null hypothesis that the rate of convulsions is less than or equal to 10% will be tested against the alternative that it is greater than 10%. Similarly, the rate of hepatic and renal toxicities will be compared to thresholds of 8% and 10% respectively. The expected threshold for cardiac toxicity is 2% and a formal monitoring plan was not developed. If the calculated rates are significantly greater than the threshold rates (as defined in section 5.3.2), the NHLBI will be consulted to determine if the Data Safety and Monitoring Board should convene to review the data.

3.4. Definition of Morphologic Complete Remission

Complete remission (CR) will be defined as all of the following according to the revised recommendations of the international working group [61]:

1. A bone marrow aspirate containing spicules with < 5% blasts with a count of at least 200 nucleated cells and no Auer rods seen. If spicules are absent in the aspirate, a bone marrow biopsy should confirm that < 5% blasts are present.
2. No evidence of a persistently abnormal leukemic population by flow cytometry.
3. ANC > 1000/ μ L and platelet count > 100,000/ μ L.
4. No extramedullary leukemia.
5. No blasts in peripheral blood.

CHAPTER 4

4. PATIENT ENROLLMENT AND EVALUATION

4.1. Enrollment and Randomization

4.1.1 Screening and Enrollment Procedures

Patients will be registered using the BMT CTN Electronic Data Capture System (AdvantageEDCSM). The following procedures should be followed:

1. An authorized user at the clinical center completes the initial screening by entering patient demographics, Segment O information (date informed consent signed) and Segment A information (inclusion/exclusion criteria and HLA typing information) of the Eligibility form preferably within 10 days prior to the initiation of the conditioning regimen and no later than 72 hours prior to transplant (Day 0).
2. If patient is eligible, an ALT test should be obtained within 72 hours of Day 0.
3. An authorized user at the clinical center completes the enrollment process by entering Segment B (dates of conditioning and proposed transplant, ALT value, baseline GM sample and CT scan) of the Eligibility Form within 72 hours of Day 0.
4. If the patient is eligible, a study number and random treatment assignment is generated.

If a connection is interrupted during a randomization session, the process is completely canceled and logged. A backup manual registration and randomization system will also be available to provide for short-term system failure or unavailability.

4.1.2 Randomization

Patients will be randomized within 72 hours prior to Day 0. Patients will be stratified by center and donor type (related vs. unrelated). Patients will be assigned to either voriconazole or fluconazole in a 1:1 ratio.

4.2. Study Monitoring

4.2.1. Follow-up Schedule

The Follow-up Schedule for scheduled study visits is outlined in Table 4.2.1. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide.

Follow-up Assessments: The timing of follow-up visits is based on the date of allogeneic blood or marrow transplant. Following randomization, the Transplant Center can print a Patient Visit Schedule listing target dates for assessments. Weeks 1-14, Day 120, and Day 150 visits are

primarily for fungal assessments. The subsequent visits are for follow-up reports. Galactomannan (GM) and investigational fungal diagnostic assays will be done up to twice weekly during the first 100 days. In addition, blood for GM and fungal diagnostic assays will be collected from patients with possible, presumptive, probable or proven infection as stated in Section 4.2.2. All efforts should be made to obtain the samples. Failure to obtain all of the samples does not constitute a protocol deviation.

Criteria for Forms Submission: Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook and User's Guide. Forms that are not submitted to the DCC within the specified time will be considered delinquent. Transplant Centers can view past due forms via the web-based electronic data capture system. A missing form will continue to appear until the form is entered into AdvantageEDC, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Data Management Handbook and User's Guide.

Reporting Patient Deaths: The Recipient Death Information must be entered into the web-based electronic data capture system within one business day of knowledge of a patient's death. If the cause of death is unknown at that time, it need not be recorded at that time. However, once the cause of death is determined, the form should be updated.

CIBMTR Data Reporting: All transplant centers will be required to pre-register all of their transplant patients, not just those participating in BMT CTN protocols, with the Center for International Blood and Marrow Transplant Research (CIBMTR). The IBMTR Day 100 Report Form (including the Core, Graft and Disease Inserts) and IBMTR Follow-up Form (including the Core and Disease Inserts) for patients participating in the BMT CTN protocols will be submitted directly to the CIBMTR at the times specified in the Data Management Handbook and User's Guide.

Table 4.2.1
FOLLOW-UP SCHEDULE

Assessment Time	Target Day¹ (Days Post-BMT)
1 week ²	7 days
2 week	14 days
3 week	21 days
4 week	28 days
5 week	35 days
6 week	42 days
7 week	49 days
8 week	56 days
9 week	63 days
10 week	70 days
11 week	77 days
12 week	84 days
13 week	91 days
100 day	100 days
120 day	120 days ³
150 day	150 days ³
6 month	180 days ³
9 month	270 days ³
12 month	365 days ³

¹ Target day range = ± 2 days up to Day 100, ± 14 days for Day 120 and 150, and ± 28 days for Day 180, 270 and 365 post-transplant.

² Samples for galactomannan (GM) assays will be drawn twice weekly on two non-consecutive days, preferably three days apart (e.g., Mondays and Thursdays), during the first 60 days, once weekly during Days 61-100 unless ONE of the following is met: (1) patient received a T cell depleted transplant and received post-transplant GVHD prophylaxis, or (2) patient is on steroids, or (3) patient has or has had acute GVHD requiring systemic therapy. These select patients will have GM samples collected twice weekly during Days 61-100. Any patient that has possible, presumptive, probable or proven infection will have additional samples collected for GM assays as described in Section 4.2.2. Investigational fungal diagnostic blood samples will be collected at each of these time points.

³ Data will be collected by telephone contact with the patient and/or treating physician if patient is unable to return to the transplant center.

4.2.2. Assessments

Pre-transplant required for study

1. History, physical examination, weight, height, and BSA according to institutional practice (preferably within one week of initiation of the conditioning regimen). Vital signs within 48 hours of initiation of the conditioning regimen.
2. Serum creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total bilirubin within 30 days of initiation of the conditioning regimen (preferably within 7 days)
3. A CT scan of the chest (with contrast) and/or sinuses (without contrast) must be obtained within three weeks (one week preferable) prior to initiation of conditioning regimen if the patient has respiratory or sinus symptoms, or a history of pulmonary infection within the preceding three months
4. Patients with signs or symptoms of respiratory infection or hepatosplenic candidiasis (e.g., elevated alkaline phosphatase) should be evaluated with appropriate CT scans within three weeks (one week preferable) prior to initiation of conditioning regimen
5. A pregnancy test must be obtained in women of childbearing age within 30 days prior to start of the conditioning regimen (test must be negative to be eligible for study)
6. Karnofsky or Lansky performance status within one week of initiation of the conditioning regimen
7. Whole blood (5 mL) to be cryopreserved at the transplant center for future testing (e.g., infectious disease testing) if consent obtained. This should be collected in a red top tube and processed per Appendix C.
8. Whole blood or stem cell product from donor (5 mL) for SNPs assay if consent obtained. This should be collected in a green top tube and processed per Appendix C.
9. EKG to exclude prolonged QTc syndrome

Pre-transplant generally performed for transplant eligibility

1. CBC (including hemoglobin and hematocrit), differential, platelet count within 48 hours of initiation of the conditioning regimen
2. Serum creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total bilirubin within one week of initiation of conditioning regimen
3. Urinalysis < 48 hours of initiation of the conditioning regimen
4. ABO and Rh typing
5. Serum chemistries including sodium, potassium, chloride, bicarbonate, blood urea nitrogen, phosphate, calcium, albumin, and total protein \leq one week of initiation of conditioning regimen and \leq 72 hours of Day 0
6. Cardiac evaluation: echocardiogram or MUGA with ejection fraction (or shortening fraction if appropriate) in accordance with institutional policies

7. Pulmonary function evaluation: pulmonary function tests in accordance with institutional policies
8. Serology for CMV, HSV, HIV, toxoplasmosis, varicella, hepatitis B-surface antigen, hepatitis B-core antibody and hepatitis C in accordance with institutional policies
9. Sample of peripheral blood (3 mL) from donor and recipient to be stored for chimerism studies at the home institution. This should be collected in a purple top tube and processed according to local institutional practices.
10. A lumbar puncture is recommended if the patient has a history of CNS disease.

Post-transplant required for study

1. Pre-engraftment: CBC at least 3 times weekly until neutrophil engraftment (once $ANC \geq 500/mm^3$ for three consecutive days). Differential if $WBC \geq 500/mm^3$.
2. CBC and Absolute CD4 counts measured at Day 100 post-transplant.
3. Peripheral blood samples (5 mL) for analysis of fluconazole or voriconazole concentrations collected at Day 14 and Day 28, and at onset of possible, presumptive, probable or proven infection (prior to start of amphotericin B or caspofungin) and at time of any suspected study drug toxicities delineated in Section 2.4.6. Blood is to be collected during one of the windows relative to study drug administration as delineated in Appendix C. Pediatric patients less than 12 years old will provide 2.5 mL of blood.
4. Serum creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total bilirubin, on a weekly basis during the first 100 days or more frequently if dictated by the condition of the patient, and if the patient is still on study drug at Day 120, 150 and 180.
5. Blood samples (5 mL for adult, 2.5 mL for pediatric patients less than 12 years old) will be obtained twice weekly on two non-consecutive days, preferably three days apart (e.g., Mondays and Thursdays), from Day 0 to Day 60 for diagnostic galactomannan testing and processed in accordance with Appendices C and D. In addition, blood samples will be obtained once weekly from Days 61 to 100 unless ONE of the following is met: (1) patient received a T cell depleted transplant and received post-transplant GVHD prophylaxis, or (2) patient is on steroids, or (3) patient has or has had acute GVHD requiring systemic therapy. Blood samples from these patients will be collected twice weekly from Days 61 to 100. In addition, at least two samples within the week before (and one within the last 48 hours) should be collected at initiation of empirical antifungal therapy for possible infection. Collection of blood for diagnostic galactomannan (GM) assays should be continued twice weekly in patients receiving empirical antifungal therapy (per Section 2.4.10) for possible invasive fungal infection.
6. Collection of blood for diagnostic GM samples should be continued once to twice weekly until Day 100 in patients who have been prematurely withdrawn from study drug for reasons other than presumptive, probable or proven fungal infection (see Section 2.4.6.1).
7. For patients prematurely withdrawn from study drug due to presumptive, probable or proven aspergillus infection, monitoring of treatment blood GM samples (5 mL for adult, 2.5 mL for pediatric patients < 12 years old) should be collected at onset and twice

weekly for four weeks and then once every two weeks for eight weeks (for a total of 12 samples) to monitor response. Collection of blood for diagnostic GM samples after treatment of presumptive, probable or proven infection is at the discretion of the investigator.

8. Blood samples (10 mL for adult; 5.0 mL for pediatric patients less than 12 years old) for investigational fungal diagnostic (IFD) assays will be collected at the same frequency as the diagnostic GM blood samples. Collection of IFD blood samples for patients with presumptive, probable or proven fungal infection will be at the same frequency as the monitoring of treatment GM samples and then 1-2x weekly until Day 100.
9. The center may make adjustments in blood volumes collected from patients less than 12 years old to meet the institution's Human Investigation Committee Guidelines.
10. For any patient receiving a bronchoalveolar lavage, a 1 mL sample should be taken and stored in a cryovial at -70°C .

Post-transplant generally performed for transplant recipient

1. CMV surveillance should be performed according to institutional policy.
2. Post-engraftment: CBC and platelet count 3 times weekly until discharge from initial hospitalization; Post-discharge: CBC and platelet count weekly until PRBC and platelet transfusion independent, and at Day 100, 180, and 365. Differential should be done if WBC is between $500\text{-}1500/\text{mm}^3$ at any time post-engraftment and at Day 100, 120, 150, 180 and 365.
3. Vital signs taken at least weekly up to Day 100 post-transplant and obtained from local health provider at Day 120, 150, 180 and 365.
4. Clinical assessment weekly until Day 100, then by telephone contact at Day 120, 150, 180 and 365 to ascertain whether any signs, symptoms or clinical events have occurred that suggest infection.
5. Serum chemistries including sodium, potassium, chloride, bicarbonate, blood urea nitrogen, phosphate, calcium, and albumin, total protein on a weekly basis during the first 100 days or more frequently if dictated by the condition of the patient, and at Day 120, 150 and 180.
6. Serum creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total bilirubin at Day 120, 150 and 180.
7. Karnofsky/Lansky assessments by history and physical examination at Day 180 and 365.
8. Cyclosporine (or tacrolimus) blood levels should be assessed closely during the first one to two weeks of study and doses adjusted in accordance with institutional policy. This is important for patient safety because of drug interactions (see Section 2.4.7). Measurement of cyclosporine or tacrolimus levels three times or more during the first week may be necessary initially until drug levels are optimized. Clinical and laboratory assessments of cyclosporine or tacrolimus toxicities should also be monitored closely.
9. Sample of peripheral blood (3 mL) or bone marrow from patient for chimerism studies to document engraftment.

4.2.3. Fungal Infection (possible, presumptive, probable or proven)

1. Specimens from sites of suspected infection and blood will be obtained and analyzed for fungi as clinical symptoms warrant. All isolates of yeast or mould organisms from blood or tissue, if available, should be obtained by the PI, stored at the local institution and shipped to the Study Repository in accordance with Appendix C. Antifungal susceptibility testing will be conducted by the University of Florida.
2. CT scans of chest and sinuses should be obtained at diagnosis and preferably at least after 6 and 12 weeks of therapy and otherwise as clinically appropriate.
3. For patients with possible aspergillus infection, at least two blood samples within the week before (and one within the last 48 hours) should be collected for diagnostic galactomannan assays upon initiation of empirical antifungal therapy with amphotericin B or caspofungin for possible infection. Collection of blood for diagnostic galactomannan assays should be continued twice weekly in patients receiving empirical antifungal therapy (per Section 2.4.10) for possible invasive fungal infection. These samples should be shipped immediately to a certified reference lab. Investigational fungal diagnostic samples should be collected at the same frequency as the diagnostic galactomannan samples.
4. For patients with presumptive, probable or proven aspergillus infection, monitoring of treatment galactomannan samples should be collected at onset and twice weekly for four weeks and then once every two weeks for eight weeks (for a total of 12 samples) to monitor response. These samples should be frozen and batched shipped quarterly to the repository. Investigational fungal diagnostic samples should be collected at the same frequency as the monitoring of treatment galactomannan samples and then 1-2x weekly until Day 100.
5. Peripheral blood sample (5 mL for adult patients, 2.5 mL for pediatric patients less than 12 years old) for analysis of fluconazole or voriconazole concentrations to be collected and processed as per Appendix C.
6. The center may make adjustments in blood volumes collected from patients less than 12 years old to meet the institution's Human Investigation Committee Guidelines.
7. Paraffin blocks of tissue specimens demonstrating fungal infection should be saved and shipped to the Study Repository in accordance with Appendix C.

4.2.4. Discontinuation of Study Drug

1. Upon discontinuation of study drug, all patients will be assessed with regards to drug efficacy in terms of the fungal definition criteria described in Section 3.1.1.
2. A physical examination including vital signs will be performed within 72 hours of discontinuing study drug.
3. If previously positive, CT scans of chest or sinus should be performed within a 72-hour window of study drug discontinuation and if abnormality suspected or proven to be fungal in nature is still present then pertinent studies should be repeated approximately

two weeks following study drug completion and subsequently repeated until resolution of clinical or radiographic signs of infection, generally at no more than 6 week intervals.

4. Other follow-up diagnostic tests should be obtained as deemed appropriate by each center's principal investigator.
5. Peripheral blood sample (5 mL for adult patients, 2.5 mL for pediatric patients less than 12 years old) for analysis of fluconazole or voriconazole concentrations (as per Appendix C).
6. Any patient removed from study drug still should continue to have all study assessments performed up through Day 365.

4.2.5. GVHD Monitoring

GVHD should be monitored in accordance with BMT Clinical Trials Network guidelines as specified in the Manual of Procedures, i.e., weekly until Day 70.

Follow-up GVHD assessments should be obtained at Day 90, 120, 180 and 365.

4.2.6. Serious Adverse Event Reporting

Unexpected grade 3-5 Adverse Events (AEs) will be reported through an expedited AE reporting system via the web-based electronic data capture system, AdvantageEDC. Unexpected grade 4-5 AEs must be reported within 24 hours of knowledge of the event. Unexpected grade 3 AEs must be reported within 3 business days of knowledge of the event. Toxicities will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 at regular intervals as defined on the Form Submission Schedule. Decision to discontinue or re-administer the study drug will be made on a case-by-case basis after discussion with the Medical Monitor or one of the Protocol Chairs.

Table 4.2.2 REQUIRED ASSESSMENTS

	X = Required O = Generally Performed	Days Post Transplant													
		Baseline ⁷	7	14	28	30	42	60	90	100	120	150	180	270	365
Pregnancy test (females only)	X														
CT scan of the chest ¹	X														
CT scans of sinus and abdomen ²	X	X-At time of possible, presumptive, probable or proven fungal infection, Refer to Section 4.2.3													
History, physical exam, weight, height, BSA, and Karnofsky/Lansky performance status	X											O ³		O ³	
CBC ⁴ , differential ⁵ and platelet count ⁴	X	X-Refer to Section 4.2.2 for frequency								O	O	O	O		O
CD4 Counts										X					
EKG	X														
Vital signs and clinical assessment	X	Generally performed weekly until Day 100								O	O	O		O	
Serum chemistries	X	X-Refer to Section 4.2.2 for frequency but at least one per week until Day 100								X ⁹	X ⁹	X ⁹			
Diagnostic galactomannan assay ⁸ and investigational fungal diagnostic (IFD) assay ¹⁰	X	X-Refer to Section 4.2.4 for frequency; twice weekly until Day 60 and at least once weekly Day 61-100. Also prior to and during empirical trials of amphotericin B or caspofungin for possible fungal infection.													
Monitoring of treatment galactomannan assay ⁸ and IFD assay		X-At onset and twice weekly for 4 weeks and then once every 2 weeks for 8 weeks for a total of 12 samples for patients with presumptive, probable or proven aspergillus infection. IFD continue 1-2x weekly until Day 100.													
Whole blood sample: 5 mL (Pharmacokinetics)			X	X	X-Day 14 and 28 and at onset of possible, presumptive, probable or proven fungal infection or serious suspected drug toxicity as described in Section 2.4.6.										
Donor whole blood or stem cell product 5 mL for SNPs	X														
Whole blood 5 mL ⁶	X														
Bronchoalveolar Lavage		X-At any time procedure is performed													
Fungal Isolates		X-At time of possible, presumptive, probable or proven infection and every seven days if available following the initiation of antifungal therapy or if infection relapses after therapy discontinued													
Paraffin Blocks of Infected Tissues		X-At time of infection													

¹ Obtain within 6 weeks (one week preferable) prior to randomization if the results of a baseline galactomannan assay are not available prior to randomization.

² Refer to Section 4.2.2.4 for conditions.

³ Physical exam only.

⁴ Post-discharge CBC & platelet count must be done weekly until PRBC and platelet transfusion independent and at Day 100, 180, 365.

⁵ Differential must be done if WBC = 500-1500/mm³ at any time post-engraftment and at Day 100, 180, 270, 365.

⁶ To be cryopreserved at the transplant center for future testing.

⁷ Baseline refers to prior to conditioning. Hepatic function tests should be repeated within 72 hours of start of study drug. See Section 4.2.2.

⁸ Refer to Section 4.2.2 for frequency in patients with possible, presumptive, probable or proven infections.

⁹ If patient is on study drug until Day 180.

¹⁰ Investigational fungal diagnostic assays are not required at baseline.

CHAPTER 5

5. STATISTICAL CONSIDERATIONS

5.1. Study Design

The study is designed as a Phase III, randomized, blinded, multicenter, prospective comparative study of fluconazole versus voriconazole in the prevention of fungal infections in allogeneic transplant recipients. The target enrollment is 600 patients.

Accrual

It is estimated that three years of accrual will be necessary to enroll the targeted sample size. Both Core and non-Core Centers will enroll patients on this study. Accrual will be reported by race, ethnicity, gender, and age (pediatric patients will be defined as < 18 years).

Randomization

Patients will be registered using the electronic data capture system, AdvantageEDC. Only patients who have completed the screening process as described in Chapter 4 will be eligible for randomization. All patients will be randomized within 3 days prior to the day of transplant. Randomization will be performed in a 1:1 ratio using random block sizes for the voriconazole and fluconazole arms. Randomization will be stratified by transplant center and donor type (sibling versus unrelated donor).

Primary Endpoint

The primary endpoint is the fungal-free survival proportion at 180 days post-transplant. The primary analysis will be performed using the intent-to-treat principle so that all randomized patients will be included in the analysis. Death or the occurrence of a presumptive, probable or proven invasive fungal infection by Day 180 will be considered events for this endpoint.

Primary Hypothesis

The primary hypothesis of the study is that the voriconazole treatment will improve Day 180 fungal-free survival as compared to the fluconazole treatment.

$$H_0: p_v = p_f$$

$$H_a: p_v \neq p_f$$

5.2. Sample Size and Power Considerations

Fungal-free survival at 180 days will be compared between the standard and experimental therapy arms using the standardized difference in the Kaplan Meier estimates of survival. The final analysis will be performed after all patients have been followed for a minimum of 180 days

post-transplant. At this time point, all individuals will have been completely observed for the primary outcome. However, the Kaplan Meier test will be used rather than a binomial test of proportions in order to ensure consistency with the results of interim analysis conducted with incomplete follow-up. The sample size of 300 patients per group is sufficient to maintain type I error of 5% across all planned interim analyses (see below) while providing > 80% statistical power for a two-sided test to detect an increase in the proportion surviving fungal-free at 180 days from 0.50 in the standard therapy arm to 0.62 in the experimental arm.

5.3. Interim Analysis and Stopping Guidelines

Interim analyses for efficacy will be conducted at times coincident with regularly scheduled meetings of the NHLBI-appointed Data and Safety Monitoring Board (DSMB) at approximately six month intervals. Monitoring of key safety endpoints will be conducted monthly, and if rates significantly exceed pre-set thresholds, the NHLBI will be consulted to determine if the DSMB should be advised and if an ad-hoc meeting will be convened. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures.

5.3.1. Interim Analysis for Efficacy

Analyses will be performed as described below for the primary endpoint. Toxicity, adverse events, and other safety endpoints will be monitored regularly and reported to the DSMB at each interim analysis.

At the time of each interim analysis, the test statistic based on the Kaplan-Meier proportion will be compared to the critical value shown below. All patients randomized prior to the time of the interim analyses will be used to compute the Kaplan-Meier estimate. If the test statistic exceeds the critical value, the DSMB will discuss the continuation of the trial.

At each interim analysis time point, a two-sided test to detect either an increase or decrease in the proportion of patients with fungal-free survival at 180 days will be performed, as described above for the final analysis. In order to preserve the over-all type I error rate at 5%, the critical value for the test statistic will be inflated above 1.96, the value that would be used if no repeated testing were used. Equivalently, the nominal p-value at which an observed difference is declared significant will be reduced below 0.05. The actual critical values and nominal p-values will be computed using statistical methods for group sequential testing with O'Brien Fleming boundaries.

As an example, Table 5.3.1a shows the critical values and nominal p-values for tests conducted at 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 years after the study opens to enrollment. The column labeled "followed to 180 days" shows the average number of individuals who have reached the 180-day post-transplant follow-up time point, assuming uniform accrual over a three-year period. The fraction of patients followed to 180 days, as compared to a denominator comprised of the total sample of 600, quantifies the "statistical information" from which the critical values, nominal p-values and cumulative type I error are computed.

Table 5.3.1a Critical Values and Nominal P-Values

Calendar Time	Followed to 180 Days	Critical Value	Nominal P-value	Cumulative Type I Error
1.0	100	5.3660	.00000	.00000
1.5	200	3.7107	.00021	.00021
2.0	300	2.9697	.00284	.00305
2.5	400	2.5386	.00905	.01210
3.0	500	2.2522	.01605	.02815
3.5	600	2.0447	.02185	.05000

In practice, the rate of accrual or timing of DSMB meetings may not be as anticipated. To permit necessary flexibility in scheduling interim analyses, the critical values will be recomputed to correspond to the actual available statistical information using the “use-function” approach of Lan and DeMets.

Operating Characteristics of the Design

Under the assumption that time to infection or death is exponentially distributed, the statistical power to reject the null hypothesis of equal 180 day survival is shown below under a variety of scenarios.

Table 5.3.1b – Power to Reject the Null Hypothesis under Various Scenarios

N per Arm	Proportion Surviving Fungal-Free at Day 180		Power at Interim and Final Analyses By Year of Scheduled Analysis						Overall Power
	Standard	Experiment	1.0	1.5	2.0	2.5	3.0	3.5	
275	.50	.50	.0000	.0003	.0030	.0114	.0167	.0239	.0553
275	.50	.62	.0000	.0337	.1847	.2609	.2053	.1198	.8044
300	.50	.50	.0000	.0005	.0039	.0099	.0170	.0218	.0531
300	.50	.62	.0006	.0400	.2061	.2736	.2085	.1068	.8355
325	.50	.50	.0000	.0002	.0036	.0102	.0155	.0224	.0519
325	.50	.62	.0001	.0475	.2251	.2829	.2088	.1021	.8665

* from simulation with 10,000 replications, assuming exponential time to failure

5.3.2. Guidelines for Safety Monitoring

The rates of neural, hepatic and renal toxicities will be monitored up to 100 days post-transplant, separately in each treatment arm for all transplanted patients. Patients will be analyzed according to their randomized treatment assignment. Refer to Section 3.3 for definitions of these safety monitoring endpoints. Monitoring will be performed monthly until enrollment to that treatment arm is closed. We expect approximately 20 patients to be on study at the time of the first look. Each month, the null hypothesis that the rate of convulsions is less than or equal to 10% will be tested against the alternative that it is greater than 10%. Similarly, the rate of hepatic and renal toxicities will be compared to thresholds of 8% and 10% respectively.

An extension to the Sequential Probability Ratio Test (SPRT) will be used for each endpoint, as described in greater detail below and in Appendix I. This sequential testing procedure conserves type I error across all of the monthly examinations for a single endpoint, but not across the multiple safety endpoints. Thus for a single endpoint, the type I error is approximately 5%, and across all three safety endpoints, the study-wise type I error is < 15% per arm for a total of 30% study-wide.

The rationale for not conserving type I error across multiple safety endpoints is twofold. First, adjusting the size of the test for multiple comparisons would reduce statistical power to detect adverse outcomes, which seems imprudent. Secondly, the procedure is a guideline for requiring additional review by the Data and Safety Monitoring Board, and is not a formal “stopping rule” that would mandate automatic closure of study enrollment.

The SPRT can be represented graphically. At each monthly interim analysis, the total time on study is plotted against the total number of endpoints, (e.g., patients with convulsions). The continuation region of the SPRT is defined by two parallel lines. Only the lower boundary will be used for monitoring each treatment arm to protect against poor 100-day rates of convulsions. If the graph falls below the lower boundary, the SPRT rejects the null hypothesis, and concludes that there are more convulsions than predicted by the observed time on study. Otherwise, the SPRT continues until enrollment to the treatment arm reaches the target goal.

This procedure assumes an exponential distribution for the time until failure during the first 100 days, but censors follow-up time after 100 days. Only endpoints that occur on or before the patient has been followed for 100 days post-randomization are counted. Total time on study is computed as time from randomization to event, or to 100 days, whichever comes first, summed for all individuals on study. Death is a competing risk for the event, and time on study will be censored at the time of death.

The usual measures of performance of an SPRT are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$ and of accepting H_1 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_i)$. The tests to be used in this protocol were developed from the following SPRTs:

- A SPRT contrasting 10% versus 15% 100-day rate of convulsions, with nominal type I and II errors of 9% and 15%, respectively. The common slope of the parallel lines is 2.076 and the intercepts are -10.755 and 8.635.
- A SPRT contrasting 8% versus 13% 100-day rate of hepatic toxicity, with nominal type I and II errors of 7% and 15%, respectively. The common slope of the parallel lines is 2.513 and the intercepts are -12.233 and 8.939.
- A SPRT contrasting 10% versus 15% 100-day rate of use of dialysis, with nominal type I and II errors of 9% and 15%, respectively. The common slope of the parallel lines is 2.076 and the intercepts are -10.755 and 8.635.

Note that since the test uses only the lower boundary, and is truncated by a finite sample size, both the size and power of the test will be lower than nominal levels.

