METHODS

Inclusion criteria

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

1. Patients who were to 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 had to have a 5 or 6 of 6 HLA-matched donor. The match could be determined at serologic level for HLA-A and HLA-B loci. For sibling donors, matching could be determined at serologic level for HLA-DR; for unrelated donors, matching for HLADRB1 had to 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.
5. Patients with one of the following underlying diseases:
   a) AML, with or without a history of myelodysplastic syndrome, in first or second complete remission or in early relapse (< 30% blasts in bone marrow with no circulating blasts in peripheral blood and no extramedulary leukemia); or
   b) ALL, in first or second complete remission; or
   c) AUL (acute undifferentiated leukemia) in first or second complete remission; or
   d) Acute biphenotypic leukemia in first or second complete remission; 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 (≥ 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: < 5 × 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 O_2 saturation > 85% of room air
8. Patients had to 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 had to 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).

Pediatric doses (Children aged < 12 years old):
Oral doses were given as follows: In patients weighing > 20 kg, voriconazole was administered at a dose of 100 mg twice daily. Patients weighing < 20 kg received oral voriconazole at a dosage of 50 mg twice daily. If intravenous administration was needed, voriconazole was dosed at 4 mg/kg (total body weight) every 12 hours (not to exceed the equivalent oral dose) for the duration of intravenous therapy.

For oral administration, patients weighing > 20 kg received fluconazole at a dose of 200 mg once daily. For those weighing < 20 kg, a dose of 100 mg once daily was used. When intravenous administration was used, fluconazole was given at 6 mg/kg/day total body weight (with maximal IV dose not to exceed the equivalent oral dose per weight) once daily.

Dose reduction for renal impairment: In the setting of renal dysfunction, fluconazole doses were 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 were made based on the method of Cockgroft and Gault (Cockgroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 16:31–41, 1976) for adult males [CrCl = (140− age in years) × kg weight (ideal) /serum creatinine ×72]. For adult females, the equation was multiplied by 0.85. Ideal body weight (IBW) was 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, 2.3 kg/inch was subtracted. For children, the Schwartz calculation was used (CrCl = k value X height (cm)/plasma creatinine). K values for children and adolescent girls = 0.55, adolescent boys = 0.70. No dose adjustments for renal dysfunction were made in the voriconazole arm. For patients with moderate renal insufficiency (estimated CrCl < 50 mL/min), accumulation of the voriconazole IV vehicle can occur. Thus, oral voriconazole was administered to those patients unless a benefit/risk assessment justified the use of IV voriconazole. However, if CrCl declined to 25 mL/min and persisted for more than 14 days and the patient had to receive IV drug and was unable to switch to oral drug, then the study drug was discontinued. Study drug was to be discontinued in the setting of hemodialysis. To maintain the blind in patients receiving intravenous study drug, for patients randomized to receive voriconazole who met the renal impairment criteria, the volume of voriconazole was adjusted to mimic the volume the patient would have received if randomized to fluconazole. To maintain the blind in patients receiving oral study drug, the number of voriconazole capsules taken in the morning was adjusted to mimic the number the patient would receive if randomized to fluconazole.

Empiric antifungal therapy: Patients who developed a possible invasive fungal disease, but did not meet the criteria for failure of prophylaxis, could be treated empirically with an amphotericin B formulation or caspofungin. Those patients remained on study and continued to be assessed for more definitive evidence of fungal infection. During empirical therapy, the study drug was continued. Empirical antifungal therapy was intended for patients strongly suspected to have invasive fungal infections where exhaustive diagnostic evaluation excluded other etiologies but

also failed to demonstrate a presumptive, probable or proven invasive fungal infection. It was generally intended for patients in whom the criteria for possible invasive fungal infection was met. The guidelines for empirical therapy for the most common situations are below. In no case was it to be continued beyond 14 consecutive days. During an empirical trial of antifungal therapy, efforts were to continue to be undertaken to ascertain the etiology of the fever through cultures and biopsies of pertinent tissue specimens.

Required tests prior to use of empirical antifungal therapy: All focal lesions suspicious for fungi were to be biopsied if clinically possible. Cultures of urine and blood were to be obtained. Isolator culture methods were preferred if available. At least two blood samples for the diagnostic galactomannan assay were to be obtained during the seven days (with at least one within 48 hours) before institution of empirical antifungal therapy and were to continue to be drawn during the empirical therapy.

Pre-engraftment requirements and amphotericin B/caspofungin dose: If the patient was neutropenic, had persistent fever (≥ 38°C) of unknown origin refractory to at least 96 hours of broad-spectrum antibiotics or fever that recurred after 96 hours of antibiotics, and a specific etiology could not 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) was permitted. This could be continued until resolution of neutropenia (ANC > 500) but was to not be continued beyond 72 hours after resolution of neutropenia and not longer than 14 consecutive days.

Post-engraftment requirements and amphotericin B/caspofungin dose: If the patient was post-engraftment and met the criteria for possible infection and attempts to document etiology had failed, an empirical trial of an amphotericin B formulation or caspofungin was permitted. Continued radiographic procedures, cultures, biopsies, and other pertinent diagnostic evaluations were to be performed in an effort to elucidate the suspected infection. If these measures failed, then the empirical trial was to be terminated after no more than 14 days.

Non-study drug prophylaxis: Patients were to be prematurely withdrawn from study treatment prior to day 100 (or 180) if:

1. There was evidence at any time during the study of a presumptive, probable or proven invasive fungal infection as defined by criteria.
2. Any of the following toxicities occurred. However, if another etiology was judged by the local PI to be the likely cause of the toxicity and an interval of no more than 14 days had lapsed, then the study drug could be resumed at original dose. If the same toxicity recurred, the patient was permanently withdrawn from the study treatment.
    a) For hepatic toxicity: If ALT exceeded ten times the upper limit of normal and was felt to be at least possibly related to study drug, the study drug was to be held until the causality clarified and until the toxicity resolved to a Grade II or less. If ALT