The actual operating characteristics of the truncated test, shown in Table 5.3.2, were determined in a simulation study that assumed uniform accrual of 300 individuals over a three-year time period, and exponential time to failure after randomization. Since 100,000 replications were used, the estimates have two digits of precision.

Table 5.3.2
Operating Characteristics of Sequential Testing Procedure
from a Simulation Study with 100,000 Replications

Neural Toxicity (Convulsions)

True 100-Day Rate	10%	15%	20%
Probability Reject Null	0.06	0.79	1.00
Mean Month Stopped	35.8	21.8	10.7
Mean # Endpoints in 100 days	28.4	25.2	15.3
Mean # Patients Enrolled	290.9	179.8	89.1

Hepatic Toxicity

True 100-Day Rate	8%	13%	18%
Probability Reject Null	0.05	0.81	1.00
Mean Month Stopped	36.1	21.2	10.2
Mean # Endpoints in 100 days	22.9	21.3	13.0
Mean # Patients Enrolled	292.7	175.3	85.3

Renal Toxicity (Use of Dialysis)

True 100-Day Rate	10%	15%	20%
Probability Reject Null	0.06	0.79	1.00
Mean Month Stopped	35.8	21.8	10.7
Mean # Endpoints in 100 days	28.4	25.2	15.3
Mean # Patients Enrolled	290.9	179.8	89.1

For example, the testing procedure for convulsions rejects the null hypothesis in favor of the alternative 6% of the time when the true 100 day convulsion rate is 10%, and 79% of the time when the rate is 15%. This corresponds to a type I error rate of $\alpha = .06$ and a type II error rate of $\beta = .21$. When the true 100 day convulsion rate is 15%, on average, the Data and Safety Monitoring Board will be consulted 21.8 months after opening, when 25.2 convulsions have been observed in 179.8 patients.

Since the expected rate of serious cardiac events is 2%, a formal monitoring rule will not be used.

5.4. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, performance status, HLA match, primary disease, risk status, prior history of fungal infections, source of stem cells, donor age, donor gender, and

donor ethnicity. Between group comparisons will be performed for continuous variables via a t-test and for categorical variables, via the chi-square test.

5.5. Analysis of Secondary Endpoints

- 1. Frequency of invasive fungal infections by Day 180:** The Cochran-Mantel Haenszel (CMH) test will be used to compare the proportion of patients with invasive fungal infection between the treatment groups.
- 2. Time to invasive fungal infection:** Cumulative incidence curves will be computed by treatment. A stratified log-rank test will be used to compare the two arms. Also, a Cox proportional hazards model will be fit to control for important prognostic variables.
- 3. Patient survival:** Survival distribution will be estimated by the Kaplan- Meier curve. The two treatment arms will be compared using the stratified log-rank test. A Cox proportional hazard model will be fit to control for important prognostic variables. All patients will be followed for a minimum of one year post transplant for mortality.
- 4. Empirical use of amphotericin B or caspofungin:** This will be assessed by measurement of proportions of patients with empirical use of amphotericin B or caspofungin and total number of days on amphotericin B or caspofungin and compared between the two treatment groups via the CMH and 2-way ANOVA respectively. Rank (or other) data transformation may be used for the number of days on amphotericin B or caspofungin for the 2-way ANOVA. The impact of use of amphotericin B or caspofungin on the incidence of systemic fungal infections will be examined using logistic regression. Duration of amphotericin B or caspofungin and the proportion of patients who received > 14 days of consecutive amphotericin B or caspofungin use will be assessed.
- 5. Time to acute or chronic graft-versus-host disease (GVHD):** Cumulative incidence curves will be computed by treatment. A stratified log-rank test will be used to compare the two arms. Also, a Cox proportional hazards model will be fit to control for important prognostic variables.
- 6. Exploratory analyses of quantitative aspects of galactomannan assay in diagnosis and treatment of aspergillus in the presence of different patient conditions:** The objectives of the analyses are (1) to explore quantitative aspects of the assay to optimize sensitivity and specificity in the setting of different host factors and manifestations of aspergillus, (2) to determine if cases first detected by the galactomannan assay have improved treatment outcomes as compared to cases first documented by other diagnostic tests, and if galactomannan kinetics are predictive of clinical response to antifungal therapy.

Objective 1

It is hypothesized that the performance of the assay will be dependent on circulating fungal burden, which is potentially impacted by multiple host factors, and various manifestations of aspergillus. Specifically, it is anticipated that pulmonary infections

may be less likely detected by GM than disseminated infection, and sinus infections will be the least likely to be detected by GM early.

Host factors that will be considered include: age, neutropenia or ANC > 500, receipt of mould-active antifungal therapy, primary graft failure versus engrafters, and receipt of voriconazole vs. fluconazole. Manifestations of aspergillus that will be considered include: (1) site of infection (lung, sinus, extra pulmonary); (2) stage of infection (localized versus disseminated); and, (3) causative aspergillus species.

Patients who develop aspergillus infection will be classified according to whether the infection was first detected by GM positivity or by conventional means. The effects of the host and infection factors listed above on the probability of first detection by GM will be explored by conditional logistic regression modeling. If infection status is not ascertained in all patients, more complex models that incorporate non-response will be considered.

The proportion of patients diagnosed with aspergillus infection, and the median time to first detection of the first episode of aspergillus, will be compared according to whether the infection was first detected by GM positivity or conventional means. However, the results are not expected to differ because the GM result will be reported in real time to clinicians and will trigger a clinical work-up.

It is anticipated that one-third of cases of “possible” aspergillosis will be upgraded to “probable” by incorporation of the GM assay. To test this hypothesis, the proportion of cases reclassified from “possible” to “probable,” and its associated confidence interval, will be estimated. More generally, the rates of reclassification will be investigated using methods appropriate for ordinal responses on paired samples.

The performance (e.g., sensitivity and specificity) of diagnostic criteria that incorporate the GM assay will be compared to a “gold standard” of the conventional diagnostic criteria, using Receiver Operator Curve (ROC) methodology. The semiquantitative GM index will be compared with baseline to determine what relative increase in GM index occurs at the time of diagnosis by other diagnostic tests. Other thresholds for determining a positive GM response will be investigated.

Objective 2

It is hypothesized that cases first detected by GM positivity will be less advanced infections and will be more likely associated with survival than those detected by other conventional diagnostic means. For cases detected by conventional means, 67% die. It is believed that the case fatality rate for GM-detected cases will be lower.

A survival model with two time-dependent indicator variables, representing the absorbing states of first diagnosis by GM positivity, and first diagnosis by conventional means will be employed. The hypothesis that GM-detected cases will

have improved survival will be tested using a likelihood ratio test to compare the coefficients for these terms.

In patients treated for aspergillosis, the temporal change in galactomannan level will be tested as a guide to response to treatment and compared with clinical, cultural, and radiographic parameters of response.

7. **Engraftment:** Rate of primary graft failure will be estimated using cumulative incidence estimates. The treatment arms will be compared using the stratified log-rank test. Neutrophil and platelet engraftment will be computed with cumulative incidence estimates. The number of autologous recoveries will be reported.
8. **Freedom from possible, presumptive, probable or proven infection, death or withdrawal of study drug.** Cumulative incidence curves will be computed by treatment. A stratified log-rank test will be used to compare the two arms. Also, a Cox proportional hazards model will be fit to control for important prognostic variables.

Two-sided tests will be used throughout. A p-value of 0.05 or less will be considered statistically significant.

5.6. Safety Analysis

All entered patients will be included in the safety analysis:

1. **Serious Adverse Events:** All reported serious adverse events potentially associated with study drug will be carefully examined with respect to the severity and relationship to study drug. Adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events Version 3.0. The incidence for each reported study drug associated adverse event delineated in Section 2.4.5 will be presented for each group.
2. **Clinical Laboratory Tests:** Laboratory tests will be performed during the study. Descriptive statistics will be calculated for each laboratory test. Abnormal laboratory test results will be summarized in the report. Laboratory data will be cross-classified into below, within, and above the reference range according to their values at screening and during the study.

APPENDIX A
REFERENCES

APPENDIX A

REFERENCES

1. Wingard JR. Fungal infections after bone marrow transplant. *Biol Blood Marrow Transplant* 5:55-68, 1999.
2. Goodman JL, Winston DJ, Greenfield RA: A controlled trial of Fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. *N Eng J Med* 326:845, 1992.
3. Rotstein C, Bow EJ, Laverdiere M, et al. Randomized placebo-controlled trial of Fluconazole prophylaxis for neutropenic cancer patients: benefit based on purpose and intensity of cytotoxic therapy. *Clin Infect Dis* 28:331, 1999.
4. Slavin MA, Osborne B, Adams R, et al. Efficacy and safety of Fluconazole prophylaxis for fungal infections after marrow transplantation – a prospective, randomized, double-blind study. *J Infect Dis* 171:1545, 1995.
5. Marr KA, Seidel K, Slavin M, et al. Outcome of blood and marrow transplantation in patients who received prolonged Fluconazole prophylaxis: long-term follow-up of a randomized, placebo-controlled trial. *Blood* 96:12055, 2000.
6. Hansen JA, Gooley TA, Martin PJ, et al. Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. *N Eng J Med* 338:962, 1998.
7. Recommendations of CDC, the Infectious Diseases Society of America, and the American Society of Blood and Marrow Transplantation. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *Biol Blood Marrow Transplant* 6:659-734, 2000.
8. Wingard JR, Chairman of the IBMTR/ASBMT/NIH State of Science BMT Meeting, Infectious Complications Working Group, April 1, 2000, personal communication.
9. Marco F, Pfaller MA, Messer S, et al. In vitro activities of Voriconazole (UK-109-496) and four other antifungal agents against 394 clinical isolates of *Candida* sp. *Antimicrob Agents Chemother* 42:161, 1998.
10. Johnston D, Zhou X, Fukuoka T, et al. Voriconazole is highly active against the 14 α -demethylase of *Candida krusei*. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 26-29, 1999, San Francisco, CA, abstract 2073.
11. Clancy CJ, Fothergill, Rinaldi MG, et al. SCH56592 and Voriconazole against emerging yeasts: in vitro efficacy compared with Fluconazole and Itraconazole. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 26-29, 1999, San Francisco, CA, abstract 1518.

12. Pai MP, Prause JL, Danziger LH, et al. In vitro activity of Voriconazole and other azoles against *Candida* isolates. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17-20, 2000, Toronto, Canada, poster presentation.
13. Schlamm HT, Corey L, Brown J, et al. Voriconazole for salvage treatment of invasive aspergillosis. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17-20, 2000, Toronto, Canada, abstract 304.
14. Perfect J, Lutsar I, Gonzalea-Ruiz A. Voriconazole (VORI) for the treatment of resistant and rare fungal pathogens. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17-20, 2000, Toronto, Canada, abstract 303.
15. Green BA, Breen A, Corey L. Voriconazole for salvage therapy of fungal infections in patients with hematological malignancies and stem cell transplantation. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17-20, 2000, Toronto, Canada, Abstract 41.
16. Johnston D, Cannom RRM, Filler SG. Amino acid substitutions in the aspergillus fumigatus ERG 11 gene product render it resistant to itraconazole yet susceptibility to Voriconazole is maintained. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17-20, 2000, Toronto, Canada. Abstract.
17. Torre-Cisneros J, Gonzalez-Ruiz A, Hodges MR. Voriconazole for the treatment of *S.apiospermum* and *S.prolificans* infection. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17-20, 2000, Toronto, Canada, Abstract 305.
18. Sabo JA, Abdel-Rahman SM. Voriconazole: a new triazole antifungal. *Ann Pharmacother* 34:1032, 2000.
19. Dupont B, Ally R, Burke J, et al. A double-blind, randomized, multicenter trial of Voriconazole (VORI) vs Fluconazole (FLU) for the treatment of esophageal candidiasis (EC) in immunocompromised adults. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17-20, 2000, Toronto, Ontario, Canada, Abstract 706.
20. Walsh TJ, Pappas P, Winston D, et al. Voriconazole versus liposomal amphotericin B for empirical antifungal therapy of persistently febrile neutropenic patients: a randomized, international, multicenter trial. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17-20, 2000, Toronto, Ontario, Canada, abstract L-1.
21. Denning DW, Patterson TF, Herbrecht R, et al. Utility of criteria to assess outcome of invasive aspergillosis: consistency of response assessments in a clinical trial. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17-20, 2000, Toronto, Canada, abstract 1325.
22. Winston DJ, Maziarz RT, Chandrasekar PH, et al. Intravenous and oral itraconazole versus intravenous and oral fluconazole for long-term antifungal prophylaxis in allogeneic hematopoietic stem-cell transplant recipients. A multicenter, randomized trial. *Ann Intern Med.* 2003; 138:705-713.
23. Marr, K, Crippa F, Leisenring W, et al. Intraconazole vs Fluconazole for antifungal prophylaxis in allogeneic HSCT recipients: results of a randomized trial. *Blood* 2002; 11: (311), 215a (Abstract 806).

24. Jantunen E, Ruutu P, Hiskanen L, et al. Incidence and risk factors for invasive fungal infections in Allogeneic BMT patients. *Bone Marrow Transplant* 19:801, 1997.
25. Wald A, Leisenring W, van Burik J-A, et al. Epidemiology of aspergillus infections in a large cohort of patients undergoing bone marrow transplantation. *J Infect Dis* 175:1459, 1997.
26. Grow WB, Moreb JS, Roque D, et al. Late onset of invasive aspergillosis infection in bone marrow transplant patients at a university hospital. *Blood* 94:341a, Abstract #1524,1999.
27. Ribaud P, Chastang C, Latge JP, et al. Survival and prognostic factors of invasive aspergillosis after allogeneic bone marrow transplantation. *Clin Infect Dis* 28:322-330, 1999.
28. Morrison VA, Haake RJ, Weisdorf DJ. The spectrum of non-candida fungal infections following bone marrow transplantation. *Medicine* 72:78, 1993.
29. Marr KA, Balajee A, Leisenring W, et al. Utility of galactomannan detection for the diagnosis of invasive aspergillosis in HSCT recipients. *Blood* 96:786a, Abstract 3400, 2000.
30. Iwen PC, Reed EC, Armitage JO, et al. Nosocomial invasive aspergillosis in lymphoma patients treated with bone marrow or peripheral stem cell transplants. *Infect Control Hosp Epidemiol* 14:131, 1993.
31. Wald A, Leisenring W, van Burik J-A, et al. Epidemiology of aspergillus infection in a large cohort of patients undergoing bone marrow transplantation. *J Infect Dis* 175:1459, 1997.
32. Maertens JA, Verhaegen J, Lagrou K, et al. Screening for circulating galactomannan as a non-invasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 97:1604-1610, 2001.
33. Salonen J, Lehtonen O-P, Terasjarvi M-R, et al. Aspergillus antigen in serum, urine and bronchoalveolar lavage specimens of neutropenic patients in relation to clinical outcome. *Scand J Infect Dis* 32:485-490, 2000.
34. Denning DW. Early diagnosis of invasive aspergillosis. *Lancet* 355:423, 2000.
35. Becker MJ, de Marie S, Willemsse D, et al. Quantitative galactomannan detection is superior to PCR in diagnosing and monitoring invasive pulmonary aspergillosis in an experimental rat model. *J Clin Microbiol* 38:1434-1438, 2000.
36. Ally R, Schurman D, Kreisel W, et al. A randomized, double-blind, double-dummy, multicenter trial of voriconazole and fluconazole in the treatment of esophageal candidiasis in immunocompromised patients. *Clin Infect Dis* 33:1447-1454, 2001.
37. Denning DW, Ribaud P, Milpied N, et al. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. *Clin Infect Dis* 34:563-571, 2002.
38. Walsh TJ, Lutsar I, Driscoll T, DuPont B, Roden M, Gharamani P, Hodges M, Groll AH, and Perfect JR: Voriconazole in the treatment of aspergillosis, scedosporiosis, and other invasive fungal infections in children. *Ped Infect Dis J* 21: 240-248, 2002.
39. Herbrecht R, Denning DW, Patterson TF, et al. Open, randomized comparison of voriconazole (VRC) and amphotericin B (AmB) followed by other licensed antifungal

- therapy of invasive aspergillosis (IA). Abstract J-680. p. 378 41st Annual Intersci Conf Antimicrob Agents Chemother. 2001.
40. McGinnis MR, Nordoff N, Li RK, et al. *Sporothrix schenckii* sensitivity to voriconazole, itraconazole and amphotericin. *Med Mycol* 39:369-371, 2001.
 41. Fernandez-Torres B, Carrillo AJ, Martin E, et al. In vitro activities of 10 antifungal drugs against 508 dermatophyte strains. *Antimicrob Agents Chemother* 45:2524-2528, 2001.
 42. Carrillo AJ, Guarro J. In vitro activities of four novel triazoles against *scedosporium* spp. *Antimicrob Agents Chemother* 45:2151-2153, 2001.
 43. Meletiadis J, Meis JF, Mouton JW, et al. In vitro activities of new and conventional antifungal agents against clinical *scedosporium* isolates. *Antimicrob Agents Chemother* 46:62-68, 2001.
 44. Walsh TJ, Pappas P, Winston DJ, et al. Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. *N Engl J Med* 346:225-234, 2002.
 45. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31-41, 1976.
 46. Ghahramani P, Purkins L, Kleinermans, et al. Voriconazole potentiates warfarin-induced prolongation of prothrombin time. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17029, 2000, Toronto, Canada, abstract 846.
 47. Ghahramani P, Purkins L, Wood ND, et al. Drug interactions between Voriconazole and phenytoin. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17029, 2000, Toronto, Canada, abstract 847.
 48. Ghahramani P, Wood ND, Purkins L, et al. Effect of omeprazole on the pharmacokinetics of Voriconazole. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17029, 2000, Toronto, Canada, abstract 843.
 49. Ghahramani P, Purkins L, Wood ND, et al. The pharmacokinetics of Voriconazole and its effect on prednisolone disposition. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17029, 2000, Toronto, Canada, abstract 842.
 50. Ghahramani P, Purkins L, Wood ND, et al. Effects of rifampicin and rifabutin on the pharmacokinetics of Voriconazole. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17029, 2000, Toronto, Canada, abstract 844.
 51. Ghahramani P, Purkins L, Kleinermans D, et al. Voriconazole does not affect the pharmacokinetics of digoxin. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17029, 2000, Toronto, Canada, abstract 849.
 52. Ghahramani P, Romero AJ, Wood ND, et al. The effect of Voriconazole on the pharmacokinetics of cyclosporin. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 17029, 2000, Toronto, Canada, abstract 845.
 53. Ascoglu S, Res JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 34:7-14, 2002.

54. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A. National Committee for Clinical Laboratory Standards, Wayne, Pa, 1997.
55. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi. Proposed standard M38-P. National Committee for Clinical Laboratory Standards, Wayne, Pa, 1998.
56. Marr KA, Carter RA, Crippa F, et al. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 34:909-917, 2002.
57. Purkins L, Wood N, Chahramani P, et al. Pharmacokinetics and safety of voriconazole following intravenous to oral-dose escalation regimens. *Antimicrob Agents Chemother* 46:2546-2553, 2002.
58. Lazarus HM, Blumer JL, Yanovich S, et al. Safety and pharmacokinetics of oral voriconazole in patients at risk of fungal infection: a dose escalation study. *J Clin Pharmacol* 42:395-402, 2002.
59. Romero AJ, Pogamp PL, Nilsson L-G, et al. Effect of voriconazole pharmacokinetics on cyclosporine in renal transplant patients. *Clin Pharmacol Ther* 71:226-234, 2002.
60. Walsh TJ, Wood N, Milligan P, et al. Pharmacokinetics of intravenous voriconazole in children after single and multiple dose administration. Abstract A17. In: 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, September 22 to 25, 2001. Washington, DC: American Society for Microbiology, 2001:2.
61. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, et al. Revised recommendations of the international working group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. *J Clin Oncol* 21(24): 4642-9, 2003.

APPENDIX B-1
CONSENT FORMS

PARTICIPANT INFORMED CONSENT

IRB #

Informed Consent to Participate in Research



If you are a parent, as you read the information in this Consent Form, you should put yourself in your child's place to decide whether or not to allow your child to take part. Therefore, for the rest of the form, the word "you" refers to your child.

If you are a child, adolescent or adult who is reading this form, the word "you" refers to you.

You are being asked to take part in a research study. This form tells you about the study. The Principal Investigator (the person in charge of this research) or a co-worker of the Principal Investigator will also describe this study to you and answer all of your questions. Before you decide whether or not to take part, read the information below and ask questions about anything you do not understand. Your taking part is entirely your choice.

1. Name of the Subject ("Study Subject")

2. Title of Research Study

A Randomized Double-blind Trial of Fluconazole Versus Voriconazole for the Prevention of Invasive Fungal Infections in Allogeneic Blood and Marrow Transplant Patients

3. Principal Investigator Contact Information

IRB #

Contact information for emergencies after hours or on weekends or holidays:

Call (xxx) xxx-xxxx, the in-patient Bone Marrow Transplant Unit. Ask to speak to the Charge Nurse.

4. Sponsor and Source of Funding or Other Material Support

The sponsor of this study, The National Institutes of Health (NIH), is providing the study drugs and research tests free of charge to study subjects through the Blood and Marrow Transplantation Clinical Trials Network (BMT CTN), a group of 32 U.S. transplant centers.

5. What is the purpose of this study?

You are being asked to take part in this research study because you are having a blood or bone marrow transplant (BMT) using cells from another person.

One of the most common side effects of BMT is a decrease in the number or strength of infection fighting cells (white blood cells) in the body. Infections are caused by bacteria, viruses or fungi. When white blood cell numbers are low or the cells are weak, the body can't fight infections. For this reason, all BMT patients take drugs to prevent infection by these germs.

Fungal infections are one of the worst forms of infection. One of the drugs used to both prevent and treat fungal infections is called fluconazole. Fluconazole prevents some, but not all, types of fungal infections. Voriconazole is a newer antifungal drug that can treat more types of fungal infections than fluconazole. But, voriconazole has more side effects than fluconazole. Although voriconazole is used to treat severe fungal infections, it has not yet been tested for the prevention of fungal infections.

The purpose of this study is to compare fluconazole and voriconazole in the prevention of fungal infections in blood or marrow transplant patients where another person is the donor. About 600 patients will take part in this study at many centers around the country.

IRB #

6. What will be done if you take part in this research study?

Before starting treatment in this study, your doctor will check your general health. This will include a physical exam and taking blood (about 3 teaspoons or 10-20 mL for adult patients). A CT scan of the chest will be required if the results of the baseline galactomannan blood draw are not available. A CT scan is a type of x-ray that takes many detailed pictures. A chest CT scan results in an estimated dose of 0.8 rem, equivalent to 2.25 years of naturally occurring background radiation in the United States. A CT scan of the abdomen and/or sinuses may be required. An abdomen CT results in 1.1 rem, equivalent to 3 years. A head (sinus) CT results in 0.55 rem, equivalent to 1.7 years of background. If you are a female able to have children, a pregnancy test will also be performed. If you are pregnant, you will not be able to take part in this study.

If you choose to take part in this study, you will be randomly assigned (much like the toss of a coin) to receive either fluconazole or voriconazole. You and your study doctor will not know whether you are receiving fluconazole or voriconazole. This is called a double-blind study. In an emergency, the name of your drug can be obtained quickly. Treatment will begin on the day of your transplant (Day 0).

Since voriconazole is given twice per day and fluconazole only once per day, a placebo (a pill with no active drug or a salt water infusion) will be used to keep us from knowing which drug you are receiving. You will take the assigned drug twice a day. Whenever possible, the drug will be given as pills. If you are unable to take pills, the drug will be given over two hours by an IV infusion through your catheter. If you have been assigned to fluconazole, one of the pills (or infusions) will be a placebo since fluconazole is normally taken once daily.

You will continue the study drug until 100 days after your transplant. At around 90 to 100 days, your doctor will test your immune function and review your immunosuppressive drugs. If your risk of fungal infection is high, you will stay on study drug until 180 days. The study drug will stop by 180 days. But your doctor can give you an antifungal drug for longer if he/she believes you need it.

While you are in the study, your doctor will conduct routine and study-specific tests (such as x-rays, CT scans, or blood tests) and check you for a fungal infection. The table below shows when you will have study-required blood tests. The tests are described after the table.

IRB #

Time	Amount of Blood per Draw (adults)*	Type of Test / Purpose** Standard of Care^S / Investigational^I
Pre transplant	1 teaspoon (5 mL) 1 teaspoon (5 mL) 1 teaspoon (5 mL)	Liver function tests ^S / eligibility Galactomannan assay ^S / eligibility Future testing ^I / research
3x/week until engraftment	1 teaspoon (5 mL)	CBC ^S / blood chemistry
1x/week until Day 100. Also on Day 120, 150 and 180 if patient remains on study drug	1 teaspoon (5mL)	Liver function tests ^S / check for bad side effects
Day 14 and 28	1 teaspoon (5 mL)	Pharmacokinetics (PK) ^I / test for drug levels
2x/week, Days 1 – 60	1 tablespoon (15 mL)	Galactomannan (GM) assay ^S and Investigational Fungal Diagnostics ^I / tests for fungal infections
1x/week***, Days 61 – 100	1 tablespoon (15 mL)	GM assay ^S and Investigational Fungal Diagnostics ^I / tests for fungal infections
If you have bad side effects	1 teaspoon (5 mL)	PK ^I / test for drug levels
If doctor suspects you have a possible fungal infection	4 teaspoons (20 mL) at onset 1 tablespoon (15 mL) 2x/week for up to 14 days while on empirical antifungal therapy	PK ^I / test for drug levels, GM assay ^S and Investigational Fungal Diagnostics ^I / tests for fungal infections GM assay ^S and Investigational Fungal Diagnostics ^I / tests for fungal infections
If you get a fungal infection	4 teaspoons (20 mL) at onset 1 tablespoon (15 mL) 2x/week for 4 weeks and then once every 2 weeks for 8 weeks, for a total of 12 samples	PK ^I / test for drug levels, GM assay ^I and Investigational Fungal Diagnostics ^I / tests for fungal infections GM assay ^I and Investigational Fungal Diagnostics ^I / measure response to treatment

* Pediatric patients less than 12 years old will provide one-half of the required amounts of blood. The center may make adjustments in blood volumes collected from patients less than 12 years old to meet the institution’s Human Investigation Committee Guidelines.

** Tests that are typically done for your care are considered standard of care. These tests will be charged to you. Tests that are done solely for research purposes are considered investigational and will not be charged to you.

*** Patients will be tested twice weekly from Days 61-100 if they (1) received a T cell depleted transplant (a transplant where certain white cells have been removed to reduce the risk of GVHD) and post transplant GVHD prophylaxis (medications designed to prevent GVHD), or (2) have or have had GVHD (a condition in which the donated cells recognize the recipient’s cells as non-self and attack them) requiring systemic therapy, or (3) are taking steroids.

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Blood (1 tablespoon or 15 mL) will be taken prior to the transplant to determine eligibility for the study. A small sample (1 teaspoon or 5 mL) will be saved for future testing.

Blood samples (1 teaspoon or 5 mL) will also be taken 3x/week to determine engraftment (when the infused stem cells begin to multiply its own cells in your bone marrow). Engraftment typically occurs within 1-4 weeks post-transplant.

Weekly blood tests (about 1 teaspoon or 5 mL) will be taken for the first 100 days (and on Day 120, 150 and 180 if you remain on study drug) to check for bad side effects. Blood (1 teaspoon or 5 mL) will also be taken to measure drug levels at about Day 14 and 28, plus if you develop any bad side effects possibly related to the study drug, and at the start of a fungal infection. Usually, blood can be taken from your central venous catheter. In addition, we will also take blood (about 1 tablespoon or 15 mL), twice a week for the first 60 days of the study, and extra samples if your doctor suspects you have an infection to see if new tests can detect a serious fungal infection earlier and more easily than current tests.

Blood samples will also be taken once per week from Days 61-100 unless: (1) you received a T cell depleted transplant and received post-transplant GVHD prophylaxis or, (2) you are on steroids, or (3) you have or have had acute GVHD requiring systemic therapy. Then blood samples will be taken twice weekly from Days 61-100. Other tests will also be done to see which treatment is more effective in treating infections.

If your doctor suspects a fungal infection he/she may give you a drug (amphotericin B, Ambisome, Abelcet, Amphotec or Caspofungin) to treat it while he/she is reviewing your symptoms. You will continue to receive the study drug. If the tests do not show a fungal infection, your doctor will stop the other drug within 14 days. If your doctor believes that you need the other drug after 14 days, then he/she will continue it but the study drug will stop. You will continue to be tested as part of the study for the full year. Your doctor will still do the research tests, including the blood tests described above just as if you were taking the study drug.

If you get a fungal infection, the study drug will be stopped and another therapy will be discussed with you. This could include voriconazole if your doctor believes it to be the best choice. Your blood will be tested twice weekly for four weeks and then once every two weeks for eight weeks, for a total of 12 samples, to test your response to treatment.

This study will employ a new test approved by the FDA for detecting fungal infections in adults using blood samples. It is called the galactomannan test.

IRB #

Galactomannan is a part of the fungus that gets into the blood of infected patients. Some (1 teaspoon or 5 mL) of the blood samples described above will be used for this test.

This galactomannan test will be used with other standard tests to check you for certain kinds of fungal infection. The results will be used along with your medical history, physical check up, and other laboratory tests to judge whether you have or may have an infection. If you have a procedure on your lungs known as a bronchoscopy to test for an infection, a small part of the fluid obtained will be saved for later testing by the galactomannan test and other investigational tests to see if these new tests are better than the tests used now. The galactomannan test will also be used to test response to treatment if you develop an infection.

It is not yet clear if the galactomannan test will be better than the old tests and it is possible that it may not be as good in patients receiving one of the study drugs and certain other antifungal drugs, including amphotericin B and caspofungin. One good thing about this new test is that it requires only a sample of blood rather than a biopsy of tissue or other more uncomfortable tests. It also may show infection earlier. However, it may not be as exact. This could mean that if the test is positive and you really are not infected, you may receive treatment you don't need or if the test is negative and you really are infected, you may not receive treatment you need. For these reasons, your doctor will be using several kinds of tests for your safety. Results from this study will be used to measure the new test's value.

After your treatment is complete, we would like to keep track of your progress until 12 months after your transplant. To do this, we will contact you or your local doctor.

7. Will You Provide Samples for Research?

Genetic material is any sample of blood, tissue, fluid, etc. that is obtained from you. You will be asked to provide samples of blood, tissue and fluid to be used solely for future research and testing in laboratories where we are studying fungal infections. These samples will not require additional procedures. Your name will not be on these samples. You do not have to agree to provide these research samples to participate in the study.

If you agree, a small blood sample (1 teaspoon or 5 mL) will be drawn pre-transplant and saved for future testing. Additional blood samples (2 teaspoons or 10 mL) will be taken, if you agree, each time samples are drawn to test for galactomannan. Usually the blood can be drawn from your central venous catheter at the time of other blood collections. If this is not possible, then it would be drawn directly from a vein.

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If you agree, small samples of tissue or fluid (1 mL) will be collected post-transplant and saved for future testing. These samples will be taken from samples that are collected for diagnosis of potential fungal infections. Additional procedures are not required.

The samples collected for research purposes will be sent to laboratories that have contracts with the National Marrow Donor Program (NMDP) to conduct these research tests. They will be labeled with unique codes that do not contain information that could identify you. A link to this code does exist. The link is stored at the Data Coordinating Center for the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). The staff at the laboratories where your samples are being tested do not have a link to this code. Your samples will be stored at these laboratories until the entire sample has been used for the research tests or until the end of the study.

If any of your samples are leftover after the research studies are completed, these samples will either be destroyed or be sent to the National Heart Lung and Blood Institute (NHLBI) sample repository in Maryland. If your leftover samples are sent to the repository, they will be given an anonymous code. These leftover samples stored at the repository can never be linked to you. Any research performed on these left-over samples must first be approved by an advisory panel at the NHLBI.

If you agree to allow your blood, tissue and fluids to be kept for research, you are free to change your mind at any time. We ask that you tell [the Principal Investigator] in writing and let him/her know you are withdrawing your permission for your samples to be used for research. The mailing address is on the first page of this form. Any unused samples will be destroyed. **You are free not to take part in this future research and still take part in the other parts of the study. There will be no change in your care if you decide not to give these extra samples. Please mark your choice below for each sample:**

(1) Pre-transplant 5 mL blood sample

- I agree to have a blood sample drawn pre-transplant for future research and testing.
- I do not agree to have a blood sample drawn pre-transplant for future research and testing.

(2) Post-transplant 10 mL blood samples taken each time blood samples are taken for the galactomannan (GM) assay

- I agree to have blood samples drawn post-transplant for future research and testing.
- I do not agree to have blood samples drawn post-transplant for future research and testing.

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(3) Post-transplant tissue and fluid samples each time samples are taken post-transplant for diagnosis of fungal infections

- I agree to have tissue and fluid samples collected post-transplant for future research and testing.
- I do not agree to have tissue and fluid samples collected post-transplant for future research and testing.

Signature

Date

8. What are the possible discomforts and risks?

Cancer treatments can cause bad side effects, some of which may be life threatening or could kill you. The drugs used in this study may cause all, some, or none of the side effects listed below. Also, there is always the chance of unexpected new side effects.

The most common side effect in patients who have received voriconazole has been a change in eyesight that was often described as a “brightness” in vision that usually lasts less than one hour. Some patients treated with voriconazole have reported temporary glare or blurred vision. The effect may begin within 30 minutes after taking a dose of voriconazole and lasts an average of one-half hour but may last up to one hour. You should not drive or operate machinery during these periods. The next most common side effect was abnormal liver tests.

The following side effects have been reported in more than 1% of patients who have received voriconazole: a reaction at the site of injection, photosensitivity (a rash caused by sunlight), weakness, dizziness, headache, trouble sleeping, dry mouth, nausea, stomach pain, and low potassium levels. Other side effects that have been reported in at least one patient that was treated with voriconazole include: skin rash, skin erythema (redness), psoriasis (scaling) with eosinophilia (an abnormal blood condition), hypoglycemia (low blood sugar), hepatitis (liver disease), nausea, vomiting and jaundice (yellow skin), pancreatitis (a disease of the pancreas with pain in the stomach), decrease in the number of cells in the bone marrow (this was reported in a patient who had leukemia before starting voriconazole), and irregular heartbeat. An irregular heartbeat resulting in death occurred during treatment in a patient who had a history of an irregular heartbeat before entering the study. The patient also had a history of cancer and was on chemotherapy and many other drugs (including a potassium infusion, which may have affected the heart beat). In addition, an irregular heartbeat possibly related to taking voriconazole with the medications pimozide,

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quinidine, dofetilide, and quinupristin-dalfopristin (an antibiotic) has been reported. One patient with leukemia, who was also taking this antibiotic, was hospitalized because of faintness and palpitations (rapid heartbeats).

Voriconazole may increase or decrease levels of other commonly prescribed drugs and non-prescription drugs. Drugs known to interact with voriconazole include warfarin, phenytoin, cyclosporine, tacrolimus, sirolimus, rifabutin, omeprazole and rifampin. Other drugs that may interact with voriconazole include ritonavir, lovastatin (and other statins), benzodiazepines, sulfonamides, clarithromycin, terfenadine, astemizole, cisapride, dihydropyridine calcium channel blockers (verapamil, diltiazem), certain chemotherapy drugs (vinca alkaloids) and cabamazepine. We ask that you consult with your doctor before starting new drugs or increasing the dose of any drug including non-prescription drugs or herbs such as St. John's Wort. Grapefruit juice should be avoided. Other drugs that you may be taking may need to be changed or stopped in order for you to receive voriconazole.

The most frequent side effects after treatment with fluconazole affect the gastrointestinal tract (stomach and intestines) such as nausea and vomiting. Other side effects include dizziness, headache, skin rash, and liver problems. Serious side effects which have been reported with fluconazole include: liver damage which included deaths primarily in patients with serious medical conditions; elevated liver tests; severe skin disorders; hepatitis (liver disease), jaundice (yellow skin), irregular heartbeats or heart function changes; and acute allergic reactions.

The risks of drawing blood from a vein include discomfort at the site of puncture; possible bruising and swelling around the puncture site; rarely, an infection; and, uncommonly, faintness from the procedure.

Only you, the person for whom it has been prescribed, can take the study drug. If the drug is not packaged in a childproof container, you should keep it out of the reach of children and persons who have limited ability to read or understand. By federal law, this drug cannot be given or transferred to anyone for whom it is not prescribed.

As noted earlier, a new test, the galactomannan test, will be used along with other tests to see if you have an infection and will be used to test your response to treatment if you develop an infection. It is possible that this test may be less exact in patients taking one of the study drugs (voriconazole) when compared to the other (fluconazole). It is hoped that information from the study will help show that the new test can quickly and easily detect fungal infections.

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Throughout the study, the researchers will tell you of new information that might affect your decision to remain in the study.

If you wish to discuss the information above or any other discomforts you may experience, you may ask questions now or call the Principal Investigator or contact person listed on the front page of this form.

9a. What are the possible benefits to you?

Although this study cannot be guaranteed to be of benefit to you, it is hoped that your taking part may prevent you from getting a fungal infection. A possible advantage of this study is that one antifungal treatment may prevent fungal infections better than the other. However, you may not benefit from this treatment. The use of the galactomannan test along with other diagnostic tests may allow us to more quickly and more easily diagnose fungal infections and determine how well you respond to treatment if you develop an infection. However, there is no guarantee that this test is better than the currently available tests.

9b. What are the possible benefits to others?

Future patients may benefit from the results of this study.

10. If you choose to take part in this study, will it cost you anything?

Your cost for care on this research study will not be higher than for standard treatment for this disease. The BMT CTN will cover the cost of the study drug. You will remain responsible for the costs of standard treatment for your disease. Your insurance provider may not cover all or part of these costs. You or your family will have to pay installments based on your verified ability to pay. Any questions about these charges should be discussed with the Principal Investigator of the study.

11. Will you receive payment for taking part in this research study?

No.

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12. What if you are injured because of the study?

If you experience an injury that is directly caused by this study, only the professional medical care you receive at the [participating clinical facility] will be provided without charge. You or your insurance provider will pay hospital expenses. No other compensation is offered. If you have any questions about study-related injuries, you may call [insert name of person at institution] at [insert phone number].

13. What other options or treatments are available if you do not want to be in this study?

Taking part in this study is entirely up to you. You are free to refuse to be in the study, and your refusal will not affect current or future health care you receive at this institution. You and your doctor will discuss any other treatment options available to you.

The current standard therapy for preventing fungal infections in patients receiving bone marrow or stem cell transplant is fluconazole. Your doctor will review other treatments with you.

14a. How can you withdraw from this research study?

If you agree to be in this study, you are free to change your mind. At any time you may withdraw your consent to be in this study and for us to use your data. If you withdraw from the study, you will continue to have access to health care at [participating clinical facility]. If you decide to withdraw, we ask that you tell [the Principal Investigator] in writing; his/her mailing address is on the first page of this form. If you do withdraw your consent, there will be no penalty and you will not lose any benefits to which you are otherwise entitled. You will be asked to return any unused study drug. You will also be asked to return for a checkup before you stop your study drug. Even if you withdraw, or your doctor withdraws you, from the study, you are asked to have research tests conducted and allow the study investigators to collect that information.

If you have any questions about your rights as a study subject, you may call the Institutional Review Board (IRB) office at (xxx) xxx-xxxx.

14b. If you withdraw, can information about you still be used and/or collected?

If you withdraw from the study, we ask that you agree that we can continue using all information about you that has already been collected as part of the study prior to your withdrawal, and to continue to allow your doctor to tell us about your progress until 12 months after your transplant. You may, of course, say no.

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14c. Can the Principal Investigator withdraw you from this research study?

You can be taken off the study (with or without your consent) for any of the following reasons:

- You do not qualify to be in the study because you do not meet the study requirements. Ask your doctor if you would like more information about this.
- You need a medical treatment not allowed in this study.
- The investigator decides that continuing in the study would be harmful to you.
- The study treatments have a bad effect on you.
- You become pregnant and the study treatment could be harmful to the fetus.
- You are unable to keep appointments or take study drugs as directed.
- Other study-specific reasons; for example, if the dose of study drug you are taking has been found to be unsafe.
- The study is cancelled by the Food and Drug Administration (FDA) or the National Institutes of Health (NIH).

15. How will your privacy and the confidentiality of your research records be protected?

Study records that have your name will be kept private as required by law. You will not be identified by name in the central study records. Your records will be given a unique code number. The key to the code will be kept in a locked file in the Principal Investigator's office. Authorized persons from [the participating clinical facility], the hospital or clinic (if any) involved in this research, and the Institutional Review Board have the legal right to review your research records and will protect their confidentiality to the extent permitted by law. This research study is sponsored by and conducted with funds from the National Institutes of Health; therefore, the sponsor, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), the investigators conducting this study and the FDA also have the legal right to review your research records. Otherwise, your research records will not be shown to anyone without your consent unless required by law or a court order.

If the results of this research are published or presented at scientific meetings, your name will not be disclosed.

Information related to or resulting from your stem cell transplant will be reported to the Center for International Blood and Marrow Transplant Research (CIBMTR). The CIBMTR is a voluntary organization of basic and clinical scientists working together in an effort to gather

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information on results of stem cell and marrow transplants. This information is used to guide clinical decisions and identify ways to improve transplant outcomes. Scientific data or medical information (not identifiable with you) that could be useful to others may be presented at meetings and/or published in medical journals.

16. Expiration date for retention of records

The study results will stay in your research record at (*insert Institution*) for at least six years or until after the study is completed, whichever is longer. At that time either the research information not already in your medical record will be destroyed or your name and other identifying information will be removed from such study results. Research information in your medical record will be kept indefinitely.

17. How will the researcher(s) benefit from your being in this study?

In general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator may benefit if the results of this study are presented at scientific meetings or in the scientific press. In addition, the sponsor is paying the Principal Investigator to conduct this study.

18. HIPAA² authorization to use and disclose individual health information for research purposes

- a. Purpose: As a research participant, I authorize the Principal Investigator and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research study entitled *A Randomized, Double-blind, Trial of Fluconazole vs. Voriconazole for Prevention of Invasive Fungal Infections in Allogeneic Blood and Bone Marrow Transplant Patients*.
- b. Individual Health Information to be Used or Disclosed: My individual health information that may be used or disclosed to conduct this research includes: demographic information (e.g., age, date of birth, sex, weight), medical history (e.g., diagnosis, complications with prior treatment), physical examination findings, and laboratory test results obtained at the time of work up and after transplantation (e.g., CT scan, blood tests, biopsy results).

² HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information

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- c. Parties Who May Disclose My Individual Health Information: The researcher and the researcher’s staff may obtain my individual health information from:

(list hospitals, clinics or providers from which health care information can be requested)

- d. Parties Who May Receive or Use My Individual Health Information: The individual health information disclosed by parties listed in item c and information disclosed by me during the course of the research may be received and used by the following parties:

- Principal Investigator and the researcher’s staff
- Dr. John Wingard, Study Chairperson and staff/laboratories at University of Florida College of Medicine
- Dr. Thomas Walsh, Study Chairperson and staff/laboratories at NIH/NCI/POB
- Staff/laboratories identified in the protocol for the evaluation of other laboratory samples; e.g., Dr. Patricia Fraser/Harvard
- National Heart, Lung and Blood Institute (NHLBI) and the National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
- Blood and Marrow Transplant Clinical Trials Network (BMT CTN), data coordinating center
- U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)
- U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments.

- e. Right to Refuse to Sign this Authorization: I do not have to sign this Authorization. If I decide not to sign the Authorization, I will not be allowed to participate in this study or receive any research-related treatment that is provided through the study. However, my decision not to sign this

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authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

- f. Right to Revoke: I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of my decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about me will be collected by or disclosed to the researcher for this study.
- g. Potential for Re-disclosure: My individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected. Examples include potential disclosures for law enforcement purposes, mandated reporting or abuse or neglect, judicial proceedings, health oversight activities and public health measures.
- h. This authorization does not have an expiration date.

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ASSENT SIGNATURES: For subjects under the age of 18 years.

Assent of Minor

I have been told what I will be asked to do if I am in this study. I have been told that I don't have to be in this study. I may quit the study at any time, and no one will be mad at me. I have had a chance to discuss the study and ask questions. My questions have been answered. I agree to be in the study and do what I am asked to do so long as I continue in the study.

Signature of Minor

Date

Age (years)

Study Personnel

I have explained the purposes, procedures, and risks involved in this research study in detail to:

Print name(s) of Parents/ Authorized Consenting Party, **and**

_____, who in my opinion _____ IS/ ___ IS NOT capable of assenting to participate in this study.

Print child's name

Signature of Person Conducting Assent

Date

APPENDIX B-2
CONSENT FORMS

**DONOR INFORMED CONSENT
FOR SNP ASSAY**

IRB #

CO-PRINCIPAL INVESTIGATORS: John R. Wingard, M.D. and Thomas J. Walsh, M.D.

STUDY TITLE: A Randomized, Double-blind, Trial of Fluconazole vs. Voriconazole for Prevention of Invasive Fungal Infections in Allogeneic Blood and Bone Marrow Transplant Patients

INTRODUCTION

We invite you to take part in a research study sponsored by the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary.

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with your family, friends, or your personal physician or other health professional.

PURPOSE

During the course of this study, we will attempt to learn about genetic factors that may have an influence on infections due to invasive fungal infections. This type of infection occurs in individuals with a breakdown in the body's immune system, due to either medications, (such as corticosteroids) or in those who have had bone marrow transplantation. We are interested in studying the small variations or differences in genes, called polymorphisms or variants that could effect the body's ability to fight off invasive fungal infections. We also will study genes that help the body eliminate fluconazole and voriconazole from the blood stream. We have identified a collection of genes (twenty-six), each of which has one or more polymorphism. We would like to analyze your DNA for these twenty-six genes to see if any of these genes are associated with fungal infections. We invite you to participate in this study so that we can learn more about these genetic

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factors that may influence the susceptibility and severity of fungal infections in those who undergo blood or marrow transplantation.

PROCEDURES

Five (5) milliliters of blood or stem cell product will be used for the genetic analysis. We ask that you submit a sample of no more than 5 mL, equivalent to one teaspoon. It may be necessary to perform a buccal swab of the inside of your mouth to obtain genomic DNA if blood is unable to be drawn. If you withdraw from the study, your samples will not be used for other research studies or tested further.

Clinical information (e.g., HLA typing) about you/your child will be collected. NIH will not have access to the names of the patients enrolled in this study. The clinical information will be coded and compared to the genetic analysis of the twenty-six genes chosen for our study: *MBL2*, *CCR5*, *IL1RN*, *IL1A*, *IL1B*, *IL6*, *IL8*, *IL8RA*, *IL8RB*, *IL10*, *TNFA*, *TNFB*, *MPO*, *NRAMP1*, *CHIT1*, *FCGR2A*, *FCGR3A*, *FCGR3B*, *MICA*, *MICB*, *TLR4*, *CD14*, *HBD-1*, *IL-18* and two cytochrome P450 genes (*3A4* and *2C19*). In the laboratory, we will isolate DNA from your blood or stem cell product and test for each of these genes by standard techniques. We shall determine normal and variant sequences in DNA and compare this information to the clinical information collected.

ALTERNATIVES

You may choose not to participate in this part of the study. The decision to participate in this study will not affect the care given to you by your physicians.

RISK AND DISCOMFORT

It is possible that the information from this study could be important for family members (even though no blood and no analysis of family members are proposed). However individual results will not be reported directly to you.

There is a small risk of an infection or fainting from the blood draw. If blood is not available, a buccal swab from the inside of your mouth needs to be performed by a health care provider. This may result in minimal discomfort in the mouth during the time the cotton swab is rubbed against the inside of the cheek of your mouth.

At no time will this information be made available to those not directly involved in the study without your written consent. Patient information will not be made public. Only the results of the proposed analysis will be collected, presented and published. At no time, will

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the name or an identifier of patients be available to anyone except those conducting the study. The investigators who will conduct the genetic analysis will not have access to the names of the patients enrolled in this study. The clinical information will be coded and compared to the genetic analysis of the twenty-six genes chosen for the study.

BENEFITS

This study will increase our understanding of the factors that influence the risk for developing and treating fungal infections during bone marrow transplantation. We hope that it will eventually contribute to improvements in medical care, treatment and prevention of these types of infection. There may be no direct benefit to you or your family from this study. If there are any questions, we will attempt to answer them with the most recent information.

SAMPLES FOR RESEARCH AND FUTURE TESTING

Genetic material is any sample of blood, tissue, fluid, etc. that is obtained from you. You will be asked to provide a sample of blood, stem cell or buccal product to be used solely for future research and testing in laboratories where we are studying fungal infections. This sample will not require additional procedures. You do not have to agree to provide this research sample to participate in the study.

A portion of your 5 mL blood sample, stem cell product or buccal swab product, if you agree, will be saved for future testing and research related to infectious diseases or the immune system. These tests may include genetic analysis of your DNA beyond the 26 genes identified in this document.

The samples collected for research purposes will be sent to laboratories that have contracts with the National Marrow Donor Program (NMDP) to conduct these research tests. They will be labeled with unique codes that do not contain information that could identify you. A link to this code does exist. The link is stored at the Data Coordinating Center for the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). The staff at the laboratories where your samples are being tested do not have a link to this code. Your samples will be stored at these laboratories until the entire sample has been used for the research tests or until the end of the study.

If any of your samples are leftover after the research studies are completed, these samples will either be destroyed or be sent to the National Heart Lung and Blood Institute (NHLBI) sample repository in Maryland. If your leftover samples are sent to the repository, they will be given an anonymous code. These leftover samples stored at the repository can never be linked to

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you. Any research performed on these leftover samples must first be approved by an advisory panel at the NHLBI.

If you agree to allow your blood, stem cell or buccal product to be kept for research, you are free to change your mind at any time. We ask that you tell [the Principal Investigator] in writing and let him/her know you are withdrawing your permission for your sample to be used for research. The mailing address is on the first page of this form. Any unused sample will be destroyed. **You are free not to take part in this future research and still take part in the other parts of the study. There will be no change in your care if you decide not to give this extra sample. Please mark your choice below:**

- I agree to use of a sample of my blood, stem cell or buccal swab product for additional research or future testing.
- I do not agree to use a sample of my blood, stem cell or buccal swab product for any additional research or future testing.

Signature

Date

HIPAA³ AUTHORIZATION TO USE AND DISCLOSE INDIVIDUAL HEALTH INFORMATION FOR RESEARCH PURPOSES

1. Purpose: As a research participant, I authorize the Principal Investigator and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research study entitled *A Randomized, Double-blind, Trial of Fluconazole vs. Voriconazole for Prevention of Invasive Fungal Infections in Allogeneic Blood and Bone Marrow Transplant Patients*.

2. Individual Health Information to be Used or Disclosed: My individual health information that may be used or disclosed to conduct this research includes: Demographic information (e.g., age, date of birth, sex, weight), medical history, physical examination findings, and genetic test results.

³ HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information.

IRB #

3. Parties Who May Disclose My Individual Health Information: The researcher and the researcher's staff may obtain my individual health information from (*list hospitals, clinics or providers from which health care information can be requested*):

4. Parties Who May Receive or Use My Individual Health Information: The individual health information disclosed by parties listed in item 3 and information disclosed by me during the course of the research may be received and used by the following parties:

- Principal Investigator and the researcher's staff
- Dr. John Wingard, Study Chairperson and staff/laboratories at University of Florida College of Medicine
- Dr. Thomas Walsh, Study Chairperson and staff/laboratories at NIH/NCI/POB
- Staff/laboratories identified in the protocol for the evaluation of other laboratory samples; e.g., Dr. Patricia Fraser/Harvard
- National Heart, Lung and Blood Institute (NHLBI) and National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
- Blood and Marrow Transplant Clinical Trials Network (BMT CTN), data coordinating center
- U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)
- U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments.

5. Right to Refuse to Sign this Authorization: I do not have to sign this Authorization. If I decide not to sign the Authorization, I will not be allowed to participate in this study or receive any research-related treatment that is provided through the study. However, my decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

IRB #

6. **Right to Revoke:** I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of my decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about me will be collected by or disclosed to the researcher for this study.

7. **Potential for Re-disclosure:** My individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected. Examples include potential disclosures for law enforcement purposes, mandated reporting or abuse or neglect, judicial proceedings, health oversight activities and public health measures.

8. This authorization does not have an expiration date.

IRB #

OTHER PERTINENT INFORMATION

1. **Confidentiality.** When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or other authorized people.

2. **Policy Regarding Research-Related Injuries.** The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. **Payments.** The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health.

4. **Problems or Questions.** If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator at (xxx) xxx-xxxx.

5. **Consent Document.** Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM BELOW, A or B

A. Adult Patient's Consent.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient & Date Signed

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM _____ THROUGH _____.

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study. (Attach NIH 2514-2, Minor's Assent, if applicable)

Signature of Parent(s)/Guardian & Date Signed
If other than parent, specify relationship: _____

Child's Verbal Assent (if applicable).

The information in the above consent form has been adequately described to my child in language that my child can understand, and my child willingly agrees to participate in the study.

Signature of Parent(s)/Guardian & Date Signed

Signature of Investigator & Date Signed

Signature of Witness & Date Signed

APPENDIX C
LABORATORY PROCEDURES

APPENDIX C

LABORATORY PROCEDURES

LABORATORY SPECIMEN COLLECTION, STORAGE AND SHIPPING PROCEDURES

Standard procedures for collection, storage, and shipping of specimens will be followed according to the NMDP and the NHLBI guidelines. Samples will be given a unique alphanumeric code that contains no personal identifiers. Transplant Center Coordinators will hold the link to the code. Laboratory staff will not have access to the link.

LABORATORY CONTRACTS AND REMAINING SAMPLES

All laboratory studies will be performed at laboratories under contract with the NMDP on behalf of the BMT CTN. The laboratory contract specifies that any remaining sample must be stored at the laboratory for the duration of the study. If the investigators choose to perform additional studies on these remaining samples, a formal amendment will be made to the protocol. Any amendments to the protocol are subject to the DSMB and IRB approval process.

At the end of the study, the BMT CTN will either instruct the laboratory to destroy any remaining samples or to transfer the remaining samples to the NHLBI sample repository in Maryland. These samples will be paired with the respective donor or recipient sample and given unique bar code designations that cannot be linked back to the donor or the recipient. An NHLBI Biologic Specimen Repository Utilization Committee will advise the Institute on requests for specimens to perform research with these anonymous samples. If an investigator request for these samples is approved by the committee, the NHLBI may provide a panel of the specimens requested using unique code numbers. Laboratory test results, clinical information, etc., associated with the coded samples are provided to the investigator only after completion of his/her research protocol. Samples sent to researchers cannot be linked with any remaining sample at the repository.

PHARMACOKINETICS

Blood for analysis of fluconazole and voriconazole concentrations will be obtained on Day 14 and 28 of therapy, at onset of serious suspected study drug toxicities as delineated in Section 2.4.6 and at onset of possible, presumptive, probable or proven infection (prior to start of antifungal therapy). Blood should be drawn in relationship to administration of the study drug in one of three windows of time after the start of the infusion or administration of the oral dose: Window 1 is 1-5 hours after start of the infusion; Window 2 is 5-8 hours after start of infusion; Window 3 is 8-12 hours after start of the infusion. On the case report form, indicate the start

time of the drug and the time of the blood draw. The form will calculate from which of the three windows the blood was drawn. Testing of pharmacokinetic samples will be conducted at Dr. Tom Walsh's lab at NCI.

Collection and Processing: Collect 5 mL (2.5 mL for patients less than 12 years old) of blood in green top vacutainer; centrifuge for 20 minutes within 120 minutes (preferably 60 minutes) of collection at 450 x g or 1500 rpm; transfer plasma to a cryovial; sample may stored at 2-8°C for 24 hours prior to cryopreservation at -70°C; batch ship frozen samples on dry ice quarterly to the repository.

ANTIFUNGAL SUSCEPTIBILITY TESTING

Collection and Processing Procedures: All fungal isolates will be collected and identified to the species level at participating centers. Isolates will then be streaked onto Sabouraud dextrose agar slants, grown at 35°C until confluent, and stored at -20°C until use. Serial isolates from a given patient will be collected every seven days when possible following initiation of antifungal therapy, or if infection relapses following the discontinuation of therapy. Frozen isolates will be shipped in batch quarterly to the Study Repository in compliance with the shipping procedures specified in the BMT CTN MOP. The antifungal susceptibility testing will be performed by Dr. Nguyen at the University of Florida College of Medicine.

Assay Procedures: The antifungal susceptibility testing will be performed according to the NCCLS recommendations (NCCLS M27-A for yeast and NCCLS M38-P for moulds) [54, 55]. The susceptibility testing will be performed in batch, and repeated twice on two different days to ensure that the results are reproducible. The reference isolate *Aspergillus flavus* ATCC 204304 and the quality control strain *Candida parapsilosis* ATCC 22019 will be included in each batch of testing as control isolates.

1. Hypothesis

The hypothesis, for patients treated with fluconazole, is that breakthrough fungal infections will be caused by yeasts or moulds exhibiting elevated fluconazole minimum inhibitory concentrations (MICs). A number of these organisms, however, will exhibit low MICs of voriconazole. The hypothesis, for patients treated with voriconazole, is that breakthrough fungal infections will be caused by yeasts or moulds exhibiting elevated MICs of both fluconazole and voriconazole. These organisms will also exhibit elevated MICs of itraconazole, consistent with broad azole cross-resistance. In addition, we hypothesize that mould infections in patients receiving fluconazole will be primarily caused by *aspergillus* spp., whereas mould infections in patients receiving voriconazole will be caused by *Rhizopus* or moulds other than *aspergillus* spp.

2. Antifungal Agents

All isolates will be tested in vitro against fluconazole (Pfizer Inc., New York, NY), itraconazole (Janssen Pharmaceutical, Titusville, NJ), and voriconazole (Pfizer Inc., New York, NY). The antifungal agents will be provided by the manufacturers as assay powders.

3. Assay Medium

The medium used will be RPMI 1640 medium without sodium bicarbonate and with L-glutamine. It will be buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid, and sterilized by filtration.

4. Drug Concentration Ranges

Drug dilutions will be prepared at 100 times the final concentration in 100% dimethyl sulfoxide followed by further dilutions (1:50) in RPMI-1640 medium to yield twice the final concentrations required for the test. The final concentrations of the tested drugs will range from 0.125 to 64 µg/mL for fluconazole and 0.03 to 16 µg/mL for itraconazole and voriconazole. 100 µl of twofold serial dilutions of the antifungal drugs will be dispensed into each well of sterile flat-bottomed plastic microtitre plates. Two drug-free-medium wells will be used per row as sterility and growth controls. The plates will be prepared in batch and stored at –70°C until used.

5. Inoculum Preparations

Yeasts: Two days before the susceptibility test, the yeast strains will be streaked for isolation from the –70°C stock onto Sabouraud Dextrose Agar (SDA). These strains will be streaked again onto SDA a day before the susceptibility test and incubated at 35°C. The yeast suspension will then be prepared from five colonies of each culture to match the turbidity of a McFarland 0.5 standard as determined by a standard spectrophotometric method. Further dilutions will be made in RPMI 1640 medium to achieve a final yeast inoculum of 0.5×10^3 to 2.5×10^3 CFU/mL.

Moulds: Stock inoculum suspensions will be prepared as described in the NCCLS M38-P document from 7-day-old cultures grown on potato dextrose agar slants and adjusted spectrophotometrically to optical density that ranges from 0.09 to 0.3 (82 to 60% transmittance). This stock solution will contain mainly conidia. The nongerminated conidial inoculum suspensions will be diluted 1:50 in medium. The final concentration of stock inoculum will range from 0.5×10^6 to 4.5×10^6 CFU/mL.

6. Determination of MICs

On the day of the test, the microtitre plates containing drug will be allowed to thaw at room temperature. A 100 µl aliquot of the tested inoculum will be added to each well. The plates will then be incubated at 35°C and examined at the time points described. MIC will be determined by visual inspection. For yeasts, the MIC will be determined at both 24 and 48h according to a 0-to-4 scale, with 0 indicating an optically clear culture, 1 indicating a slightly hazy culture, 2 indicating a prominent decrease in turbidity, 3 indicating a slight decrease in turbidity, and 4 indicating no reduction in turbidity. The MIC will be defined as the lowest concentration of a drug for which the score is 2 [54]. For moulds, the MIC will be determined at 48h, and will be defined as the lowest drug concentration that yields a prominent inhibition of growth compared to the growth control well [55].

INVESTIGATIONAL FUNGAL DIAGNOSTIC ASSAYS TO DIAGNOSE ASPERGILLUS AND OTHER INFECTIONS

New assays, including antigen, antibody, metabolite, and PCR techniques, are being developed to improve diagnostic accuracy. Whole blood will be collected for storage and assay by future investigational tests. These blood samples will be collected twice weekly on two non-consecutive days, preferably three days apart (e.g., Mondays and Thursdays), during the first 60 days post-transplant and once weekly on Days 60-100 unless one of the following criteria is met: (1) patient received a T cell depleted transplant and received post-transplant GVHD prophylaxis; or, (2) patient is on steroids; or, (3) patient has or has had acute GVHD requiring systemic therapy. Investigational fungal diagnostic samples will be collected twice weekly from these select patients. Investigational fungal diagnostic samples will also be collected at onset of possible, presumptive, probable and proven infections and twice weekly from patients receiving empirical antifungal therapy for possible invasive infection. Investigational fungal diagnostic samples will also be collected from patients with presumptive, probable or proven infection twice weekly for four weeks, then once every two weeks for eight weeks and then once to twice weekly until Day 100. The investigational assays will be conducted at Dr. John Wingard's lab at the University of Florida College of Medicine.

Collection and Processing:

Serum: Collect 5 mL (2.5 mL for patients less than 12 years old) of blood in red top vacutainer; allow 15-30 minutes for clotting; centrifuge at 900 x g or 2100 rpm for 10 minutes; (clot and serum should be distinct); carefully remove the serum without disturbing the clot (leaving behind a small volume of serum if necessary) and transfer to a cryovial; sample may stored at 2-8°C for 24 hours prior to cryopreservation at -70°C; batch ship frozen samples on dry ice quarterly to the repository.

Blood: Collect 5 mL (2.5 mL for patients less than 12 years old) of blood in green top vacutainer; transfer whole blood, without centrifugation, to a cryovial; sample may stored at 2-8°C for 24 hours prior to cryopreservation at -70°C; batch ship frozen samples on dry ice quarterly to the repository.

GENETIC VARIATION STUDY SAMPLING SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)

A major goal of human genetics is to understand the contribution of genetic diversity to disease susceptibility and outcome. With the completion of a first generation map of the human genome, a catalogue of known genes and their annotated sequence variations has begun to take shape^{1,2}.

DNA sequence variations include insertions, deletions, variable numbers of repeat sequences, and single nucleotide polymorphisms (SNPs). The latter, SNPs, are the most common variants that occur and create alleles that can be investigated for their association with a specific, defined disease outcome³. Current estimates are that there could be as many as 10 million SNPs (defined on the basis of the frequency of the minor allele being greater than 1% in at least one population)⁴. Until recently, the ability to perform genetic association studies with SNPs has been limited by the paucity of known SNPs. Advances in high-throughput sequence analysis and

DNA chip arrays systems are generating an expanding set of reagents that can be used for investigating the relationship between genetic variations and disease. For the past five years, we have exclusively utilized the candidate SNP approach⁵. However, new technical developments and comprehensive SNP catalogues should not only improve the utility of SNP association studies, but also in the near future allow their use in classical linkage studies; however, for the immediate future, this approach is not viable nor is it economically feasible to search for such genetic markers. Accordingly, we will continue to concentrate on candidate SNPs to dissect their genetic contribution to complex diseases⁶. The focus will shift from the study of previously characterized SNPs of known biological importance, towards the identification of novel SNPs and haplotypes in groups of genes belonging to key pathways. Haplotypes comprise a set of genetic variants residing on one chromosome and represent ancestral segments inherited by descent from shared ancestors⁷.

In order to validate SNPs and standardize assays, the laboratory devised a program that has been adapted and supported by the Cancer Genome Anatomy Program (CGAP). This web-based resource, the SNP500Cancer (<http://snp500cancer.nci.nih.gov>). SNP500Cancer is a new database designed to publicly address the immediate needs for candidate SNPs and candidate gene approaches to mapping complex diseases. SNP500Cancer provides bi-directional sequencing information on a set of control DNA samples derived from anonymized subjects (102 Coriell samples representing four self-described ethnic groups: African/African-American, Caucasian, Hispanic and Pacific Rim). All SNPs are chosen from public databases and reports, and the choice of genes includes a bias towards nonsynonymous and promoter SNPs in genes that have been implicated in one or more cancers. The website is searchable for SNPs by gene, chromosome and by known dbSNP ID. For each analyzed SNP, gene location and over 200 bps of surrounding annotated sequence (including nearby SNPs) are provided, with frequency information in total and per sub-population. A calculation of Hardy-Weinberg Equilibrium is included. Moreover, genotyping assays (5'-exonuclease, hybridization-triggered fluorescence, and/or MALDI-TOF) were developed for SNPs with minor allele frequencies > ~5%. These assays have been performed on the same 102 samples and the results compared for concordance with the sequencing results. The website will soon post the conditions (including sequences for probes, primers, etc.) for validated genotyping assays. Estimation of haplotypes is currently provided for select genes, with the goal of expanding this effort rapidly. SNP500Cancer provides an invaluable resource for investigators world-wide to select validated candidate SNPs linked to established genotyping assays and sequence data. In addition, this project forms the cornerstone for the assay validation pipeline of the Core Genotyping Facility.

In this prospective study, the purpose is to study genetic variants in genes of innate immunity, which could contribute to fungal infections. Already, the paradigm has been established in a number of infections, such as malaria, filariasis, RSV infection and most recently in candidiasis in leukemia patients (Choi et al in press JID, 2003). Moreover, the initial rationale was based upon the search for genetic determinants of infectious complications using immune SNPs in a well-defined population receiving comparable therapy, but the design is suitable for investigating pharmacogenomic questions at a later time⁸. The choice of genes was made because on the basis of three criteria: (1) well defined SNPs altered function of the gene (either the protein sequence or expression), (2) a frequency for the variants was greater than 5% (most are between 20 and 35% for the minor alleles), which provides adequate opportunity for statistical analysis and (3)

prior association studies⁸⁻⁹. A total of 20 genes will be studied by Dr. Tom Walsh at NIH/NCI/POB. The genes were chosen from several pathways within innate immunity, specifically, because these pathways are not substantially altered by changes in lymphocyte or phagocyte populations following myelotoxic therapy. The 18 genes involved in intrinsic host defense include *MBL2*, *CCR5*, *IL1RN*, *IL1A*, *IL1B*, *IL6*, *IL8*, *IL8RA*, *IL8RB*, *IL10*, *TNFA*, *TNFB*, *MPO*, *NRAMP1*, *CHIT1*, *FCGR2A*, *FCGR3A*, and *FCGR3B*. The other two genes will be studied for the metabolism of voriconazole (*3A4* and *2C19*). The products of the 20 following genes mediate their respective functions (indicated in parentheses): *MBL2* (involved in innate host recognition of fungal pathogens); *CCR5*, *IL8*, *IL8RA*, and *IL8RB* (mediate recruitment of PMNs); *IL1RN*, *IL1A*, *IL1B*, and *IL6*, *TNFA*, *TNFB* (pro-inflammatory cytokine mediators); *IL10* (suppression of phagocytic host defenses); *MPO* (mediator of phagocytic oxidative injury); *NRAMP* (mediates intracellular phagocytic injury and divalent metal efflux); *CHIT1* (mediates chitin and fungal cell wall hydrolysis); *FCGR2A*, *FCGR3A*, and *FCGR3B* (antibody receptors); and *3A4* and *2C19* (cytochrome P450-dependent hepatic microsomal enzymes involved in metabolic clearance of voriconazole). Additional genes mediating host response and metabolism will be studied as new technology is developed.

Additional SNP analyses in six other genes involved in the innate immune response to fungal infection will be conducted by Dr. Patricia Fraser at the Center for Blood Research at Harvard Medical School. The six genes and their respective functions (indicated in parenthesis) include *MICA* and *MICB* (augment cytotoxicity mediated by $\gamma\delta$ T cells and NK cells and co-stimulate $CD8^+$ $\alpha\beta$ T cells in pathogen-specific immune response), *TLR4* and *CD14* (*TLR4* mediates cell signaling from exposure to microbial pathogens such as fungi and complexes with *CD14* which facilitates monocyte activation by *Aspergillus* hyphae), *HBD-1* (expresses antifungal properties) and *IL-18* (confers protection against pulmonary and disseminated infections).

Collection and Processing: Collect 5mL of blood or stem cell product (2.5 mL for patients less than 12 years old) from related donor in green top vacutainer; transfer whole blood, without centrifugation, to two cryovials; samples may be stored at 2-8°C for 24 hours prior to cryopreservation at -70°C; batch ship frozen samples on dry ice to the repository.

Sample Analysis

1. Dr. Tom Walsh at NIH/NCI/POB and Dr. Patricia Fraser at the Center for Blood Research at Harvard Medical School will conduct extraction of genomic DNA by standard technique under SOPs filed at their laboratories.
2. Samples will be given a numerical number and a record linking the number to patient information will be kept confidential. It will only be available for investigators participating in this study. Samples will be linked to clinical data collected under the supervision of Dr. Walsh. Genomic samples will not be shared with other investigators unless approved by an IRB amendment and informed consent is given.
3. Sample tracking will be accomplished using a numerical code assigned each consecutive patient enrolled on study. Clinical data will be entered on datasheets containing the polymorphism/variant data on the 26 genes of the study.

4. Genetic analysis will be performed on fragments amplified by the polymerase chain reaction (PCR) and subjected to analysis designed to distinguish between known alleles in the following genes: *MBL2*, *CCR5*, *IL1RN*, *IL1A*, *IL1B*, *IL6*, *IL8*, *IL8RA*, *IL8RB*, *IL10*, *TNFA*, *TNFB*, *MPO*, *NRAMP1*, *CHIT1*, *FCGR2A*, *FCGR3A*, *FCGR3B*, *3A4*, *2C19*, *MICA*, *MICB*, *TLR4*, *CD14*, *HBD-1* and *IL-18*. Each study will be confirmed at least twice before entry into the spread sheet data bank.

Data Evaluation

- 1.a Sample analysis will be performed as they are made available.
- 1.b The genomic DNA of each patient will be genotyped for allelic variations in the 26 genes: *MBL2*, *CCR5*, *IL1RN*, *IL1A*, *IL1B*, *IL6*, *IL8*, *IL8RA*, *IL8RB*, *IL10*, *TNFA*, *TNFB*, *MPO*, *NRAMP1*, *CHIT1*, *FCGR2A*, *FCGR3A*, *FCGR3B*, *3A4*, *2C19*, *MICA*, *MICB*, *TLR4*, *CD14*, *HBD-1* and *IL-18*. The genotype determination for each gene will be considered to be a separate variable for each patient.
- 1.c Data will be analyzed with respect to each gene in terms of allelic frequency and separately in terms of gene distribution (wild type, heterozygotes and homozygotes for the gene being analyzed).
- 1.d Frequencies will be compared to the general population norms- both published and established in our laboratory. Comparison for statistical significance will be evaluated within the cohort to determine if specific alleles are over or under-represented.
2. Epidemiological data on the infectious complications of all enrolled will be evaluated and entered into the database program for comparison with genotypic evaluation of individual patients. Continuous and non-continuous variable analysis will be performed.
3. The primary outcome will be a direct comparison of the allelic frequencies of a specific gene or combination of genes in the identified population to established controls (already established in our laboratory and in the literature). Secondary outcomes that will undergo statistical analysis are continuous variables (e.g., complications, response to therapy and presenting parameters).
4. After univariate analyses are performed in an exploratory mode for the individual loci, multivariate analyses of the possibly correlated outcomes will be performed. This analysis will be performed with as few as two loci and as many as all 26 loci.
5. Complex trait analysis will be performed on the database. All of the variables included in the infectious events will be analyzed with respect to cohorts of patients determined by allelic frequency.

PARAFFIN BLOCKS OF INFECTED TISSUE SPECIMENS

Paraffin blocks from tissues found to be infected should be saved and batch sent quarterly at room temperature to the Study Repository in compliance with the shipping procedures specified in the BMT CTN MOP. Dr. John Wingard at the University of Florida will analyze these specimens for identification of fungal pathogen where cultures were negative but organisms were seen microscopically.

INVESTIGATIONAL FUTURE TESTING

Blood for investigational future testing will be collected at baseline prior to initiation of conditioning. The identification and location of test(s) to be performed have not been determined.

Collection and Processing: Collect 5 mL (2.5 mL for patients less than 12 years old) of blood in red top vacutainer; allow 15-30 minutes for clotting; centrifuge at 900 x g or 2100 rpm for 10 minutes; (clot and serum should be distinct); carefully remove the serum without disturbing the clot (leaving behind a small volume of serum if necessary) and transfer equally to two cryovials; samples may stored at 2-8°C for 24 hours prior to cryopreservation at -70°C; batch ship frozen samples on dry ice quarterly to the repository.

DIAGNOSTIC GALACTOMANNAN ASSAYS

Incorporation of the “real-time” diagnostic GM assay along with classical criteria will improve accuracy and may increase the number of documented invasive aspergillosis cases (by upgrading possible cases not proven by classical criteria to proven or probable cases with the GM assay). Blood samples for the GM assays will be collected twice weekly on non-consecutive days, preferably three days apart (e.g., Mondays and Thursdays), during the first 60 days post-transplant and once weekly on Days 60-100 unless one of the following criteria is met: (1) patient received a T cell depleted transplant and received post-transplant GVHD prophylaxis; or, (2) patient is on steroids; or, (3) patient has or has had acute GVHD requiring systemic therapy. GM blood samples will be collected twice weekly from these select patients. Diagnostic GM samples should also be collected at onset of possible infections and twice weekly from patients receiving empirical trials of amphotericin B or caspofungin for possible invasive infection.

GM samples may be processed at contract laboratories or hospital laboratories associated with the transplant center if the laboratory is certified by CLIA, CAP or JCAHO. Samples to be processed at these certified laboratories must be collected twice weekly on two non-consecutive days, preferably three days apart. Results are required within 72 hours of collection. It is recommended that these certified laboratories complete Bio-Rad’s proficiency panel and use the criteria for galactomannan positivity as defined in §3.2 of the protocol, i.e., two consecutive positives on one sample.

Collection and Processing: Collect 5 mL of blood (2.5 mL for patients less than 12 years old) in gold top plastic serum separator tube; allow 15-30 minutes for clotting; centrifuge at 900 x g or 2100 rpm for 10 minutes but do not decant or transfer *unless instructed otherwise by reference*

lab; store at 2-8°C for up to 72 hours if necessary; ship sample on cold packs immediately via priority overnight to the reference lab according to the lab's instructions.

INVESTIGATIONAL MONITORING OF TREATMENT GALACTOMANNAN ASSAYS

For patients with presumptive, probable or proven aspergillus infection, monitoring of treatment GM samples will be collected at onset and twice weekly for four weeks and then once every two weeks for eight weeks for a total of 12 samples. These samples will be processed at the Fred Hutchinson Cancer Research Center at a later date. Results will not be made available to the transplant center.

Collection and Processing: Collect 5 mL of blood (2.5 mL for patients less than 12 years old) in gold or red top, plastic or glass serum separator tube; allow 15-30 minutes for sample to clot; centrifuge at 900 x g or 2100 rpm for 10 minutes (clot and serum should be distinct); carefully remove the serum without disturbing the clot (leaving behind a small volume of serum) and transfer to cryovial; sample may stored at 2-8°C prior to cryopreservation at -70°C; batch ship on dry ice quarterly to the repository.

INVESTIGATIONAL GALACTOMANNAN ASSAYS OF BAL

Whenever a bronchoscopy is performed, 1 mL of BAL (bronchoalveolar lavage) will be collected in a cryovial, frozen at -70°C, and batch shipped quarterly to the Repository. These samples will be processed at the Fred Hutchinson Cancer Research Center at a later date. Results will not be made available to the transplant center.

REPOSITORY INFORMATION

Room temperature and frozen samples should be batched and shipped quarterly to the Repository in compliance with the shipping procedures specified in the BMT CTN MOP and the BMT CTN 0101 Laboratory Sample Information Guide:

Misti Dowell
NHLBI Repository
SeraCare BioServices
217 Perry Parkway
Gaithersburg, Maryland 20877
Phone: (301) 208-8100
Fax: (301) 208-8829
Email: nhlbi@bbii.com

REFERENCES IN APPENDIX C

1. Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. *Science* 2001; 291:1304-51.
2. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature* 2001; 409:860-921.
3. Kruglyak L, Nickerson DA. Variation is the spice of life. *Nat Genet* 2001; 27:234-6.
4. Taylor JG, Choi EH, Foster CB, Chanock SJ. Using genetic variation to study human disease. *Trends Mol Med* 2001; 7:507-12.
5. Chanock S. Candidate genes and single nucleotide polymorphisms (SNPs) in the study of human disease. *Dis Markers* 2001; 17:89-98.
6. Schork NJ, Fallin D, Thiel B, et al. The future of genetic case-control studies. *Adv Genet* 2001; 42:191-212.
7. Fallin D, Cohen A, Essioux L, et al. Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. *Genome Res* 2001; 11:143-51.
8. Chanock SJ, Foster CB. SNPing away at innate immunity. *J Clin Invest* 1999; 104:369-70.
9. Foster CB, Chanock SJ. Mining variations in genes of innate and phagocytic immunity: current status and future prospects. *Curr Opin Hematol* 2000; 7:9-15.

Table C-1
LABORATORY SCHEDULE

TEST	TYPE OF SAMPLE (collection container)	TYPE OF STORAGE	DATES SAMPLES OBTAINED	SHIPPING SPECIFICATIONS	LOCATION OF TEST PERFORMED
Pharmacokinetics	5 mL peripheral blood ¹ (green top tube)	Centrifuge for 20 min within 120 min of collection; transfer plasma to cryovial Store at -70°C	Day 14 & 28 of therapy, at onset of serious suspected drug toxicities as delineated in Section 2.4.6 and at onset of possible, presumptive, probable or proven infection (prior to start of antifungal therapy).	Frozen shipment quarterly to Repository as specified in the BMT CTN MOP/BMT CTN 0101 Laboratory Sample Information Guide	Dr. Walsh's Lab at NCI
Antifungal Susceptibility Test	Fungal isolate from any infected site, if available	Streak on agar slant Store at -20°C	Serial isolates every 7 days if available following initiation of antifungal therapy, or if infection relapses following discontinuation of therapy.	Frozen shipment quarterly to Repository as specified in the BMT CTN MOP/BMT CTN 0101 Laboratory Sample Information Guide	Dr. Nguyen's Lab at University of Florida College of Medicine
Investigational Fungal Diagnostic Assays	5 mL peripheral blood ¹ (red top tube) 5 mL peripheral blood ¹ (green top tube)	Sit for 15-30 minutes to allow for clotting centrifuge for 10 min; transfer serum to cryovial May store at 2°-8° C for up to 24 hours For longer storage -70°C Transfer to cryovial without centrifugation May store at 2°-8° C for up to 24 hours For longer storage -70°C	2x/week from Day 0 to Day 60. After Day 60, samples will be collected once per week until Day 100 unless one of the following is met: (1) patient received a T cell depleted transplant and received post-transplant GVHD prophylaxis, or (2) patient is on steroids, or (3) patient has or has had acute GVHD requiring systemic therapy. These patients will provide samples 2x/week until Day 100. Samples will also be collected at onset of possible fungal infection and 2x/week in patients receiving an empirical trial of amphotericin B or caspofungin for possible invasive fungal infection. For patients with presumptive, probable or proven aspergillus infection, 2x/week for 4 weeks and then once every 2 weeks for 8 weeks for a total of 12 samples, and then 1-2x/week until Day 100.	Frozen shipment quarterly to Repository as specified in the BMT CTN MOP/BMT CTN 0101 Laboratory Sample Information Guide	Dr. John Wingard's Lab at University of Florida College of Medicine
Donor SNPs (related donor) (Single Nucleotide Polymorphisms)	5 mL peripheral blood or stem cell product from donor (green top tube)	Transfer without centrifugation equally to two 3.6 mL cryovials Store at -70°C	At baseline prior to transplant.	Frozen shipment quarterly to Repository as specified in the BMT CTN MOP/BMT CTN 0101 Laboratory Sample Information Guide	Dr. Walsh's Lab at NCI and Dr. Fraser's Lab at Harvard

TEST	TYPE OF SAMPLE (collection container)	TYPE OF STORAGE	DATES SAMPLES OBTAINED	SHIPPING SPECIFICATIONS	LOCATION OF TEST PERFORMED
Paraffin Blocks of Infected Tissues	Paraffin blocks	Room temperature	At time of infection.	Ship at room temp. quarterly to Repository as specified in the BMT CTN MOP/BMT CTN 0101 Laboratory Sample Information Guide	Dr. Wingard's Lab at the University of Florida College of Medicine
Diagnostic Galactomannan Assays²	5 mL peripheral blood ¹ (gold hemogard™ tube)	Sit for 15-30 minutes to allow for clotting Centrifuge for 10 min (but do not decant or transfer), leave in gold hemogard™ collection tube unless instructed otherwise by reference lab Store at 2°-8°C	2x/week from Day 0 to Day 60. After Day 60, samples will be collected once per week until Day 100 unless one of the following is met: (1) patient received a T cell depleted transplant and received post-transplant GVHD prophylaxis; or, (2) patient is on steroids; or, (3) patient has or has had acute GVHD requiring systemic therapy. These patients will provide samples 2x/week until Day 100. Samples will also be collected prior to and at onset of possible fungal infection and 2x/week in patients receiving an empirical trial of amphotericin B or caspofungin for possible invasive fungal infection.	Cold pack shipment PRIORITY OVERNIGHT to reference lab in compliance with shipping procedures specified in the BMT CTN MOP/BMT CTN 0101 Laboratory Sample Information Guide	Certified contract lab or certified hospital lab associated with the transplant center
Investigational Monitoring of Treatment Galactomannan Assays	5 mL peripheral blood ¹ (gold hemogard™ tube)	Sit for 15-30 minutes to allow for clotting Centrifuge for 10 min; transfer serum to cryovial May store at 2°-8°C for up to 24 hours. Freeze at -70°C	For patients with presumptive, probable or proven aspergillus infection, at onset and 2x/week for 4 weeks and then once every two weeks for a total of 12 samples.	Frozen shipment quarterly to Repository as specified in the BMT CTN MOP/BMT CTN 0101 Laboratory Sample Information Guide	Dr. Kieren Marr's Lab at Fred Hutchinson Cancer Research Center
Investigational Galactomannan Assays of BAL (bronchoalveolar lavage)	1 mL BAL (cryovial)	May store at 2°-8°C for up to 24 hours. Freeze at -70°C	Anytime bronchoscopy is performed.	Frozen shipment quarterly to Repository as specified in the BMT CTN MOP/BMT CTN 0101 Laboratory Sample Information Guide	Dr. Kieren Marr's Lab at Fred Hutchinson Cancer Research Center

TEST	TYPE OF SAMPLE (collection container)	TYPE OF STORAGE	DATES SAMPLES OBTAINED	SHIPPING SPECIFICATIONS	LOCATION OF TEST PERFORMED
Investigational Future Testing	5 mL peripheral blood ¹ (red top tube)	Sit for 15-30 minutes to allow for clotting Centrifuge for 10 min; transfer serum to two cryovials. May store at 2°-8°C for up to 24 hours. For longer storage -70°C	Baseline.	Frozen shipment quarterly to Repository as specified in the BMT CTN MOP/BMT CTN 0101 Laboratory Sample Information Guide	TBD

Notes for Table C-1:

¹ Collect 2.5 mL for pediatric patients less than 12 years old

² Diagnostic GM samples must be collected twice weekly on two non-consecutive days preferably three days apart (e.g., Monday and Thursday).

APPENDIX D

GALACTOMANNAN ASSAY

Platelia[®] Aspergillus Laboratory Protocol
with
Platelia[®] Aspergillus Kit Receipt Log
Platelia[®] Aspergillus Package Insert
Platelia[®] Aspergillus Worksheets
Platelia[®] Aspergillus Quick Reference Guide

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PLATELIA[®] ASPERGILLUS LABORATORY PROTOCOL

for

**“A Randomized Double-blinded Trial of Fluconazole vs. Voriconazole
for the Prevention of Invasive Fungal Infections in Allogeneic Blood and Marrow
Transplant Patients”**

Read the entire protocol, including the appendices, before beginning the study. Instructions for collecting, handling, and testing specimens are provided in detail in this protocol.

INTENDED USE

The Platelia[®] *Aspergillus* EIA Galactomannan Assay is a 1-stage immunoenzymatic assay utilizing the sandwich microplate technique for the detection of galactomannan in human serum. This assay is to be used and test results interpreted in conjunction with other conventional diagnostic procedures such as microbiological culture, histologic examination of biopsy samples, and other signs and symptoms for detection of aspergillus infection. Positive Platelia[®] *Aspergillus* EIA test results are an aid in the early diagnosis of Invasive Aspergillosis, and, along with other diagnostic tests and clinical signs of disease, allow for effective early treatment of disease. Negative Platelia[®] *Aspergillus* EIA test results, along with other diagnostic tests, can be used to prevent the unnecessary use of anti-fungal agents in the absence of Invasive Aspergillosis.

SUMMARY AND EXPLANATION

Aspergillus infections usually occur following inhalation of aspergillus spores, which are present in the environment. Invasive forms, which have been on the increase for more than 10 years, constitute the most serious infections. They mainly occur in neutropenic patients (following anticancer treatment) and in patients treated with immunosuppressants (organ transplantations, particularly bone marrow transplantation) and corticosteroids⁴. AIDS-related cases of aspergillus have also been reported⁵. The efficacy of treatment depends on early introduction of treatment, but the diagnosis of Aspergillosis is still very difficult. The test for anti-aspergillus antibodies is not adapted to the diagnosis of invasive Aspergillosis in immunocompromised patients. Aspergillus is rarely isolated from blood culture. The diagnosis is often based on nonspecific or late criteria (clinical symptoms, radiology, etc.) At the present time, the test for soluble aspergillus antigens in the serum appears to be the only serological method able to improve both the rapidity and specificity in the diagnosis of the infection^{6,7}. Among the various antigens present in body fluids of patients suffering from invasive Aspergillosis, carbohydrates and particularly galactomannan appear to be well-suited for the diagnosis of the infection^{8,9}.

⁴ Denning DW, Stevens DA. 1990. Anti-fungal and surgical treatment of invasive Aspergillosis: review of 2121 published cases. *Rev. Infect. Dis.* 12:1147 - 1201.

⁵ Lortholary O, Meyohas MC, Dupont B, et al. 1993. Invasive aspergillosis in patients with acquired immunodeficiency syndrome: report of 33 cases. *Am J. Med.* 95:177 - 187.

⁶ Andriolet VT. 1993. Infections with *Aspergillus* species. *Clin Infect Dis.* 17 (suppl 2) : S481-6.

⁷ De Repentigny L, Kaufman L, Cole GT, Druse K, Latge JP, Matthews RC. 1994. Immunodiagnosis of invasive fungal infections. *J. Med Vet Mycol.* 32 (S1): 239 - 252.

PRINCIPLE OF THE PROCEDURE

The Platelia® *Aspergillus* EIA is a one-stage immunoenzymatic sandwich microplate assay that detects galactomannan in human serum. The assay uses the rat monoclonal antibody EBA-2, which is directed against aspergillus galactomannan, and has been characterized during previous studies^{10 11 12}. The monoclonal antibody is used to sensitize the wells of the microplate, bind the antigen and as the detector antibody in the conjugate reagent (peroxidase-linked monoclonal antibody). Serum samples are heat-treated in the presence of EDTA in order to dissociate the immune-complexes and to precipitate serum proteins, which could possibly interfere with the EIA. The treated serum samples and conjugate are added to the wells coated with the monoclonal antibody, and incubated. A monoclonal antibody - galactomannan - monoclonal antibody / peroxidase complex is formed in the presence of aspergillus antigen. The strips are washed to remove any unbound material. Next, the substrate solution is added, which will react with the complexes bound to the well to form a blue color reaction. The enzyme reaction is stopped by the addition of acid, which changes the blue color to yellow. The optical absorbance of specimens and controls is determined with a spectrophotometer set at 450 and 620/630-nm wavelength.

⁸ De Repentigny L. 1989. Serological techniques for diagnosis of fungal infection. *Eur. J. Clin Microbiol Infect Dis.* 8 (4) : 362 - 375.

⁹ Latge JP. 1995. Tools and trends in the detection of *Aspergillus fumigatus*. *Curr Top Med Mycol*, in press.

¹⁰ Latge JP, Kobayashi H, Debeaupuis JP, Diaquin M, Sarfati J, Wieruszkeski JM, Parra E, Bouchara JP, Fournet B. 1994. Chemical and immunological characterization of the extracellular galactomannan secreted by *Aspergillus fumigatus*. *Infect Immun.* 62: 5424 -5433.

¹¹ Stynen D, Sarfati J, Symoens F, Goris A, Nolard N, Latge JP. 1991. Rat monoclonal antibodies against exocellular carbohydrate antigens of *Aspergillus* and dermatophytes. In: "Fungal Cell Wall and Immune Response", 181 -193. Ed. By J.P. Latge and D. Boucias, Springer Verlag.

¹² Stynen D, Sarfati J, Goris A, Prevost MC, Lesour M, Kamphuis H, Darras V, Latge JP. 1992. Rat monoclonal antibodies against *Aspergillus galactomannan*. *Infect Immun.* 60: 2237 - 2245.

STUDY DESIGN for Laboratory Protocol for

BMT CTN Protocol 0101 – “A Randomized Double-blinded Trial of Fluconazole versus Voriconazole for the Prevention of Invasive Fungal Infections in Allogeneic Blood and Marrow Transplant Recipients”

Objectives:

- 1) To perform exploratory analyses of quantitative aspects of the Platelia[®] *Aspergillus* EIA in detecting galactomannan antigen in serum as an aid in the diagnosis of invasive Aspergillosis in allogeneic blood and marrow transplant patients receiving prophylactic treatment with Fluconazole or Voriconazole (diagnostic phase).
- 2) To perform exploratory analyses of quantitative aspects of the Platelia[®] *Aspergillus* EIA in determining response to anti-fungal therapy in allogeneic blood and marrow transplant patients with proven or probable invasive Aspergillosis (monitoring phase).

Population:

Allogeneic hematopoietic transplant recipients randomized to receive post-transplant prophylaxis with either Fluconazole (N = 300) or Voriconazole (N = 300). The length of these studies is expected to be three years.

Study Design:

This is a randomized, double-blinded, multicenter, prospective, comparative study of Fluconazole versus Voriconazole prophylaxis in the prevention of invasive fungal infections in allogeneic hematopoietic transplant recipients. The Platelia[®] *Aspergillus* assay for detection of galactomannan is being used in conjunction with host and clinical factors (see Table 3.1.1 a and b in BMT CTN Protocol 0101) to determine the likelihood of invasive aspergillus infection.

Sample Collection and Testing:

DIAGNOSTIC PHASE

A serum specimen for the Platelia[®] *Aspergillus* assay will be collected prior to the start of the conditioning regimen. Serum blood samples will be obtained up to twice weekly from Day 0 to Day 100 for testing with the Platelia[®] *Aspergillus* assay.

The Platelia[®] *Aspergillus* EIA will be performed “real-time” for this protocol, and positive or negative assay results will be reported to the clinician. Positive results will elicit further clinical evaluation for the presence of invasive aspergillus.

The assay testing will be performed at a certified reference laboratory. Testing will be performed in all sera following the manufacturer’s package insert instructions. Results of each assay will be communicated to each site’s clinical coordinator within three business days. Index Values and positive or negative results will be reported.

MONITORING PHASE

During this monitoring phase, two serum samples will be collected within 7 days of initiation of anti-fungal therapy, with one of the samples collected 48 hours before. After anti-fungal therapy has been initiated and during therapy monitoring, a serum sample will be collected twice a week for four weeks and then once every two weeks for eight weeks. In all, 12 samples will be collected and tested with the Platelia[®] *Aspergillus* assay after the initiation of drug therapy. Samples will be frozen at -70°C and batch shipped quarterly to the Repository. These samples should be clearly marked "Monitoring of Treatment." Results of this testing will not be reported to the clinician.

Testing Algorithm:

DIAGNOSTIC PHASE

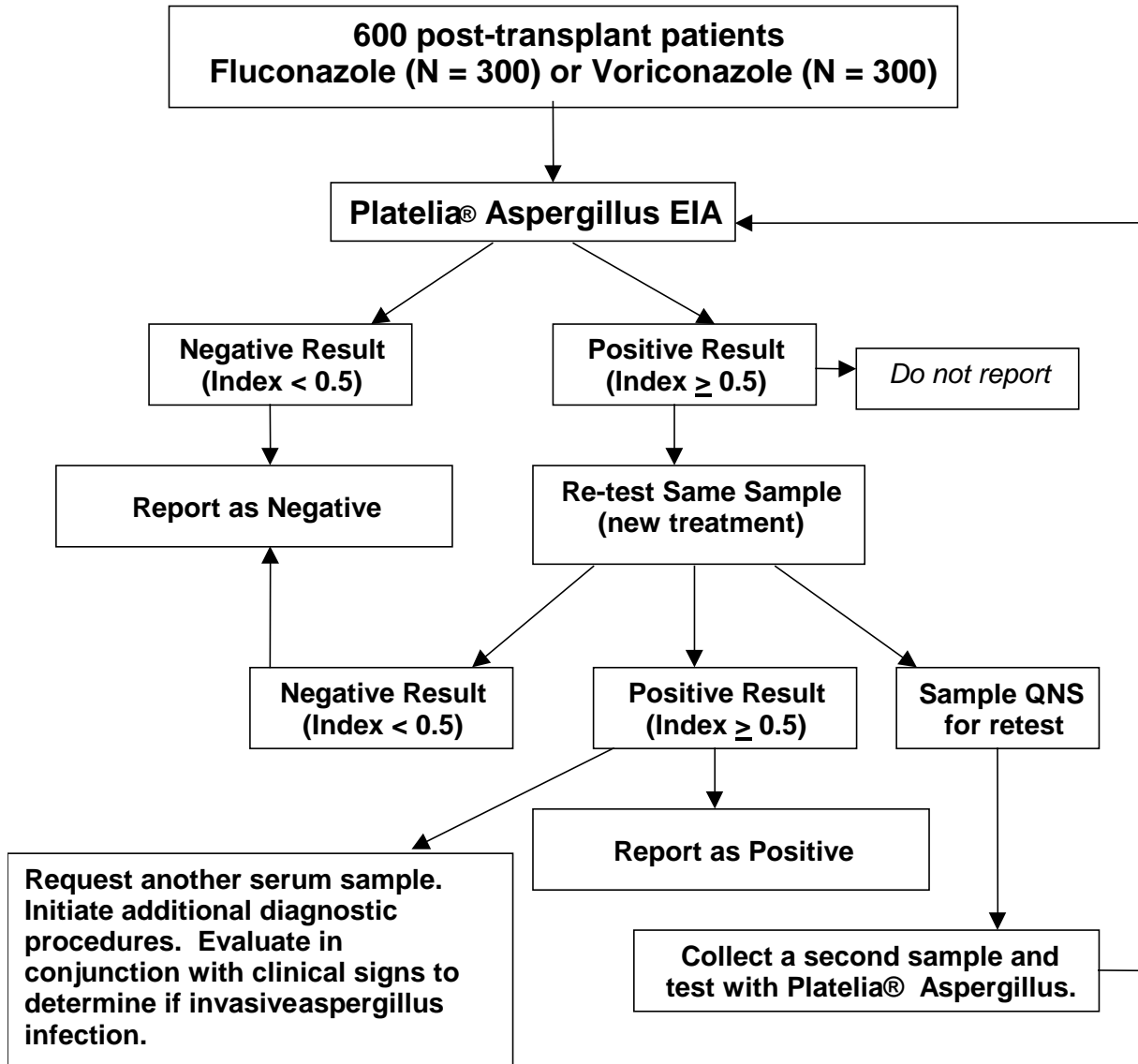
If a sample is negative in the Platelia[®] *Aspergillus* assay, the sample is reported as negative. If a sample is initially positive in the Platelia[®] *Aspergillus* assay, the result is not reported and a new treatment of the same sample will be retested as soon as possible. If the repeat test result using a new treatment is also positive, the result will be reported as positive and another serum sample will be requested for testing with the Platelia[®] *Aspergillus* assay. In addition, additional diagnostic procedures will be initiated. These will be evaluated in conjunction with clinical signs to determine the likelihood of invasive aspergillus infection.

MONITORING PHASE

The monitoring phase of the study will begin for any patient who is diagnosed with invasive aspergillosis and begins anti-fungal therapy. Samples will be collected as described above. Testing will be performed at a later date. Test results are to be blinded and not reported to the clinician.

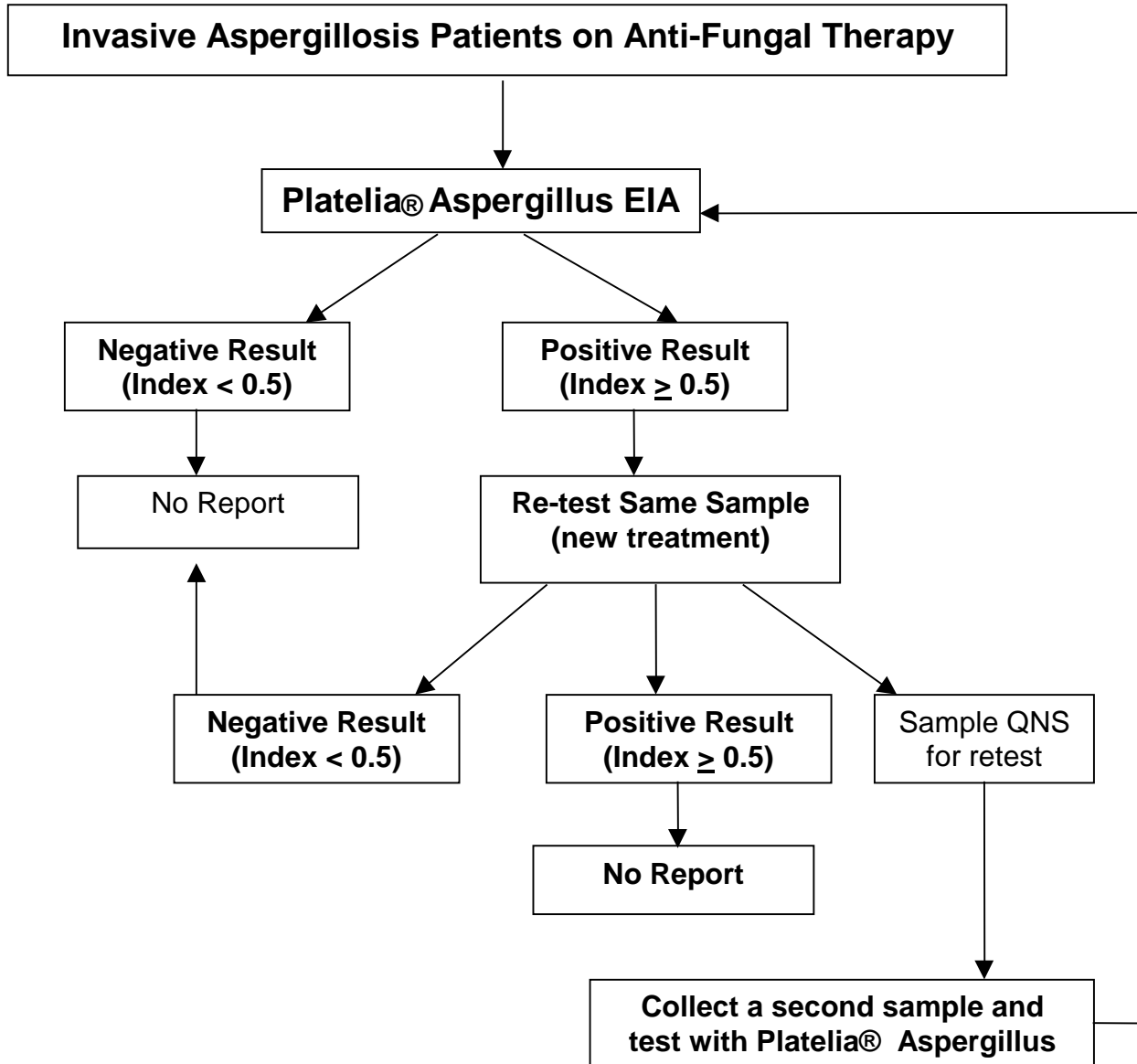
TESTING ALGORITHM

Diagnostic Phase



TESTING ALGORITHM

Monitoring Phase
(No reporting of results)



TECHNICAL INFORMATION

Store the Platelia® *Aspergillus* assay kits at 2-8 °C. The kit lot number as well as the expiration date will be on the box containing the reagents.

All printouts must be signed in blue or black ink by the person actually performing the Platelia® *Aspergillus* assay and should be sent by facsimile or electronically weekly to the study monitor:

Lisa McLaughlin	Phone: 425-498-1774
Bio-Rad Laboratories	Fax: 425-498-1651
6565 185 th N.E.	E-mail: lisa_McLaughlin@bio-rad.com
Redmond, WA 98052	

The investigator, or a responsible party designated by the investigator, must maintain an inventory record of devices/reagents received (see Attachment A to Appendix D) to assure that the materials will not be used for any purpose other than what is stated in this protocol. These records must include all dates, lot numbers, quantities received, and identification of the subject. If any portion of the device is lost, i.e. wastage, the name of the responsible individual, the date, and the quantity must be documented. The study reagents must be used only at the institution specified in this protocol.

Return the completed Platelia® *Aspergillus* Receipt Log and all unused devices/reagents in their original containers to the attention of the study monitor at Bio-Rad Laboratories at the conclusion or termination of the study. Return unused serum samples to the Repository.

MATERIALS AND METHODS

A. Materials Provided by Bio-Rad Laboratories

- Platelia® *Aspergillus* Kits
- 7 Member panel for Training / Proficiency

Platelia® Aspergillus Kit Composition [Product No. 62797 (96 Tests)]

Component	Contents	Quantity
R1 - Microwell Strip Plate	<ul style="list-style-type: none"> 96 wells coated with antigalactomannan monoclonal antibody 	1 plate containing 12 strips with 8 wells each
R2 - Wash Solution, 10X Concentrate	<ul style="list-style-type: none"> Tris NaCl buffer 1% Tween 20 0.01% sodium merthiolate 	1 x 100 mL
R3 - Negative Control	<ul style="list-style-type: none"> Freeze-dried human negative serum Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen 	2 x 1 mL
R4 – Cut-off Control	<ul style="list-style-type: none"> Freeze-dried human serum containing galactomannan Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen 	2 x 1 mL
R5 - Positive Control	<ul style="list-style-type: none"> Freeze-dried human serum containing galactomannan Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen 	2 x 1 mL
R6 - Conjugate	<ul style="list-style-type: none"> Peroxidase-labeled antigalactomannan monoclonal antibody 0.01% sodium merthiolate 	1 x 8 mL
R7 - Serum Treatment Solution	<ul style="list-style-type: none"> EDTA acid solution 	1 x 10.5 mL
R8 - Substrate buffer	<ul style="list-style-type: none"> Citric acid Sodium acetate 0.009% Hydrogen peroxide 4% Dimethylsulfoxide (DMSO) 	1 x 60 mL
R9 - Chromogen	<ul style="list-style-type: none"> Dimethylsulfoxide (DMSO) Tetramethylbenzidine (TMB)* 	1 x 1 mL
R10 - Stopping Solution	<ul style="list-style-type: none"> 1.5 N Sulphuric acid 	1 x 12 mL
Plate sealers	<ul style="list-style-type: none"> Adhesive sheets for microplates 	6

*NOTE: TMB (Tetramethylbenzidine) is a non-carcinogenic and non-mutagenic chromogen for peroxidase. Store the kit at 2-8°C. Bring all reagents to room temperature (15-30°C) before use. Return all reagents, except controls, to 2-8°C immediately after use. After reconstitution, unused Negative Control, Cut-off Control, and Positive Control must be frozen at -20°C. Return unused strips/plates to pouch and reseal. Do not remove desiccant. Strips should be used within 5 weeks of opening and resealing the pouch. After dilution, wash solution can be kept for 15 days at 2-8°C. All other reagents are stable until expiration after opening. Reagents are supplied in sufficient quantity to perform 96 determinations in a maximum of 6 batches.

B. Materials Not Provided

1. Distilled or deionized water, for dilution of Wash Solution.
2. Sterile purified water, for reconstitution of control sera.
3. Absorbent paper.
4. Disposable gloves.
5. Protective glasses.
6. Sodium hypochlorite (bleach) and sodium bicarbonate.
7. Pipettes or multi-pipettes, adjustable or fixed, to measure and dispense 50 µl, 100 µl, 300 µl, and 1000 µl.
8. 1.5 mL Eppendorf (Sarstedt Cat. # 72.692.105) or similar polypropylene tubes with airtight stoppers, able to support heating to 100°C.

9. Laboratory bench centrifuge for Eppendorf tubes capable of obtaining 10,000g (Brinkman Cat.# 22-36-280-1 or VWR Scientific Cat.# 20901-051 or equivalent).
10. Vortex agitator.
11. Boiling water bath or heating block (VWR Scientific Cat. # 13259-005).
12. Micro-plate incubator at $37 \pm 1^\circ\text{C}$.
13. Manual or semi-automatic or automatic plate washer.
14. Microplate reader equipped with 450 nm and 620/630 nm filters.
15. Printer.

PRECAUTIONS

A. Health and Safety Precautions

1. The Positive Control, Cut-off Control, and Negative Control are heat-inactivated, human serum. They have been tested and found to be negative for anti-HIV-1, anti-HIV-2, and anti-HCV antibodies, as well as HBs antigen. However, all the reagents should be handled as though capable of transmitting infection. All tests should be conducted using the precautions recommended for bloodborne pathogens, as defined by OSHA regulations.
2. Wear protective clothing and disposable gloves while handling the kit reagents and patient samples. Wash hands thoroughly after performing the test.
3. Do not pipette by mouth.
4. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
5. Avoid splashing samples or solutions containing them.
6. Biological spills not containing acid should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach (0.5% solution of sodium hypochlorite), 70% ethanol, or 0.5% Wescodyne™. Spills containing acid should be wiped dry or neutralized with sodium bicarbonate and then cleaned with one of the chemical disinfectants. Materials used to wipe up spills should be disposed of as biohazardous waste. CAUTION: Do not place solutions containing bleach in the autoclave.
7. Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Disposal should comply with all applicable waste disposal requirements.
8. CAUTION: The Stopping Solution is an acid. Wipe up spills immediately and flush the areas with water.
R36/38: Irritating to eyes and skin.
S:2-26-30: Keep out of reach of children. In case of contact with eyes, flush immediately and thoroughly with water and consult a physician. Never pour water into this product.
9. Avoid contact of Substrate Buffer, Chromogen, and Stopping Solution with eyes, skin, and mucosa (risk of toxicity, irritation, and burns).

B. Test Precautions

Please follow these guidelines in order to maintain consistency and reliability of results when using the Bio-Rad Laboratories Platelia® *Aspergillus*:

1. Do not use kit or any kit reagents after the stated expiration date.
2. Do not mix reagents from other kits that have the same or different lot numbers.
3. Store Platelia® *Aspergillus* kit reagents at 2 - 8°C. Bring all reagents to room temperature before use. Return to storage at 2 - 8°C immediately after use.
4. Mix thoroughly while reconstituting reagents, exercising care to avoid microbial contamination.
5. Do not conduct the test in the presence of reactive vapors (acids, alkalis, aldehydes) or dust, which could affect the enzymatic activity of the conjugate.
6. Use clean, disposable polypropylene plastic containers to prepare the chromogen solution. If glassware must be used, clean thoroughly and rinse with deionized water.
7. Always use a fresh disposable pipette tip for each specimen and when making reagent dilutions.
8. When aliquots of reagents are removed from their stock bottles, take care to avoid chemical and microbial contamination. Always use a fresh disposable pipette or pipette tip for these transfers.

9. To ensure adequate washing of the wells, comply with the recommended number of wash cycles and ensure that all wells are completely filled and then completely emptied.
10. Use plastic disposable containers to dispense the conjugate and substrate solution. Do not use the same container for the conjugate and substrate solutions.
11. Do not allow conjugate or substrate solutions to come into contact with metal or metallic ions.
12. Use clean, dust-free materials (tubes, tips, containers, etc.) to minimize the possibility of contamination with *aspergillus* spores in the environment. Because galactomannan is heat-stable, sterilization of material used does not guarantee the absence of contaminating antigen. Pyrogen-free materials are optimal, but standard material can be used with adequate precautions.
13. Never perform the test in the same room where *aspergillus* or other molds are being cultured or otherwise manipulated.
14. Limit exposure of solutions (sera, treatment solution, conjugate) or open containers (plates, tubes, pipettes) to free air. Do not leave microplate wells exposed to air. Perform the assay as soon as possible after removing the strips from the packet. Remove only the number of strips necessary for testing samples.
15. Add Conjugate to the microplate wells before the sample is added. Do not pour any unused conjugate back into the original container.
16. The substrate-chromogen reaction solution must be colorless. The appearance of a blue color after dilution indicates the reagent is contaminated and should not be used. Discard and prepare fresh reagent.

SPECIMEN COLLECTION / TESTING

A. Specimen Collection:

Collect a blood sample according to current practice. The test is performed on undiluted serum. Extract the serum from the clots as soon as possible to avoid hemolysis. Serum samples must be uncontaminated with fungal spores or bacteria. Transport and store samples in sealed tubes, unexposed to air. Samples can be stored at 2-8°C unopened for up to 5 days prior to testing. After initial opening, samples may be stored at 2-8°C for 48 hours prior to testing. For longer storage, store the serum at -70°C.

B. Specimen Testing:

Procedural Comments:

Test patient samples according to the Platelia® *Aspergillus* package insert (See Attachment B of Appendix D). Note: Samples should be tested in batches for best utilization of assay reagents. Each kit has reagents in sufficient quantity to perform 96 determinations in a maximum of 6 batches.

BRING ALL REAGENTS TO ROOM TEMPERATURE (15 - 30°C) BEFORE USE AND RETURN THEM IMMEDIATELY AFTER USE TO 2 - 8°C.

If samples have been frozen, thaw at room temperature and mix thoroughly (vortex) before testing. Negative, Positive, and Cut-off Controls must be run on each plate to validate the test results.

Reagent preparation and storage / Reconstitution of Controls (Negative, Cut-off, and Positive Controls)

Working Wash Solution

Prepare Working Wash Solution as needed by adding one part Concentrated Wash Solution to 9 parts deionized or distilled water. The Working Wash Solution can be stored for 15 days at 2-8°C. Prepare a sufficient amount of Working Wash Solution to complete the run (i.e., minimum of 160 mls for each 2 strips of 8 wells).

Negative Control Serum

Reconstitute the contents of one bottle of control with 1000 µl (1 mL) of sterile purified water (preferably pyrogen-free). The sera must be rehydrated just before performing the test. Mix thoroughly after allowing 2-3 minutes for re-hydration of the serum. Aliquot 300 µl into each of 3 Eppendorf tubes. Immediately freeze at -20°C the two tubes not used on the day of re-hydration. Note: control sera that have been previously rehydrated and immediately frozen at -20°C may be thawed and used without further rehydration. Frozen rehydrated controls may be stored at -20°C for up to 5 weeks. Handle the control sera in the same manner as patient specimens.

Cut-off, and Positive Control Serum

PREPARE AS DESCRIBED ABOVE FOR THE NEGATIVE CONTROL.

Substrate-Chromogen Reaction Solution

Prepare Substrate-Chromogen Reaction Solution by adding one part Concentrated Chromogen Solution, R9, to 50 parts Substrate Buffer, R8 (e.g., 200 µl of R9 + 10 mL of R8). Prepare 4 ml of Substrate-Chromogen Reaction Solution per strip. The solution is stable for 6 hours when stored in the dark at room temperature.

Treatment of Sera / Controls

1. Pipette 300 µl of each test serum and control into individual 1.5 mL polypropylene tubes.
2. Add 100 µl of treatment solution (R7) to each tube.
3. Mix tubes thoroughly by vigorous homogenization or vortex to mix thoroughly. Heat tubes in boiling water bath or heat block for 3 minutes at 100°C.
4. Centrifuge tubes at 10,000 x g for 10 minutes.
5. Test the supernatants using the following procedure. After preparation, the supernatant may be removed and stored at 2-8°C for up to 72 hours prior to testing. If analysis of the results indicate retesting is required, another aliquot of serum must be treated for testing.

EIA Procedure

1. Prepare a Platelia® *Aspergillus* Worksheet (see Attachment C of Appendix D) and indicate the identification of test sera and controls in the microplate on the platemap. Use one well for the Negative Control Serum (R3), two wells for the Cut-off Control (R4), and one well for the Positive Control Serum (R5).

See suggested platemap below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	R3	↓ 5	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
B	R4	↓ 6	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
C	R4	↓ 7	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
D	R5	↓ 8	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
E	Sample 1	↓ 9	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
F	↓ 2	↓ 10	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
G	↓ 3	↓ 11	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
H	↓ 4	↓ etc.	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅

∅ = Null strip / empty well

2. Remove the plate holder and microwell strips (R1) from the plate pouch. Return any strips to the pouch that will not be used, with the desiccant, and reseal the pouch.

3. Add 50 µl of conjugate (R6) to each well. Next, add 50 µl of treated serum supernatant to each well, as designated above. Do not add serum samples to the wells before the conjugate.
4. Cover plate with plate sealer, ensuring that entire surface is covered and watertight.
5. Incubate the microplate in a water bath or dry microplate incubator for 90 ± 5 minutes at 37°C (±1°C).
6. Remove the plate sealer. Wash the plate 5 times, using a minimum of 370 µl of Working Wash Solution. After the last wash, invert the microplate and gently tap on absorbent paper to remove remaining liquid.
7. Add 200 µl of Substrate-Chromogen Reaction Solution (R8 + R9) to each well, avoiding exposure to bright light.
8. Incubate microplate in the dark at room temperature (18 to 25°C) for 30 ± 5 minutes. Do not use adhesive film during this incubation.
9. Add 100 µl of Stopping Solution (R10) to each well, utilizing the same order for addition of Substrate Solution.
10. Thoroughly wipe the bottoms of each plate.
11. Read the optical density of each well at 450 nm (reference filter of 620/630 nm). Micro-plates must be read within 30 minutes of addition of Stopping Solution. Avoid exposure of plate to light before reading.

C. Calculation of Results

The presence or absence of galactomannan antigen in the test sample is determined by calculation of an index for each sample. The Index (I) is the OD value of the specimen divided by the mean optical density of the wells containing the cut-off control specimen.

Calculation of the Mean Cut-off Control OD:

Add the OD values of the two wells containing Cut-off control (R4) and divide the total by 2.

Calculation of an index (I) for R3 and R5 control:

Calculate the following ratio for R3 and R5 controls:

$$I = \frac{\text{OD R3 /or R5 control}}{\text{Mean Cut-off Control OD}}$$

Assay Validation

Calculate an index (I) for the Positive and Negative controls as described above. In order for the assay to be valid, the following criteria must be met:

- **Cut-off Control:** The optical density of each Cut-off control must be between 0.3 and 0.8
- **Positive Control:** Index (I) must be greater than 2.0
- **Negative Control:** Index (I) must be less than 0.4

Interpretation of Results

The presence or absence of galactomannan antigen in the test sample is determined by calculation of an index for each patient specimen. The Index (I) is the OD value of the specimen divided by the mean optical density of the wells containing the cut-off control specimen

Calculation of an index (I) for each patient sample

Calculate the following ratio for each patient sample:

$$I = \frac{\text{OD patient sample}}{\text{Mean Cut-off Control OD}}$$

- Sera with an Index less than 0.5 are considered to be negative.
- Sera with an Index greater than or equal to 0.5 are considered to be positive.

Whenever a sample has an Index ≥ 0.5 , positivity must be confirmed by re-testing the sample, including repeating the heat treatment on a new aliquot, and/or by testing another sample obtained from the patient.

D. Limitations of the Procedure

1. A negative test cannot rule out the diagnosis of invasive Aspergillosis. Patients at risk for invasive Aspergillosis should be tested twice a week.
2. The Platelia® *Aspergillus* Procedure and the Interpretation of Results must be followed when testing samples for the presence of *aspergillus* antigen. The user of the kit is advised to read the package insert carefully prior to conducting the test. In particular, the test procedure must be carefully followed for sample and reagent pipetting, plate washing, and timing of the incubation steps.
3. Failure to add specimen or reagent as instructed in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of infection or procedural error.
4. Contamination of negative patient specimen wells by positive control/patient specimen wells is possible if the contents of one well spill over into another well due to rough handling of the microplate or poor pipetting technique while adding reagents.

TRAINING / PROFICIENCY TESTING

The purpose of training is to provide the technologist with familiarity in using the Bio-Rad Platelia® *Aspergillus* and instruments. Proficiency in running the assay will be assessed and demonstrated by testing a panel of specimens provided by Bio-Rad Laboratories with the Bio-Rad Platelia® *Aspergillus* EIA kit. Testing will be done according to package insert instructions (See Attachment B of Appendix D).

Each technologist who will be testing study specimens with the Platelia® *Aspergillus* EIA must demonstrate proficiency in running the assay before testing study specimens.

Proficiency will be assessed and demonstrated by testing a 7 member -training panel provided by Bio-Rad Laboratories. Each of the 7 panel members will be pretreated by heating 300µl of serum with 100µl Treatment Solution to 100°C as instructed in the Platelia® *Aspergillus* assay package insert. After centrifugation, the supernatant will be assayed in triplicate. See the suggested plate map below. Complete testing according to package insert instructions.

The within plate accuracy and reproducibility for each panel member will be calculated by the monitor.

	1	2	3	4	5	6	7	8	9	10	11	12
A	R3	X	AF	AI	∅	∅	∅	∅	∅	∅	∅	∅
B	R4	X	AF	∅	∅	∅	∅	∅	∅	∅	∅	∅
C	R4	Z	AF	∅	∅	∅	∅	∅	∅	∅	∅	∅
D	R5	Z	AH	∅	∅	∅	∅	∅	∅	∅	∅	∅
E	W	Z	AH	∅	∅	∅	∅	∅	∅	∅	∅	∅
F	W	AC	AH	∅	∅	∅	∅	∅	∅	∅	∅	∅
G	W	AC	AI	∅	∅	∅	∅	∅	∅	∅	∅	∅
H	X	AC	AI	∅	∅	∅	∅	∅	∅	∅	∅	∅

R3 = Negative Control Serum R4 = Cut-off Control R5 = Positive Control Serum
W,X,Z,AC,AF,AH,AI = Panel Members ∅ = Empty well

The study monitor will complete the Proficiency Testing Documentation Form (page D-17) for each technologist who will be testing specimens with the Platelia® *Aspergillus* antigen assay and will indicate on the form if proficiency testing is acceptable or unacceptable. If proficiency testing is unacceptable, the technologist must repeat proficiency testing as instructed by the study monitor. If proficiency testing is acceptable, the technologist can begin testing study specimens on the date indicated on the form.

Testing of study specimens can begin when proficiency testing is acceptable and a copy of the signed and dated Proficiency Testing Documentation Form is received from the study monitor.

Proficiency Testing Documentation Form

Site: _____

Name of Technologist: _____ Date of Testing _____

Platelia® *Aspergillus* Lot # _____ Expiration Date _____

R3 = _____

R4 = 1. _____ 2. _____ Mean = _____

R5 = _____

Cutoff = mean optical density of R4 (Cut-off Control) = _____

Panel Member	Mean Index (I)	Interpretation	SD	CV%	Acceptable Range Index (I)	Acceptable CV%
W					0.10-0.21	< 25%
X					2.60-3.45	< 10%
Z					3.88-5.44	< 10%
AC					2.75-3.56	< 10%
AF					1.68-2.27	< 10%
AH					0.85-1.33	< 15%
AI					0.47-0.78	< 25%

___ Proficiency Testing **Not Acceptable**. Repeat proficiency testing.

___ Proficiency Testing **Acceptable**. Testing of specimens can begin on: _____
(Attach a copy of the printout of OD values).

Signature of the Study Monitor: _____ Date: _____

DATA COLLECTION

Platelia® *Aspergillus* Data

Data will be collected following the NIH Clinical Protocol guidelines.

Patient Clinical Data

Blood samples will be collected as part of the clinical protocol (“A Randomized Double-blind Trial of Fluconazole vs. Voriconazole for the Prevention of Invasive Fungal Infections in Allogeneic Blood and Marrow Transplant Patients”). These samples will be tested with the Platelia® *Aspergillus* assay and the results correlated with patient clinical data.

APPENDIX D

ATTACHMENT A: PLATELIA[®] ASPERGILLUS KIT RECEIPT LOG

APPENDIX D

ATTACHMENT B: PLATELIA® ASPERGILLUS PACKAGE INSERT



PLATELIA[®] ASPERGILLUS EIA

96 TESTS: 62793

480 TESTS: 62794

**THE PLATELIA[®] ASPERGILLUS EIA IS AN IMMUNOENZYMATIC SANDWICH
MICROPLATE ASSAY FOR THE DETECTION OF ASPERGILLUS
GALACTOMANNAN ANTIGEN IN SERUM**

1. INTENDED USE

The Platelia® *Aspergillus* EIA is an immunoenzymatic sandwich microplate assay for the detection of *Aspergillus* galactomannan antigen in serum.

2. INDICATIONS FOR USE

The Platelia® *Aspergillus* EIA is a test which, when used in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples and radiographic evidence, can be used as an aid in the diagnosis of Invasive Aspergillosis.

3. SUMMARY AND EXPLANATION

Aspergillus infections usually occur following inhalation of *Aspergillus* spores which are present in the environment. Invasive forms, which have been on the increase for the past 10 years, constitute the most serious infections. They mainly occur in neutropenic patients (following anti-cancer treatment) and in patients treated with immuno-suppressants (organ transplantations, particularly bone marrow transplantation) and corticosteroids².

Aspergillus is rarely isolated from blood culture. The diagnosis is often based on nonspecific diagnostic or radiological evidence (clinical symptoms, CT scan, chest x-ray, etc.).

At the present time, the test for soluble *Aspergillus* antigen in serum appears to be a serological method able to aid in the diagnosis of Invasive Aspergillosis^{1,3,6,17,21}.

4. PRINCIPLE OF THE PROCEDURE¹¹

The Platelia® *Aspergillus* EIA is a one-stage immunoenzymatic sandwich microplate assay which detects galactomannan in human serum. The assay uses the rat monoclonal antibody EBA-2, which is directed against *Aspergillus* galactomannan, and has been characterized during previous studies^{8,12}. The monoclonal antibody is used, (1) to coat the wells of the microplate and bind the antigen, and (2) as the detector antibody in the conjugate reagent (peroxidase-linked monoclonal antibody).

Serum samples are heat-treated in the presence of EDTA in order to dissociate the immune complexes and to precipitate serum proteins that could possibly interfere with the test⁷. The treated serum samples and conjugate are added to the wells coated with the monoclonal antibody, and incubated. A monoclonal antibody - galactomannan - monoclonal antibody / peroxidase complex is formed in the presence of *Aspergillus* antigen.

The strips are washed to remove any unbound material. Next, the substrate solution is added, which will react with the complexes bound to the well to form a blue color reaction. The enzyme reaction is stopped by the addition of acid, which changes the blue color to yellow. The optical absorbance of specimens and controls is determined with a spectrophotometer set at 450 and 620/630 nm wavelength.

5. REAGENTS

Platelia® *Aspergillus* product No. 62793 (96 Tests)
product No. 62794 (480 Tests)

Store the kit at 2-8°C. Bring all reagents to room temperature (18-25°C) before use. Return all reagents, except controls, to 2-8°C immediately after use. After reconstitution, unused Negative

Control, Cut-off Control, and Positive Control must be frozen at -20°C. Return unused strips/plates to pouch and reseal. Do not remove desiccant. Strips should be used within 5 weeks of opening and resealing the pouch. After dilution, wash solution can be kept for 15 days at 2-8°C. All other reagents are stable until expiration after opening. Reagents are supplied in sufficient quantity to perform 96 determinations in a maximum of 6 batches or 480 tests in a maximum of 12 batches.

Component		Contents	Quantity	
			96 Test Kit	480 Test Kit
R1	Microwell Strip Plate	<ul style="list-style-type: none"> • 96 wells (12 strips of 8 wells each) coated with antigalactomannan monoclonal antibody 	1 Plate / 12 x 8 Wells	5 Plates / 12 x 8 Wells
R2	Wash Solution 10X Concentrate	<ul style="list-style-type: none"> • Tris NaCl buffer • 1% Tween[®] 20 • 0.01% thimerosal 	1 x 100 mL	3 x 100 mL
R3	Negative Control Serum	<ul style="list-style-type: none"> • Freeze-dried human negative serum • Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBsAg 	2 x 1 mL	4 x 1 mL
R4	Cut-off Control Serum	<ul style="list-style-type: none"> • Freeze-dried human serum containing galactomannan • Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBsAg 	2 x 1 mL	4 x 1 mL
R5	Positive Control Serum	<ul style="list-style-type: none"> • Freeze-dried human serum containing galactomannan • Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBsAg 	2 x 1 mL	4 x 1 mL
R6	Conjugate	<ul style="list-style-type: none"> • Anti-galactomannan monoclonal antibody/peroxidase labeled • 0.01% thimerosal 	1 x 8 mL	4 x 8 mL
R7	Treatment Solution	<ul style="list-style-type: none"> • EDTA acid solution 	1 x 10.5 mL	5 x 10.5 mL
R8	Substrate Buffer	<ul style="list-style-type: none"> • Citric acid • Sodium acetate • 0.009% Hydrogen peroxide • 4% Dimethylsulfoxide (DMSO) 	1 x 60 mL	3 x 60 mL
R9	Chromogen	<ul style="list-style-type: none"> • Dimethylsulfoxide (DMSO) • Tetramethylbenzidine (TMB)* 	1 x 1 mL	3 x 1 mL
R10	Stopping Solution	<ul style="list-style-type: none"> • 1.5 N Sulphuric acid (H₂SO₄) 	1 x 12 mL	5 x 12 mL
	Plate sealers	<ul style="list-style-type: none"> • Adhesive sheets for microplates 	1 x 6 sheets	2 x 6 sheets

***Note:** TMB (Tetramethylbenzidine) is a non-carcinogenic and non-mutagenic chromogen for peroxidase.

6. WARNINGS FOR USERS

1. For *in vitro* diagnostic use.
2. For professional use only.
3. Use of this test kit with samples other than human serum is not recommended.
4. The Positive Control, Cut-off Control, and Negative Control are heat-inactivated, human serum. They have been tested and found to be negative for anti-HIV-1, anti-HIV-2, and anti-HCV antibodies, as well as HBsAg with French approved tests. However, all the reagents should be handled as though capable of transmitting infection. All tests should be conducted using the precautions recommended for blood borne pathogens, as defined by OSHA regulations.
5. Wear protective clothing and disposable gloves while handling the kit reagents and patient samples. Wash hands thoroughly after performing the test.
6. Do not pipette by mouth.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Avoid splashing samples or solutions containing them.
9. Biological spills not containing acid should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach (0.5% solution of sodium hypochlorite), 70% ethanol, or 0.5% Wescodyne™. Spills containing acid should be wiped dry or neutralized with sodium bicarbonate and then cleaned with one of the chemical disinfectants. Materials used to wipe up spills should be disposed of as biohazardous waste. CAUTION: Do not place solutions containing bleach in the autoclave.
10. Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Disposal should comply with all applicable waste disposal requirements.
11. CAUTION: The Stopping Solution is an acid. Wipe up spills immediately and flush the areas with water.



Xi-irritant

R36/38: Irritating to eyes and skin.

S:2-26-30: Keep out of reach of children. In case of contact with eyes, flush immediately and thoroughly with water and consult a physician. Never pour water into this product.

12. Avoid contact of Substrate Buffer, Chromogen, and Stopping Solution with eyes, skin, and mucosae (risk of toxicity, irritation, and burns).

7. PRECAUTIONS FOR USERS

1. **FROZEN SERUM SAMPLES STORED IN UNKNOWN CONDITIONS MAY GIVE FALSE POSITIVE RESULTS DUE TO CONTAMINATION WITH FUNGUS AND/OR BACTERIA.**
2. Do not use kit or any kit reagents after the stated expiration date.
3. With the exception of the 10x Wash Solution (R2) and the Stopping Solution (R10), do not mix reagents from other kits that have the same or different lot numbers. Note: Platelia[®] 10X Wash Solution (R2) may be used on any Platelia[®] brand assay.
4. Bring all reagents to room temperature for at least 15 minutes before use.
5. Mix thoroughly while reconstituting reagents, exercising care to avoid microbial contamination.
6. Do not conduct the test in the presence of reactive vapors (acids, alkalis, aldehydes) or dust, which could affect the enzymatic activity of the conjugate.
7. Use clean, disposable polypropylene plastic containers to prepare the chromogen solution. If glassware must be used, clean thoroughly and rinse with deionized water.
8. For manual pipetting of controls and specimens, use individual pipette tips to prevent carryover of samples.
9. To ensure adequate washing of the wells, comply with the recommended number of wash cycles and ensure that all wells are completely filled and then completely emptied. Washing should not be performed manually with a squeeze bottle.
10. Do not use the same container for the conjugate and substrate solutions.
11. Do not allow conjugate or substrate solutions to come into contact with metal or metallic ions.
12. Avoid exposing the Chromogen or the Substrate-Chromogen Reaction Solution to strong light during storage or incubation. Do not allow the chromogen solutions to come into contact with an oxidizing agent.
13. Avoid contact of the Stopping Solution with any oxidizing agent. Do not allow the Stopping Solution to come into contact with metal or metallic ions.
14. Use clean, dust-free materials (tubes, tips, containers, etc.) to minimize the possibility of contamination with *Aspergillus* spores from the environment. Because galactomannan is heat-stable, sterilization of material used does not guarantee the absence of contaminating antigen. Pyrogen-free materials are optimal, but standard material can be used with adequate precautions.
15. Limit exposure of solutions (sera, treatment solution, conjugate) or open containers (plates, tubes, pipettes) to the air.
16. Do not pour any unused conjugate back into the original container.
17. The Substrate-Chromogen Reaction Solution must be colorless. The appearance of a blue color after dilution indicates the reagent is contaminated and should not be used. Discard and prepare fresh reagent.

8. REAGENT PREPARATION AND STORAGE

Wash Solution

Prepare Working Wash Solution as needed by adding one part Concentrated Wash Solution to 9 parts deionized or distilled water. The Working Wash Solution can be stored for 15 days at 2-

8°C. Prepare a sufficient amount of Working Wash Solution to complete the run (i.e., minimum of 160 mls for each 2 strips of 8 wells).

Negative Control Serum

Reconstitute the contents of one bottle of control with 1000 µl (1 mL) of sterile, purified water. The sera must be rehydrated just before performing the test. Mix thoroughly after allowing 2-3 minutes for rehydration of the serum. Aliquot 300 µl into each of 3 Eppendorf tubes. Immediately freeze at -20°C any remaining Eppendorf tubes that will not be used after rehydration.

Note: Control sera that have been previously rehydrated and immediately frozen at -20°C may be thawed and used without further rehydration. Frozen rehydrated controls may be stored at -20°C for up to five weeks. Handle the control sera in the same manner as patient specimens.

Cut-off Control Serum and Positive Control Serum

Prepare as described above for Negative Control Serum.

Substrate-Chromogen Reaction Solution

Prepare Substrate-Chromogen Reaction Solution by adding one part Concentrated Chromogen Solution, R9, to 50 parts Substrate Buffer, R8 (e.g., 200 µl of R9 + 10 mL of R8). Prepare 4 mL of Substrate-Chromogen Reaction Solution per strip. The solution is stable for 6 hours when stored in the dark at room temperature.

9. SPECIMEN COLLECTION

Collect blood samples according to standard laboratory procedures. The test is performed on serum. Serum samples must be uncontaminated with fungal spores and/or bacteria. Transport and store samples in sealed tubes, unexposed to air. Unopened samples can be stored at 2-8°C for up to 5 days prior to testing. After initial opening, samples may be stored at 2-8°C for 48 hours prior to testing. For longer storage, store the serum at -70°C.

10. PROCEDURE

Materials provided

See REAGENTS section.

Materials required but not provided

1. Distilled or deionized water, for dilution of Wash Solution.
2. Sterile, purified water for reconstitution of control sera.
3. Absorbent paper.
4. Disposable gloves.
5. Protective glasses.
6. Sodium hypochlorite (bleach) and sodium bicarbonate.
7. Pipettes or multipipettes, adjustable or fixed, to measure and dispense 50 µl, 100 µl, 300 µl, and 1000 µl.
8. 1.5 mL Eppendorf (Sarstedt Cat. #72.692.105) or similar polypropylene tubes with airtight stoppers, able to support heating to 100°C.

9. Laboratory bench centrifuge for polypropylene tubes capable of obtaining 10,000g (Brickman Cat. #22-36-280-1 or VWR Scientific Cat. #20901-051 or equivalent).
10. Round, floating micro-centrifuge rack for 1 L beaker (VWR Scientific Cat. # 60986-100 or Nalgene #5974-1015 or equivalent).
11. Vortex agitator.
12. Boiling waterbath.
13. Microplate incubator at $37 \pm 1^\circ\text{C}$.
14. Manual or semi-automatic plate washer.
15. Microplate reader equipped with 450 nm and 620/630 nm filters.

Procedural Comments

1. Negative, Positive, and Cut-off Controls must be tested on each run to validate the test results.

Treatment of the sera

1. Pipette 300 μL of each test serum and control into individual 1.5 mL polypropylene tubes.
2. Add 100 μL of Treatment Solution (R7) to each tube.
3. Mix tubes thoroughly by vigorous homogenization or vortexing to mix thoroughly. Heat tubes for 3 minutes in boiling waterbath at 100°C .
4. Carefully remove hot tubes from the boiling waterbath and place in a centrifuge. Centrifuge tubes at 10,000 x g for 10 minutes.
5. Test the supernatants using the following procedure. After preparation, the supernatant may be removed and stored at $2-8^\circ\text{C}$ for up to 72 hours prior to testing. If analysis of the results indicates retesting is required, another aliquot of serum must be treated for testing.

EIA Procedure

1. Bring reagents to room temperature ($18 - 25^\circ\text{C}$) for at least 15 minutes before use.
2. Prepare Wash Solution, Substrate-Chromogen Reaction Solution, and Negative, Positive, and Cut-off Controls.
3. Prepare a chart for identification of test sera and controls in the microplate. Use one well for the Negative Control Serum (R3), two wells for the Cut-off Serum (R4), and one well for the Positive Control Serum (R5).
4. Remove the plateholder and microwell strips (R1) from the plate pouch. Return any strips that will not be used to the pouch, with the desiccant, and reseal the pouch.
5. Add 50 μL of Conjugate (R6) to each well. Next, add 50 μL of treated serum supernatant to each well, as designated above. Do not add serum samples to the wells before the conjugate.
6. Cover plate with plate sealer, or other means to prevent evaporation, ensuring that entire surface is covered and watertight.
7. Incubate the microplate in a dry microplate incubator for 90 ± 5 minutes at 37°C ($\pm 1^\circ\text{C}$).
8. Remove the plate sealer. Wash the plate 5 times, using a minimum of 370 μL of Working Wash Solution. After the last wash, invert the microplate and gently tap on absorbent paper to remove remaining liquid.

9. Add 200 µl of Substrate-Chromogen Reaction Solution (R8 + R9) to each well, avoiding exposure to bright light.
10. Incubate microplate in the dark at room temperature (18 to 25°C) for 30 ± 5 minutes. **Do not use adhesive film during this incubation step.**
11. Add 100 µl of Stopping Solution (R10) to each well, utilizing the same order for addition of Substrate Solution. Mix well.
12. Thoroughly wipe the bottoms of each plate.
13. Read the optical density of each well at 450 nm (reference filter of 620/630 nm). Microplates must be read within 30 minutes of addition of Stopping Solution.

11. QUALITY CONTROL (VALIDITY CRITERIA)

Cut-off Control: The O.D. of each Cut-off Control Serum must be ≥ 0.3 and ≤ 0.8.

Positive Control: The index of the Positive Control Serum must be greater than 2.

$$I = \frac{\text{OD Positive Control (R5)}}{\text{Mean Cut-off Control OD}} > 2$$

Negative Control: The index of the Negative Control Serum must be less than 0.4.

$$I = \frac{\text{OD Negative Control (R3)}}{\text{Mean Cut-off Control OD}} < 0.4$$

Failure of any of the controls to meet the validity criteria described above renders the assay invalid, and patient specimen results should not be reported. The operator may decide to repeat the assay, after reviewing the procedure, or may contact the manufacturer for assistance. If a repeat assay is performed, then a new aliquot of the same sample should be used in the repeat assay.

Example Calculation:

Sample	Absorbance (OD)
Negative Control (R3) OD	0.117
Cut-off Control (R4) OD	0.596
	0.576
Positive Control (R5) OD	2.602

Calculations

Mean Cut-off Control Value

To calculate the mean Cut-off Control (R4) OD, add the OD values for each Cut-off Control replicate together and divide the result by 2:

$$(0.596 + 0.576) \div 2 = 0.586$$

Negative Control Index

To calculate the index of the Negative Control, divide the OD of the Negative Control by the mean Cut-off Control OD:

$$I = \frac{0.117}{0.586} = 0.20$$

Positive Control Index

To calculate the index of the Positive Control, divide the OD of the Positive Control by the mean Cut-off Control OD:

$$I = \frac{2.602}{0.586} = 4.44$$

Validity**In the above example:**

- Each Cut-off Control OD is ≥ 0.3 and ≤ 0.8 , indicating that the Cut-off Control is valid.
- The index of the Negative Control is < 0.4 , indicating that the Negative Control is valid.
- The index of the Positive Control is > 2 , indicating that the Positive Control is valid.

The test run in this example is considered to be valid since the results meet the validity criteria for each control.

12. INTERPRETATION OF RESULTS

The presence or absence of *Aspergillus* antigen in the test sample is determined by calculation of an index for each patient specimen. The Index (I), is the OD value of the specimen divided by the mean optical density of the wells containing Cut-off Control Serum.

Calculation of the mean Cut-off Control optical density:

Add the optical densities of the two wells containing Cut-off Control Serum (R4) and divide the total by 2.

Calculation of an index (I) for each test serum:

Calculate the following ratio for each test serum:

$$I = \frac{\text{OD sample}}{\text{Mean Cut-off Control OD}}$$

Sera with an index < 0.5 are considered to be negative for galactomannan antigen.

Note: A negative result may indicate that the patient's result is below the detectable level of the assay.

Note: Negative results do not rule out the diagnosis of Invasive Aspergillosis. Repeat testing is recommended if the result is negative, but the disease is suspected.

Sera with an index ≥ 0.5 are considered to be positive for galactomannan antigen.

Note: The Platelia® Aspergillus EIA is intended to be used as an aid in the diagnosis of Invasive Aspergillosis. Positive results obtained with the Platelia® Aspergillus EIA should be considered in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples and radiographic evidence.

For all positive patients, it is recommended that a new aliquot of the same sample be repeated as well as collection of a new sample from the patient for follow-up testing.

Note: An absorbance value of less than 0.000 OD may indicate a procedural or instrument error which should be evaluated. That result is invalid and the specimen must be re-run.

Example Calculation:

Sample	Absorbance (OD)
Negative Control (R3) OD	0.117
Cut-off Control (R4) OD	0.596 0.576
Positive Control (R5) OD	2.602
Patient Sample #1	0.134
Patient Sample #2	0.436
Patient Sample #3	1.196

Calculations

Refer to the Quality Control (Validity Criteria) section for an example of calculations to determine the validity of the assay controls.

Mean Cut-off Control Value

To calculate the mean Cut-off Control (R4) OD, add the OD values for each Cut-off Control replicate together and divide the result by 2:

$$(0.596 + 0.576) \div 2 = 0.586$$

Patient Sample #1

To calculate the index of Patient #1, divide the OD of Patient Sample #1 by the mean Cut-off Control OD:

$$I = \frac{0.134}{0.586} = 0.23$$

In this example, Patient Sample #1 is negative, since the Index of 0.23 is < 0.5 .

Patient Sample #2

To calculate the index of Patient #2, divide the OD of Patient Sample #2 by the mean Cut-off Control OD:

$$I = \frac{0.436}{0.586} = 0.74$$

In this example, Patient Sample #2 is positive, since the Index of 0.74 is ≥ 0.5 .

Patient Sample #3

To calculate the index of Patient #3, divide the OD of Patient Sample #3 by the mean Cut-off Control OD:

$$I = \frac{1.196}{0.586} = 2.04$$

In this example, Patient Sample #3 is positive, since the Index of 2.04 is ≥ 0.5 .

13. LIMITATIONS OF THE PROCEDURE

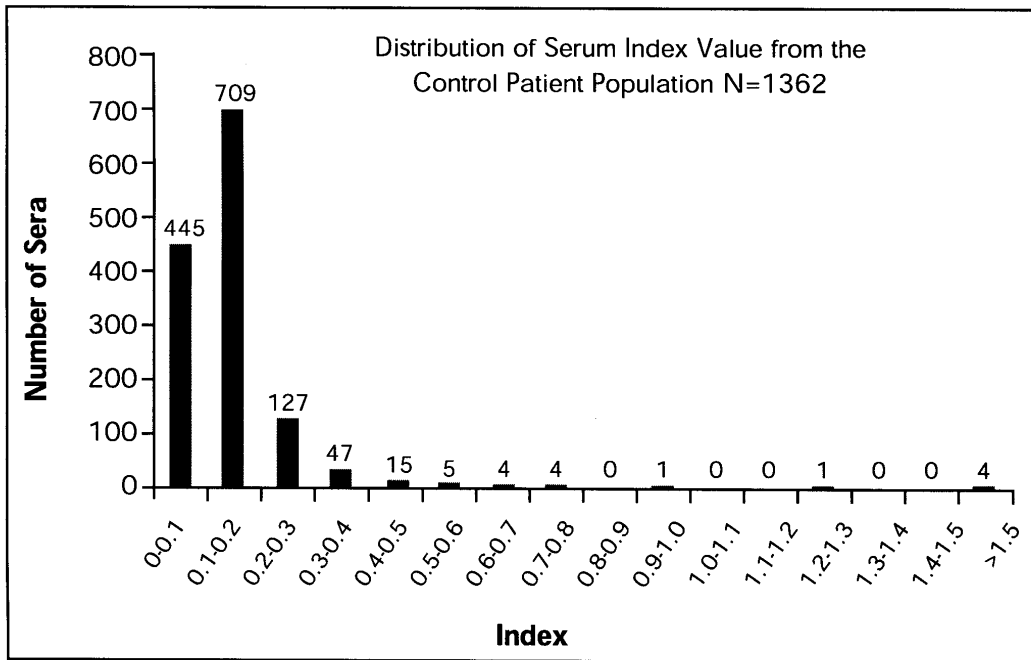
1. **A negative test cannot rule out the diagnosis of Invasive Aspergillosis. Patients at risk for Invasive Aspergillosis should be tested twice a week.**
2. The Platelia® *Aspergillus* Procedure and the Interpretation of Results must be followed when testing samples for the presence of *Aspergillus* antigen. The user of the kit is advised to read the package insert carefully prior to conducting the test. In particular, the test procedure must be carefully followed for sample and reagent pipetting, plate washing, and timing of the incubation steps.
3. Failure to add specimen or reagent as instructed in the procedure could result in a falsely negative test. Repeat testing of additional samples should be considered where there is clinical suspicion of Invasive Aspergillosis or procedural error.
4. Contamination of negative patient specimen wells by positive control/patient specimen wells is possible if the contents of one well spill over into another well due to rough handling of the microplate or poor pipetting technique while adding reagents.
5. The performance of the Platelia® *Aspergillus* EIA has not been evaluated with neonate or pediatric serum samples.
6. The Platelia® *Aspergillus* EIA may exhibit reduced detection of galactomannan in patients with chronic granulomatous disease (CDG) and Job's syndrome^{19,20}.
7. The concomitant use of mold-active anti-fungal therapy in some patients with Invasive Aspergillosis may result in reduced sensitivity with the Platelia® *Aspergillus* EIA.
8. The Platelia® *Aspergillus* EIA has not been evaluated for use with plasma or other sample types such as urine, BAL, or CSF.
9. The performance of the Platelia® *Aspergillus* EIA has not been established for manual reading and/or visual result determination.
10. Other genera of fungi such as *Penicillium* and *Paecilomyces* have shown reactivity with the rat, EBA-2 monoclonal antibody used in the assay for the detection of *Aspergillus* galactomannan¹⁵. These species are rarely implicated in invasive fungal disease.

14. EXPECTED VALUES

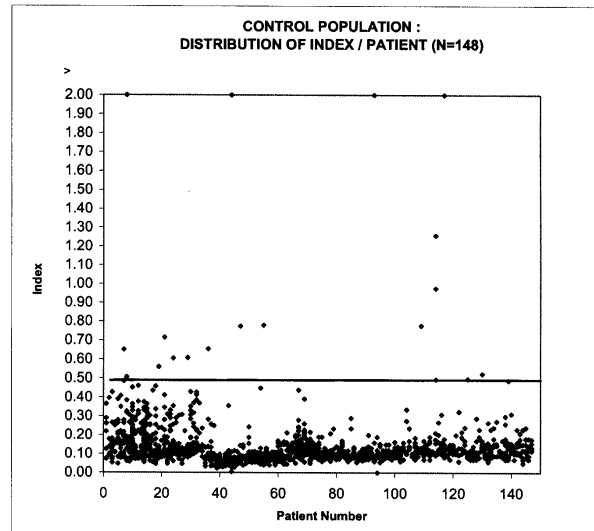
The expected prevalence of Invasive Aspergillosis varies with the patient population; rates from 5-20% have been reported^{2,5}. A clinical study was conducted on a total of 1890 serum samples from 179 bone marrow transplant (BMT) and leukemic patients diagnosed with and without Invasive Aspergillosis, at three testing centers in North America to determine the performance characteristics of the Platelia[®] *Aspergillus* EIA. The average prevalence rate for this study was 14%. The distribution of index values for these populations is represented in the following charts.

Patients diagnosed with Invasive Aspergillosis (control population)

A total of 1362 frozen serum samples obtained from 148 bone marrow transplant (BMT) and leukemic patients at three testing centers in North America were tested with the Platelia[®] *Aspergillus* EIA test. The distribution of index values is shown in the following chart.

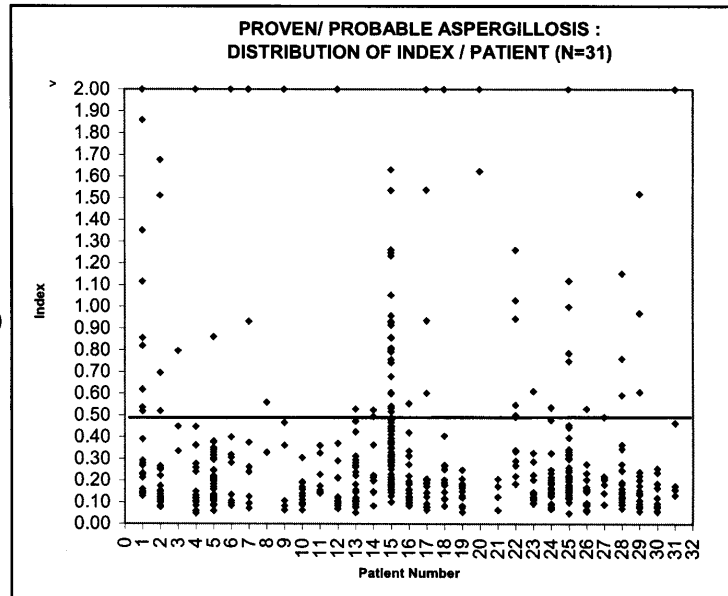


This scatter plot depicts galactomannan assay results for the 1362 serum samples from 148 control patients in this study (patients undergoing immunosuppressive therapy for HSCT, or to treat hematological malignancy).



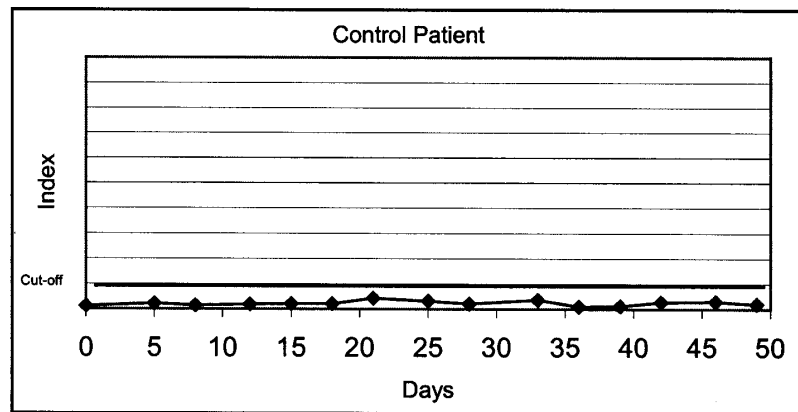
Patients diagnosed with Invasive Aspergillosis

This scatter plot depicts galactomannan assay results for the 528 serum samples from 31 patients in this study diagnosed with proven or probable Invasive Aspergillosis as defined by EORTC/NIAID definitions. Not every serum sample from each patient is expected to be positive. The expected prevalence of Invasive Aspergillosis varies with the patient population; rates from 5-20% have been reported^{2,5}. The prevalence rate for this study was 14%.

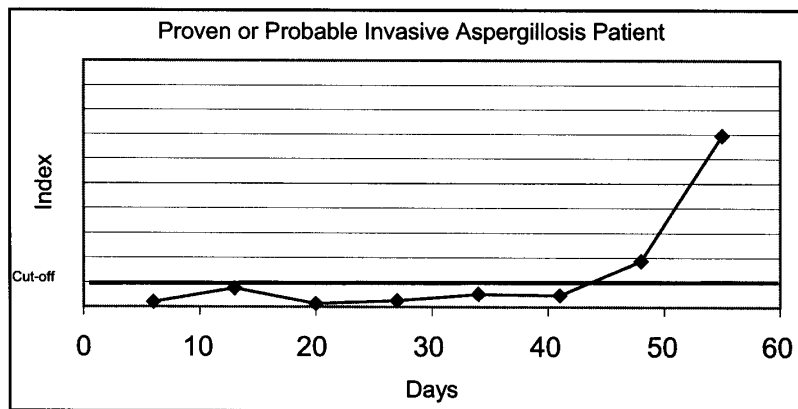


The following graphs represent examples of a patient without clinical signs or symptoms of Invasive Aspergillosis (negative for *Aspergillus*) and a patient with proven or probable Invasive Aspergillosis (positive for *Aspergillus*) respectively.

Negative Patient



Positive Patient



15. SPECIFIC PERFORMANCE CHARACTERISTICS

A. Reproducibility Studies

Inter-assay and Intra-assay variability for the Platelia® *Aspergillus* EIA were determined in a study using a panel of 6 pooled patient serum samples (one negative, one low positive, two positive, and two high positive) obtained from actual clinical trial sites. Each of the 6 panel members was tested in triplicate (x3) on 3 different days, on one lot, at two sites (total number of replicates at each site = 9). Each of the 6 panel members was tested in duplicate (x2) on 3 different days, on 1 lot, at a third site (total number of replicates at the third site = 6). One (1) operator performed all precision testing at each site. The data were analyzed according to the National Committee for Clinical Laboratory Standards (NCCLS). The mean optical density (D) and mean index value, standard deviation (SD), percent coefficient of variation (%CV), within run precision (intra-assay) and within site (inter-assay) precision for each panel member at each site are illustrated below in the following tables.

Site 1

Panel Member	Neg		Low Pos		Pos #1		Pos #2		High Pos #1		High Pos #2		Neg Control		CO Control		Pos Control	
	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index
N	9	9	9	9	9	9	9	9	9	9	9	9	3	3	6	6	3	3
Mean	0.052	0.09	0.445	0.74	0.702	1.17	0.931	1.563	1.227	2.06	2.887	4.83	0.046	0.08	0.606	1.00	2.216	3.67
Within Run (intra-assay) ¹ SD	0.002	0.00	0.022	0.03	0.059	0.09	0.044	0.08	0.051	0.09	0.089	0.17	N/A	N/A	0.02	0.03	N/A	N/A
% CV	N/A	N/A	4.8%	4.4%	8.4%	7.6%	4.7%	5.1%	4.2%	4.4%	3.1%	3.6%	N/A	N/A	3.7%	3.4%	N/A	N/A
Total (inter-assay) ² SD	0.036	0.04	0.051	0.08	0.070	0.14	0.044	0.25	0.058	0.29	0.169	0.58	N/A	N/A	0.102	0.03	0.317	0.12
% CV	N/A	N/A	11.5%	10.4%	10.0%	11.6%	4.7%	15.7%	4.7%	14.3%	5.9%	11.9%	N/A	N/A	16.9%	2.8%	14.3%	3.3%

Site 2

Panel Member	Neg		Low Pos		Pos #1		Pos #2		High Pos #1		High Pos #2		Neg Control		CO Control		Pos Control	
	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index
N	9	9	9	9	9	9	9	9	9	9	9	9	3	3	6	6	3	3
Mean	0.040	0.10	0.280	0.70	0.364	0.89	0.602	1.49	0.801	2.01	1.361	3.43	0.074	0.18	0.415	1.00	1.197	2.97
Within Run (intra-assay) ¹ SD	0.006	0.01	0.041	0.09	0.023	0.07	0.045	0.11	0.046	0.10	0.047	0.11	N/A	N/A	0.00	0.01	N/A	N/A
% CV	N/A	N/A	14.5%	13.0%	6.4%	7.6%	7.5%	7.1%	5.7%	4.8%	3.5%	3.2%	N/A	N/A	1.1%	1.1%	N/A	N/A
Total (inter-assay) ² SD	0.006	0.03	0.058	0.19	0.083	0.18	0.057	0.28	0.042	0.53	0.079	1.00	N/A	N/A	0.094	0.01	0.068	0.54
% CV	N/A	N/A	20.8%	27.0%	22.7%	19.8%	9.5%	18.7%	5.3%	26.5%	5.8%	29.2%	N/A	N/A	22.7%	0.9%	5.7%	18.2%

Site 3

Panel Member	Neg		Low Pos		Pos #1		Pos #2		High Pos #1		High Pos #2		Neg Control		CO Control		Pos Control	
	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index
N	6	6	6	6	6	6	6	6	6	6	6	6	3	3	6	6	3	3
Mean	0.049	0.10	0.388	0.81	0.652	1.36	0.830	1.73	1.158	2.41	2.378	4.96	0.059	0.12	0.480	1.00	1.652	3.45
Within Run (intra-assay) ¹ SD	0.003	0.01	0.009	0.02	0.082	0.17	0.068	0.14	0.094	0.20	0.126	0.25	N/A	N/A	0.028	0.06	N/A	N/A
% CV	N/A	N/A	2.4%	2.4%	12.5%	12.2%	8.2%	8.2%	8.1%	8.2%	5.3%	5.1%	N/A	N/A	5.8%	5.8%	N/A	N/A
Total (inter-assay) ² SD	0.012	0.03	0.078	0.13	0.068	0.15	0.104	0.25	0.082	0.15	0.111	0.34	N/A	N/A	0.028	0.04	0.056	0.23
% CV	N/A	N/A	20.0%	15.8%	10.5%	11.1%	12.5%	14.3%	7.1%	6.2%	4.7%	6.8%	N/A	N/A	5.8%	4.1%	3.4%	6.6%

N/A = not applicable

¹NCCLS EP5-A, Vol. 19, No. 2, Page 24, Equation (C2)

²NCCLS EP5-A, Vol. 19, No. 2, Page 25, Equation (C3) and Equation (C4)

B. Cross Reactivity

A study to evaluate the effect of potentially interfering medical conditions unrelated to Invasive Aspergillosis was performed with one lot of the Platelia® *Aspergillus* EIA kit. The following serum samples were tested for cross-reactivity with the Platelia® *Aspergillus* EIA. A total of 151 sera were tested.

Pathology	#Samples Tested	# Positives
Rheumatoid Factor	10	0
ANA Positive	10	0
IgG Hypergammaglobulinemia	10	0
IgM Hypergammaglobulinemia	10	0
Cancer*	11	0
Non-Viral Cirrhosis (primary biliary; alcohol induced; drug induced)	10	0
Multiple Transfusions	10	0
Multiparous Females	10	0
HAV	10	0
HCV	10	0
Rubella	10	0
CMV	10	0
Syphilis (RPR+)	10	0
Toxoplasmosis	10	0
Mycoplasma	10	0

*One each of bladder, breast (2), colon, endometrial, lung, prostate, renal, and squamous (3).

C. Clinical Testing

Clinical testing to evaluate the sensitivity, specificity, and predictive value of the Platelia® *Aspergillus* EIA was conducted at three sites located in the U.S. and Canada. The study was conducted retrospectively using a total of 1890 serum samples collected from 179 patients from the following populations*:

- patients without signs of Invasive Aspergillosis (control patients)
- patients with probable Invasive Aspergillosis
- patients with proven Invasive Aspergillosis

* The Invasive Fungal Infection Cooperative Group (IFICG) of the European Organization for Research (EORTC) and the Mycosis Study Group (MSG) of the National Institute of Allergy and Infectious Diseases (NIAID) have defined criteria for diagnosis of Invasive Aspergillosis (IA) in patients with hematologic malignancy or hematopoietic stem cell transplant.

Proven Invasive Aspergillosis is defined by positive microbiological culture obtained by sterile procedure from the site affected, and histopathological demonstration of the appropriate morphological forms in a host with symptoms attributed to the fungal infection.

Probable Invasive Aspergillosis is defined as a least one microbiological criterion, **and** one major or two minor clinical criteria from a site consistent with infection, in a host with symptoms attributed to the fungal infection.

Possible Invasive Aspergillosis is defined as at least one microbiological criterion, **or** one major or two minor clinical criteria from a site consistent with infection, in a host with symptoms attributed to the fungal infection.

Given the relative rarity of Probable and Proven Invasive Aspergillosis, we offer the following definition of clinical sensitivity and specificity for the purposes of this study.

SENSITIVITY

Results from this study have been analyzed in terms of patient sensitivity. Sensitivity testing was conducted using the Platelia[®] *Aspergillus* EIA at three sites on a combined total of 31 Bone Marrow Transplant (BMT) and Leukemia patents diagnosed with Proven or Probably Invasive Aspergillosis.

1. Proven Aspergilliosis (as defined by IFICG / EORTC; see above)

Combined Sites **N = 11 (patients)**

11 patients:

- 6 patients diagnosed with Proven Invasive Aspergillosis of the lung
- 5 patients diagnosed with Proven Invasive Aspergillosis of the sinus

Sensitivity: 81.8% (9/11).

Note: The 95% confidence interval could not be calculated due to insufficient sample size.

2. Probable Aspergillosis (as defined by IFICB / EORTC; see above)

Combined Sites **N = 20 (patients)**

20 patients:

- 16 patients diagnosed with Probable Invasive Aspergillosis of the lung
- 4 patients diagnosed with Probable Invasive Aspergillosis of the sinus

Sensitivity: 80.0% (16/20)

Note: The 95% confidence interval could not be calculated due to insufficient sample size.

3. Combined Proven and Probable Aspergillosis (as defined by IFICG / EORTC; see above)

Combined Sites **N = 31 (patients)**

31 patients:

- 22 patients diagnosed with Proven or Probable Invasive Aspergillosis of the lung
- 9 patients diagnosed with Proven or Probable Invasive Aspergillosis of the sinus

Sensitivity: 80.7% (25/31). The 95% confidence interval is 64.0 – 97.3%

SPECIFICITY

Specificity testing was conducted using the Platelia® *Aspergillus* EIA at three sites on a combined total of 1362 samples obtained from 148 Bone Marrow Transplant (BM) and Leukemia patients without signs of Invasive Aspergillosis (control patients).

Site 1 N = 33 patients

449 sera obtained from:

- 16 BMT patients without signs of Invasive Aspergillosis
- 11 BMT patients colonized with *Aspergillus* and/or *Candida* sp.
- 1 BMT patient diagnosed with Invasive Fusariosis
- 3 BMT patients diagnosed with Candidemia
- 1 patient with blood cultures positive for *Lecythophora mutabilis*
- 1 patient diagnosed with Invasive *Pseudoallescheria boydii*

Site 1	Specificity	95% Confidence Interval
Patients (27/33)	81.8%	66.1 – 97.5%
Patients after repeat testing (31/33)		

Site 2 N = 77 patients

560 sera obtained from:

- 67 Leukemic patients without signs of Invasive Aspergillosis
- 8 Leukemic patients with Fungemia (*Candida*, *Fusarium*, *Trichosporon*, or *Aureobasidium*)
- 1 Leukemic patient diagnosed with Probable *Fusarium* pneumonia
- 1 Leukemic patient diagnosed with *Candida* pneumonia

Site 2	Specificity	95% Confidence Interval
Patients (71/77)	93.4%	87.1 – 99.8%
Patients after repeat testing (74/77)		

Site 3 N = 38 patients

353 sera obtained from:

- 28 BMT patients without signs of Invasive Aspergillosis
- 5 Leukemic patients receiving a second course of cytotoxic therapy
- 5 BMT patients being treated for Graft Versus Host Disease

Site 3	Specificity	95% Confidence Interval
Patients (34/38)	89.5%	77.8 – 100%
Patients after repeat testing (38/38)		

Combined Sites N = 148 patients*

Combined Sites	Specificity	95% Confidence Interval
Patients (132/148)	89.2%	83.8 – 94.6%
Patients after repeat testing (143/148)		

PREDICTIVE VALUE

Positive and negative predictive values have been analyzed for the patient population in this study, based on the actual average 14% prevalence rate observed in this study. Positive and negative predictive values have been calculated for both the initial test result and after repeat testing.

Actual Prevalence of 14%	PPV	NPV
Patients	54.8%	96.6%
Patients after repeat testing	68.3%	95.5%

The expected prevalence of Invasive Aspergillosis varies with the patient population; rates from 5-20% have been reported^{2,5}. For patient populations on the lower end of the published prevalence, the positive and negative prevalence have been re-calculated using a 5% prevalence rate.

Calculated Prevalence of 5%	PPV	NPV
Patients	12.5%	96.0%
Patients after repeat testing	31.3%	96.3%

*Note: A total of 1362 sera obtained from 148 patients were tested. 1343 of the 1362 sera were initially negative, resulting in a sample agreement of 98.6% with a 95% confidence interval of 97.9 – 99.3%. On repeat testing, 1355 of the 1362 sera were negative.

16. APPENDIX D BIBLIOGRAPHY

1. **de Repentigny L., L. Kaufman, G.T. Cole, D. Kruse, J.P. Latge, R.C. Matthews.** 1994. Immunodiagnosis of Invasive Fungal Infections. *J Med Vet Mycol.* **32:** 239-252.
2. **Denning, D.W.** 1998. Invasive Aspergillosis. *Clin. Infect. Dis.* **26:** p. 781-803.
3. **Erjavec, Z. and P.E. Verweij,** 2002. Recent progress in the diagnosis of fungal infections in the immunocompromised host. *Drug Resist. Updat.* **5:** p. 3-10.
4. **Herbrecht, R., V. Letscher-Bru, C. Oprea, B. Lioure, J. Waller, F. Campos, O. Villard, K.L. Liu, S. Natarajan-Ame, P. Lutz, P. Dufour, J.P. Bergerat, and E. Candolfi.** 2002. *Aspergillus* galactomannan detection in the diagnosis of Invasive Aspergillosis in cancer patients. *J. Clin. Oncol.* **20:** p. 1898-1906.
5. **Herbrecht, R., D. Denning, T. Patterson, J. Bennett, R. Greene, J. Oestmann, W. Kern, K. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R. Rubin, J. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P. Chandrasekar, M. Hodges, H. Schlamm, P. Troke, B. DePauw.** 2002. Voriconazole Versus Amphotericin B for Primary Therapy of Invasive Aspergillosis. *N Engl J Med.* **347, 6:** p. 408-415.
6. **Latge, J.P.** 1995. Tools and trends in the detection of *Aspergillus fumigatus*. *Curr Top Med Mycol* **6,** p. 245-281.
7. **Latge, J.P.** 1999. *Aspergillus fumigatus* and Aspergillosis. *Clin Microbiol Rev* **12[2],** 310-50.
8. **Latge, J.P., H. Kobayashi, J.P. Debeaupuis, M. Diaquin, J. Sarfati, J.M. Wieruszkeski, E. Parra, J.P. Bouchara, and B. Fournet.** 1994. Chemical and immunological characterization of the extracellular galactomannan of *Aspergillus fumigatus*. *Infect. Immun.* **62:** p. 5424-5433.
9. **Maertens, J., J. Verhaegen, H. Demuyneck, P. Brock, G. Verhoef, P. Vandenberghe, J. Van Eldere, L. Verbist, and M. Boogaerts.** 1999. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive Aspergillosis. *J. Clin. Microbiol.* **37:** p. 3223-3228.
10. **Maertens, J., J. Verhaegen, K. Lagrou, J. Van Eldere, and M. Boogaerts.** 2001. Screening for circulating galactomannan as a noninvasive diagnostic tool for Invasive Aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* **97:** p. 1604-1610.
11. **Stynen D, A. Goris, J. Sarfati, and J.P. Latge.** 1995. A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with Invasive Aspergillosis. *J. Clin Microbiol.* **33:** 497-500.
12. **Stynen D., J. Sarfati, A. Goris, M.C. Prevost, M. Lesourd, H. Kamphuis, V. Darras, and J.P. Latge.** 1992. Rat monoclonal antibodies against *Aspergillus* galactomannan. *Infect. Immun.* **60:** p. 2237-2245.

13. **Sulahian, A., F. Boutboul, P. Ribaud, T. Leblanc, C. Lacroix, and F. Derouin.** 2001. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of Invasive Aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. *Cancer* **91**: p. 311-318.
14. **Sulahian, A., M. Tabouret, P. Ribaud, J. Sarfati, E. Gluckman, J.P. Latge, and F. Derouin.** 1996. Comparison of an enzyme immunoassay and latex agglutination test for detection of galactomannan in the diagnosis of Invasive Aspergillosis. *Eur. J. Clin. Microbiol. Infect. Dis.* **15**: p. 139-145.
15. **Swanink, C.M., J.F. Meis, A.J. Rijs, J.P. Donnelly, and P.E. Verweij.** 1997. Specificity of a sandwich enzyme-linked immunosorbent assay for detecting *Aspergillus* galactomannan. *J Clin. Microbiol.* **35**: p. 257-260.
16. **Verweij, P.E., E.C. Dompeling, J.P. Donnelly, A.V. Schattenberg, and J.F. Meis.** 1997. Serial monitoring of *Aspergillus* antigen in the early diagnosis of Invasive Aspergillosis. Preliminary investigations with two examples. *Infection* **25**: p. 86-89.
17. **Verweij, P.E. and J.F. Meis.** 2000. Microbiological diagnosis of Invasive Fungal Infections in transplant recipients. *Transpl. Infect. Dis.* **2**: p. 80-87.
18. **Verweij, P.E., D. Stynen, A.J. Rijs, B.E. de Pauw, J.A. Hoogkamp-Korstanje, and J.F. Meis.** 1995. Sandwich enzyme-linked immunosorbent assay compared with Pastorex latex agglutination test for diagnosing Invasive Aspergillosis in immunocompromised patients. *J. Clin. Microbiol.* **33**: p. 1912-1914.
19. **Verweij, P.E., C.M. Weemaes, J.H. Curfs, S. Bretagne, J.F. Meis.** 2000. Failure to detect circulating *Aspergillus* markers in a patient with chronic granulomatous disease and Invasive Aspergillosis. *J Clin. Microbiol.* **38**: p. 3900-3901.
20. **Walsh, T.J., R.L. Schaufele, T. Sein, J. Gea-Banacloche, M. Bishop, N. Young, R. Childs, J. Barrett, H.L. Malech, and S.M. Holland.** 2002. Reduced expression of galactomannan antigenemia in patients with Invasive Aspergillosis and chronic granulomatous disease or Job's syndrome. Abstracts of the 40th Annual Meeting of the Infectious Diseases Society of America. Arlington, VA. P. 105; Abstr. 345.
21. **Yeo, S.F. and B. Wong.** 2002. Current Status of Nonculture Methods for Diagnosis of Invasive Fungal Infections. *Clin. Microbiol. Rev.* **15**: p. 465-484.

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APPENDIX D

ATTACHMENT C: PLATELIA® ASPERGILLUS WORKSHEETS

(THIS PAGE TO BE HAND-WRITTEN)

Clinical Trial Site: _____

Run No: _____ Date Performed: _____ Technologist: _____

Platelia Aspergillus Kit Lot #: _____ Expiration Date: _____

KIT REAGENTS

REAGENT	LOT #	COMMENTS
R1 Microwell Plates		Date pouch opened:
R2 Concentrated Wash		Date diluted:
R3 Negative Control Serum		Date reconst: Fresh (circle one) Frozen
R4 Threshold Control Serum		Date reconst: Fresh (circle one) Frozen
R5 Positive Control Serum		Date reconst: Fresh (circle one) Frozen
R6 Conjugate		
R7 Serum Treatment Solution		
R8 Peroxidase Substrate Buffer		
R9 Chromogen		
R10 Stopping Solution		

INSTRUMENTATION USED

INSTRUMENT	MANUFACTURER	SERIAL NO.	COMMENTS
Boiling water bath or heat block			Temp:
Centrifuge for Eppendorf tubes			Revolution: xG
Washer			
Incubator			Temp:
Reader			Wavelength: /

PROCEDURE TIMING VERIFICATION

ASSAY STEP	START TIME	FINISH TIME	ACCEPTABLE
TREATMENT OF SERA			
Heat in water bath or heat block			3 minutes
Heat in water bath or heat block			3 minutes
Heat in water bath or heat block			3 minutes
Centrifuge at 10,000 g			10 minutes
Centrifuge at 10,000 g			10 minutes
Centrifuge at 10,000 g			10 minutes
EIA PROCEDURE			
Incubation of conjugate and treated sera			90 minutes
Substrate-chromogen incubation			30 minutes +/- 5
Stopping solution added		N/A	N/A
Plate read		N/A	within 30 min of stop

(THIS PAGE TO BE HAND-WRITTEN)

Clinical Trial Site: _____

Run No: _____ Date Performed: _____ Technologist: _____

Platelia Aspergillus Kit Lot #: _____ Expiration Date: _____

	1	2	3	4	5	6
A						
B						
C						
D						
E						
F						
G						
H						

	7	8	9	10	11	12
A						
B						
C						
D						
E						
F						
G						
H						

APPENDIX D

ATTACHMENT D: QUICK REFERENCE GUIDE

PLATELIA® *Aspergillus* EIA Quick Reference Guide

1 Treatment of sera (controls and samples)
300 µl serum - 100 µl R7

300 µl 100 µl

Serum

2 Vortex then heat for 3 minutes at 100°C

100°C
212°F

3 Centrifuge 10 minutes at 10,000g

Test the supernatant

4 Chart for distribution and identification of controls and test samples

		1	2	3
R3	A	●	○	○
R4	B	●	○	○
R4	C	○	○	○
R5	D	●	○	○
	E	○	○	○

5 Add 50 µl of Conjugate R6

50 µl

R6

6 Add 50 µl of treated controls and test samples supernatant

50 µl

7 Incubate at 37°C for 90 minutes

90 mn 37°C

8 Prepare Washing Solution: dilute to 1:10

16 ml R2 + 144ml H₂O for 2 strips

dist. water

9 Aspirate and wash 5 times

10 Prepare Substrate-Chromogen Reaction Solution

100 µl 5 ml

100 µl R9 + 5ml R8 for 2 strips

11 Add 200 µl Substrate-Chromogen Reaction Solution

200 µl

12 Incubate at 18-25°C for 30 minutes in the dark

30 mn 18 - 25°C

13 Add 100 µl Stopping Solution R10; wipe microplate bottom carefully

100 µl

R10

14 Bichromatic reading (450 nm and 620/630 nm)

15 Interpretation of results

Calculate the index *i* for each sample

$$i = \frac{OD \text{ sample}}{OD \text{ R4 (cut-off control)}}$$

- Positive sample if $i \geq 0.5$
- Negative sample if $i < 0.5$

62793 Bio-Rad Platelia® *Aspergillus* EIA 96 test kit

BIO-RAD Clinical Diagnostics Group

503831

APPENDIX E
HUMAN SUBJECTS

APPENDIX E

HUMAN SUBJECTS

Subject consent: Candidates for the study will be identified as described in Section 2.0 of the protocol. The PI or his/her designee at each transplant center will contact the candidates and enroll them onto the study. The study coordinator at each center will provide the patient with information about the purpose of the study and obtain consent. A template of the consent form will be provided by the network to each center. Each center will customize the template according to their local requirements and submit it for review by the local IRB. Each center must provide evidence of IRB approval.

Confidentiality: Confidentiality will be maintained by individual names being masked and assigned a patient identifier code. The code relating the patient's identity with the ID code will be kept separately at the center. The ID code will be transmitted to the network.

Participation of women and minorities, children and other populations: Women and ethnic minorities will be included in this study. The lower age limit of participants is age 2.

Accrual will be monitored within each center with the expectation that the enrolled patient population is representative of the transplanted patient population at each center. Representation will be examined by comparing gender, race, ethnicity and age distributions. Accrual of minority patients will be expected to be in proportion to the number of minority patients transplanted at each center. The DCC and NHLBI will discuss enrollment anomalies with the centers.

APPENDIX F

**VORICONAZOLE AND FLUCONAZOLE
DILUTION AND INFUSION INSTRUCTIONS**

APPENDIX F**VORICONAZOLE AND FLUCONAZOLE
DILUTION AND INFUSION INSTRUCTIONS****A. VORICONAZOLE DILUTION AND INFUSION INSTRUCTIONS**

Voriconazole is manufactured and supplied by Pfizer Pharmaceuticals, Groton, Connecticut, USA and will be supplied by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). Voriconazole is packaged as a lyophilized powder for injection, 200 mg in 30 mL vials, with each vial also containing 3200 mg sulfobutyl ether beta-cyclodextrin sodium (SBECD). Prior to reconstitution vials should be stored at room temperature 15°-30°C. The drug does not contain a preservative, and is suitable for single use only. If not used immediately, the in-use storage times and conditions prior to use are the responsibility of the individual user and should not be longer than 24 hours at 2°-8°C (37°-46°F).

RECONSTITUTION FOR PEDIATRIC DOSES - Under aseptic conditions:

1. Voriconazole should be reconstituted with 19 mL Sterile Water for Injection, USP. Each mL will contain 10 mg voriconazole.
2. Use the table below to determine the number of vials needed for each dose.
3. The volume of 10 mg/mL concentrated voriconazole solution required based on the patients weight should be calculated (see below) and withdrawn into a syringe.

VORICONAZOLE - Patients < 12 years		
Body Weight (kg)	Dose	Volume of Voriconazole Concentrated (10 mg/mL) Solution Required for ~4 mg/kg Dose (Number of Vials)
≤ 10	50 mg	5 mL (1)
10 – 14.99	50 mg	5 mL (1)
15 – 19.00	100 mg	10 mL (1)
20 – 24.99	100 mg	10 mL (1)
25 – 29.99	100 mg	10 mL (1)
30 – 34.99	150 mg	15 mL (1)
35 – 39.99	150 mg	15 mL (1)
≥ 40	200 mg	20 mL (1)

***NOTES:**

1. Dose capped for children weighing > 40 kg.
2. It is expected that weight fluctuations during study periods will not be substantial enough to warrant dosing changes.

4. This volume should be withdrawn and injected into an empty Viaflex[®] bag, and then further diluted with an approved solution (these solutions will not be provided by the BMT CTN).
5. Approved diluent: Sodium Chloride 0.9%, USP.
6. The final volume is dependent upon the age of the patient. For **pediatric** patients the final *concentration* of voriconazole will vary, with the final volume varying according to body weight.

Body Weight (kg)	VORICONAZOLE - Patients < 12 Years		
	Volume concentrated solution (10mg/mL)	Additional Fluid for Final Reconstitution	Total Volume
≤ 10	5.0 mL	45 mL	50 mL
10 – 14.99	5.0 mL	45 mL	50 mL
15 – 19.99	10.0 mL	40 mL	50 mL
20 – 24.99	10.0 mL	65 mL	75 mL
25 – 29.99	10.0 mL	65 mL	75 mL
30 – 34.99	15.0 mL	85 mL	100 mL
35 – 39.99	15.0 mL	85 mL	100 mL
≥ 40	20.0 mL	80 mL	100 mL

NOTE: for children < 30 kg, a single vial of voriconazole 200 mg may be used to prepare both AM and PM doses. In such circumstances doses should be prepared at the same time and given an expiration of 24 hours under refrigerated conditions.

Administration: Voriconazole is to be administered over 2 hours.

RECONSTITUTION FOR ADULT (patients aged 12 years or greater) DOSES - Under aseptic conditions:

1. Voriconazole should be reconstituted with 19 ml Sterile Water for Injection, USP. Each mL will contain 10 mg voriconazole.
2. Use the table below to determine the number of vials needed for each dose.
3. The volume of 10 mg/mL concentrated voriconazole solution required based on the patients weight should be calculated (see below) and withdrawn into a syringe.

Volume of Voriconazole Concentrated (10 mg/mL) Solution required:
All adult patients (i.e., those 12 years of age or greater) will receive a standard intravenous dose of voriconazole of 200 mg twice daily. The volume of concentrated solution to make each dose is 20mL.

4. This volume should be withdrawn and injected into an empty Viaflex[®] bag, and then further diluted with an approved solution (these solutions will not be provided by the BMT CTN).

5. Approved diluent: Sodium Chloride 0.9%, USP.
6. The final volume is dependent upon the age of the patient.

Age	VORICONAZOLE – Adult Patients (≥ 12 years)		
	Volume concentrated solution (10 mg/mL)	Additional Fluid for Final Reconstitution	Total Volume
≥ 12 years (Normal Renal Function)	20.0 mL	180 mL	200 mL
≥ 12 years (CrCl < 50 mL/min)	20.0 mL	80 mL	100 mL

Notes: Voriconazole should not be infused into the same catheter as other drug infusions, including parenteral nutrition. Infusion of blood products and infusion of electrolyte supplementation must not occur simultaneously with voriconazole infusion.

B. FLUCONAZOLE DILUTION AND INFUSION INSTRUCTIONS

Fluconazole (Diflucan[®]) is manufactured by Pfizer, Inc., Groton, Connecticut and will be supplied by the Blood and Marrow Transplant Clinical Trials Network. Fluconazole for injection is packaged in Viaflex[®] Plus plastic containers containing 200 mg of fluconazole in a volume of 100 mL in sodium chloride (concentration 2 mg/mL).

RECONSTITUTION FOR PEDIATRIC (< 12 years of age) PATIENTS:

1. The dose of fluconazole required should be calculated, and the corresponding volume of fluid should be withdrawn from the manufacturer's Viaflex[®] bag (see below for volumes).

FLUCONAZOLE – Patients < 12 years			
Body Weight (kg)	Volume of Fluconazole Concentrated (2 mg/mL) Solution Required for 6 mg/kg Dose in Pediatrics (Number of Bags)	Additional Fluid for Final Reconstitution	Total Volume
< 10	30 mL (1)	20 mL	50 mL
10 – 14.99	30 mL (1)	20 mL	50 mL
15 – 19.99	45 mL (1)	5 mL	50 mL
20 – 24.99	60 mL (1)	15 mL	75 mL
25 – 29.99	75 mL (1)	Nil	75 mL
30 – 34.99	90 mL (1)	10 mL	100 mL
≥ 35	100 mL (2)	Nil	100 mL

2. This solution should be injected into an empty Viaflex[®] bag, and further diluted as directed in the table above with an approved diluent (see below)
3. Approved diluent: Sodium Chloride 0.9%, USP.

RECONSTITUTION FOR PEDIATRIC (< 12 years of age) PATIENTS WITH CREATININE CLEARANCE <50mL/min/1.73m²:

FLUCONAZOLE – Patients < 12 years (CrCl <50 mL/min/1.73m²)			
Body Weight (kg)	Volume of Fluconazole Concentrated (2 mg/mL) Solution Required for 3 mg/kg Dose in Pediatrics (Number of Bags)	Additional Fluid for Final Reconstitution	Total Volume
< 10	15 mL (1)	35 mL	50 mL
10 – 14.99	15 mL (1)	35 mL	50 mL
15 – 19.99	22.5 mL (1)	27.5 mL	50 mL
20 – 24.99	30 mL (1)	45 mL	75 mL
25 – 29.99	37.5 mL (1)	37.5 mL	75 mL
30 – 34.99	45 mL (1)	55 mL	100 mL
≥ 35	50 mL (1)	50 mL	100 mL

RECONSTITUTION FOR PEDIATRIC (< 12 years of age) PATIENTS WITH CREATININE CLEARANCE < 20mL/min/1.73m²:

FLUCONAZOLE – Patients < 12 years (CrCl < 20 mL/min/1.73m²)			
Body Weight (kg)	Volume of Fluconazole Concentrated (2 mg/mL) Solution Required for 1.5 mg/kg Dose in Pediatrics (Number of Bags)	Additional Fluid for Final Reconstitution*	Total Volume
< 10	7.5 mL (1)	42.5 mL	50 mL
10 – 14.99	7.5 mL (1)	42.5 mL	50 mL
15 – 19.99	11.25 mL (1)	38.75 mL	50 mL
20 – 24.99	15 mL (1)	60 mL	75 mL
25 – 29.99	18.75 mL (1)	56.25 mL	75 mL
30 – 34.99	22.5 mL (1)	77.5 mL	100 mL
≥ 35	25 mL (1)	75 mL	100 mL

- This solution should be injected into an empty Viaflex[®] bag, and further diluted as directed in the table above with an approved diluent (see below)
- Approved diluent: Sodium Chloride 0.9%, USP.

RECONSTITUTION FOR ADULT (≥ 12 years of age) PATIENTS:

- A total volume of 200 mL should be withdrawn from the manufacturer's Viaflex[®] bag. This solution is to be transferred into an empty sterile Viaflex[®] bag (250 mL bag).
- No further dilution is required.

Administration: Doses should be administered over 2 hours for both adults and pediatrics.

C. PLACEBO SOLUTION INSTRUCTIONS FOR FLUCONAZOLE-RANDOMIZED PATIENTS





















PLACEBO – Patients < 12 years	
Body Weight (kg)	Volume of Sodium Chloride
< 10	50 mL
10 – 14.99	50 mL
15 – 19.99	50 mL
20 – 24.99	75 mL
25 – 29.99	75 mL
30 – 34.99	100 mL
≥ 35	100 mL

**PLACEBO SOLUTION FOR FLUCONAZOLE RANDOMIZED
ADULT (≥ 12 years of age) PATIENTS**

PLACEBO – Patients ≥ 12 years	
	Volume of Sodium Chloride
≥ 12 years age (Normal Renal Function)	200 mL
≥ 12 years age (CrCl < 50 mL/min)	100 mL

APPENDIX G
DOSING TABLES







ORAL DOSING TABLE

Age	Drug	Route	Renal Function	Dosage	Pills in AM	Pills in PM
Adult	fluconazole	PO	normal	400 mg/d		
	voriconazole	PO	normal	200 mgQ12H		
Adult *	fluconazole	PO	< 50 mg/min	200 mg/d		
	voriconazole	PO	< 50 mg/min	200 mgQ12H		
Age < 12 and ≥ 20 kg	fluconazole	PO	normal	200 mg/d		
	voriconazole	PO	normal	100 mgQ12H		
Age < 12 and ≥ 20 kg *	fluconazole	PO	< 50 mg/min	100 mg/d		
	voriconazole	PO	< 50 mg/min	100 mgQ12H		
Age < 12 and < 20 kg	fluconazole	PO	normal	100 mg/d		
	voriconazole	PO	normal	50 mgQ12H		

* RENAL INSUFFICIENCY based on calculated creatinine clearance of < 50 mL/min

RENAL INSUFFICIENCY in a patient age < 12 and < 20kg should be treated by IV infusion in order to achieve a dosage of 50 mg/d fluconazole. To maintain blind, voriconazole patient should be treated by IV.

LEGEND:

Real Drug	
200mg flu	
200mg vori	
100mg flu	
50mg vori	
Placebo	
200 mg flu / 200 mg vori	
100 mg flu / 50 mg vori	

IV DOSING TABLE

Age	Drug	Route	Renal Function	Agents	Number of Doses/Dose [#]	Time
Adult [≥ 12]	Fluconazole	IV	Normal	1 FLUC NS	400 mg/200 mL 200 mL NS	AM PM
	Voriconazole	IV	Normal	1 VORI 1 VORI	200 mg/200 mL 200 mg/200 mL	AM PM
Adult [≥ 12]	Fluconazole	IV	< 50mL/min	1 FLUC NS	200 mg/100 mL 100 mL NS	AM PM
	Voriconazole	IV	< 50mL/min	1 VORI 1 VORI	200 mg/100 mL 200 mg/100 mL	AM PM
Pediatric [< 12 years]	Fluconazole	IV	Normal	1 FLUC NS	6 mg/kg/weight adjusted volume* Weight adjusted volume*	AM PM
	Voriconazole	IV	Normal	1 VORI 1 VORI	4 mg/kg/weight adjusted volume* 4 mg/kg/weight adjusted volume*	AM PM
Pediatric [< 12 years]	Fluconazole	IV	< 50mL/min	1 FLUC NS	3 mg/kg/weight adjusted volume* Weight adjusted volume*	AM PM
	Fluconazole	IV	< 20mL/min	1 FLUC NS	1.5 mg/kg/weight adjusted volume* Weight adjusted volume*	AM PM
	Voriconazole	IV	< 50mL/min	1 VORI 1 VORI	4 mg/kg/weight adjusted volume* 4 mg/kg/weight adjusted volume*	AM PM

[#] Doses to be rounded up to the nearest 5kg bracket.

* See Appendix F for volume guidelines.

If the patient is an outpatient, and requires study IV drug for more than 2 weeks and cannot tolerate oral study drug, the patient must be taken off study drug.

APPENDIX H

ORDER FORM FOR DOSE ADJUSTMENT OF STUDY DRUG

DATE	TIME	PHYSICIAN'S ORDERS (Provider ID # Required)	SMS #
		Orders for IRB#*****, A Randomized , Double-blind	
		Trial of Fluconazole vs. Voriconazole for the Prevention of Invasive Fungal Infections in Allogeneic Blood and Marrow Transplant Patients.	
		1. Subject meets inclusion/exclusion criteria and is enrolled in IRB # ***	
		2. Patient's serum creatinine:_____mg/dL, patient weight (kg):_____	
		3. Calculate Creatinine Clearance using Cockcroft-Gault equation in 2.4.3 protocol for adults and Schwartz equation for pediatrics or 24-hour urine Creatinine Clearance.	
		Creatinine Clearance (mL/min):_____mL/min	
		4. Dosage adjustments:	
		If CrCl ≥ 50 mL/minute – no dose adjustment required	
		If CrCl < 50 mL/minute - adjust the doses of study medications as follows:	
		Fluconazole: dose reduction of 50% PO and IV.	
		Initial dose:_____mg, new dose _____mg. OR	
		If CrCl < 20 mL/min/1.73m ² for pediatric patients (i.e. < 12 years)	
		Fluconazole: dose reduction of 75% PO and IV.	
		Initial dose:_____mg, new dose _____mg.	
		Voriconazole: no dose adjustment PO. Accumulation of the voriconazole IV vehicle can occur. Oral voriconazole should be administered to these patients unless a benefit/risk assessment justifies the use of IV voriconazole.	
		Medications to be reconstituted in the same volume of fluid to maintain the blind.	

APPENDIX I

DERIVATION OF THE SPRT FOR UNCENSORED EXPONENTIAL SURVIVAL TIMES

APPENDIX I

DERIVATION OF A SEQUENTIAL TEST STATISTIC FOR CENSORED
EXPONENTIAL DATA**Background – The Sequential Probability Ratio Test**

Let $f(., \theta)$ be the density function for random variable X . According to Neyman and Pearson, the most powerful test of $H_0 : \theta = \theta_0$ versus $H_1 : \theta = \theta_1$ decides in favor of H_1 or H_0 if $L_n > c_\alpha$ or $L_n < c_\alpha$, respectively, where $L_n = \prod_i^n f(x_i; \theta_1) / f(x_i; \theta_0)$ is the likelihood ratio, and c_α is determined to have the size α . When the sample size is not fixed in advance, further improvement is possible by using Wald's Sequential Probability Ratio Test (SPRT). The SPRT continues to sample as long as $B < L_n < A$ for some constant $B < 1 < A$, stops sampling and decides in favor of H_1 as soon as $L_n > A$, and stops sampling and decides in favor of H_0 as soon as $L_n < B$.

The usual measures of performance of such a procedure are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$, and of accepting H_0 when $\theta = \theta_1$, respectively, and the expected sample size $E(N | \theta_j) \equiv E_j(N)$. Wald and Wolfowitz showed that among all tests, sequential or not, for which $\Pr_0(\text{reject } H_0) \leq \alpha$ and $\Pr_1(\text{reject } H_0) \leq \beta$, and for which $E_j(N)$ are finite, $j=0,1$, the SPRT with error probabilities α and β minimizes $E_0(N)$ and $E_1(N)$. If, in addition, the x_1, x_2, \dots are independent and identically distributed (i.i.d.) with density function $f(x, \theta)$, with monotone likelihood ratio in $\tau(x)$, then any SPRT for testing θ_0 against $\theta_1 (> \theta_0)$ has non-decreasing power function.

For the SPRT with error probabilities α and β , the SPRT boundaries are given approximately by $A = (1 - \beta) / \alpha$ and $B = \beta / (1 - \alpha)$. The operating characteristics of the SPRT are given by $O(\theta, \alpha, \beta, \theta_0, \theta_1) = (A^{h(\theta)} - 1) / (A^{h(\theta)} - B^{h(\theta)})$ where $h(\theta)$ is the non-trivial solution to the equation $\int (f(x; \theta_1) / f(x, \theta_2))^{h(\theta)} f(x; \theta) dx = 1$.

The formula $E(N; \theta) = [(1 - O(\theta)) \log A + O(\theta) \log B] / E(z; \theta)$ provides the average sample number for an arbitrary θ . The sample size distribution is very highly skewed, $\text{Var}(N) \approx [E(N)]^2$. Thus we will consider a truncated test with maximum sample size of N_0 and simulate to obtain the operating characteristics of the test.

Derivation of the SPRT for Uncensored Exponential Survival Times

For example, we wish to construct a sequential test for the composite null hypothesis that the rate of convulsions at 100 days is less than or equal to 10% versus the alternative hypothesis that it is greater than or equal to 10%. For the derivation of the uncensored SPRT, we will require that the type I error of the test be less than 5%, and that the test provide 80% power to reject the null hypothesis under a specified alternative that the true rate is 15%. A maximum sample size of 300 patients will be permitted.

Let us assume that the survival times, T_1, T_2, \dots, T_n , are completely observed (uncensored) and are i.i.d. with exponential density function $f(T, \theta) = \theta e^{-\theta T}$. These assumptions will be relaxed to incompletely observed data subsequently. In the exponential parameterization, a 100-day survival rate of 90% translates into a mean survival of 2.599 years ($\theta_0 = .3848$), and 85% translates into a mean survival of 1.685 years ($\theta_1 = .5936$).

The SPRT is derived with reference to a simple null and alternative hypothesis, in this case, $H_0 : \theta = \theta_0 = .3848$ versus $H_1 : \theta = \theta_1 = .5936$. However, since the log-likelihood ratio for the exponential,

$$\log \prod_i^n f(x_i; \theta_1) - \log \prod_i^n f(x_i; \theta_0) = n(\log(\theta_1) - \log(\theta_0)) - (\theta_1 - \theta_0) \sum_i^n T_i,$$

is a monotone function of $\sum_i^n T_i$, the power of the test is non-decreasing in θ . Thus the SPRT is a one-sided level .05 test of a composite null ($H_0 : \theta \leq \theta_0 = .3848$) versus a composite alternative ($H_1 : \theta \geq \theta_0 = .3848$), with power of $1 - \beta = .80$ at the selected alternative $\theta = \theta_1 = .5936$.

The SPRT can be represented graphically. The continuation region is bounded by two parallel lines with common slope $(\log \theta_0 - \log \theta_1) / (\theta_0 - \theta_1) = 2.076$, and intercepts $\log A / (\theta_0 - \theta_1) = -13.28$ and

$\log B / (\theta_0 - \theta_1) = 7.462$, for the lower and upper bounds, respectively. As each individual unit is put on

trial and observed to fail, the cumulative sum of failure times, $\sum_i^n T_i$, is recomputed, and plotted against the current sample size, n . When this graph crosses the lower boundary, the null hypothesis is rejected.

The maximum sample size of 300 patients requires that the SPRT be truncated. We choose to truncate the SPRT by declaring that if the test has failed to terminate after 300 patients, that the null hypothesis will be accepted. Since the probability that the untruncated SPRT would reject the null at a sample size of 300 is negligible, it makes little difference how the final boundary value is selected, and this rule is chosen for simplicity.

Derivation of a Modified SPRT for Censored Exponential Data

The assumption of uncensored exponential survival times is flawed. However, we consider it reasonable to assume the hazard for convulsions is constant over the first 100 days post-transplant, and we will restrict our attention to this time interval. Furthermore, it is not practical to conduct a clinical study by putting each individual on trial, and waiting until that individual is observed to fail. We relax our assumptions as follows. Firstly, each individual's time on study will be computed as time from transplant

to failure, or to the 100 day time point, whichever comes first. Secondly, we will put individuals on trial as soon as they become available, without waiting for the previous individual to fail.

Let us consider the impact of relaxing these assumptions one at a time. In a fixed sample size trial with uncensored exponential failure times, mean survival time is estimated by the sample mean of the failure times, or total time on study divided by the number of individuals enrolled. When censoring is introduced, the estimate becomes the total time on study divided by the number of observed (non-censored) failures. This suggests that in an exponential SPRT test modified to incorporate censoring, we replace the observed failure times, T_1, T_2, \dots, T_n , with censored failures times, x_1, x_2, \dots, x_n , and the current sample size, n , with the number of observed failures, d .

Now we relax the second assumption, and put individuals on trial as soon as they become available, without waiting for the previous individual to fail. Assume that three years are required for accrual of 300 patients to the study, and that the final analysis takes place 100 days after the last patient is entered. Putting all of this together, we propose a modified truncated SPRT, where at any interim time point, s , ranging from 0 to 3 years 100 days, the sum of observed time on study, $\sum_i^n X_i(s)$ is plotted against the number of observed failures, $d(s)$. In practice, monitoring will be scheduled monthly after the start of enrollment to the study. A further modification to the SPRT was to only use the lower boundary for stopping since the primary focus of the monitoring is to protect against unacceptable 100-day convulsion rates.

Operating Characteristics of the Modified SPRT Test for Censored Exponential Data

Recall that the uncensored SPRT targeted a drop in convulsion-free survival at Day 100 from 90% to 85%, with type I and II errors of 5% and 20%. Since only the lower boundary is used for monitoring, the continuation region of the test was bounded below by a line with a slope of 2.076 and intercept of -13.28 . The effect of truncation is to reduce the power of the test. In order to compensate for this, we raise the lower boundary to make it easier to cross. Under the further assumption of uniform accrual over a three year period, and monthly interim analyses over the course of the study, the operating characteristics of the modified SPRT were obtained from a simulation study. These simulation show that an intercept of -10.555 , corresponding to setting parameters α and β to 9% and 15%, result in empirical type I and II error rates of 5% and 20%.

Table I-1 Operating Characteristics of Sequential Testing Procedures from a Simulation Study with 100,000 Replications

Neural Toxicity (Convulsions)			
True 100-Day Rate	10%	15%	20%
Probability Reject Null	0.06	0.79	1.00
Mean Month Stopped	35.8	21.8	10.7
Mean # Endpoints in 100 days	28.4	25.2	15.3
Mean # Patients Enrolled	290.9	179.8	89.1

While the motivation for this testing procedure is largely heuristic rather than theoretical, the simulation results validate the approach. When the true rate of convulsions on or before Day 100 was 10%, the test crossed the lower boundary in 5977 of 100,000 replications, for an estimated type I error rate of 6%. When the true rate of convulsions on or before Day 100 was 15%, the test failed to cross the boundary in the in 21483 of 100,000 replications, for an estimated type II error rate of 21%. The test is almost certain (100%) to reject the null hypothesis when the true rate is 20%, and on average, the boundary will be crossed at 10.7 months, with probability less than 1% of crossing in the first 3 months.

It is interesting to note that the SPRT derived above for exponential failure times with censoring at 100 days, has operating characteristics which are similar to those of a more traditional SPRT, derived for binomial variates with success probability equal to the 100 day failure rate. Using time to failure rather than a simple binary indicator of failure, leads to little improvement in power when failure times are censored relatively soon after entry on study. We speculate that if the constant hazard rate over the first 100 days were high, the exponential test would reject faster than the binomial test, but have not conducted simulation studies to demonstrate this.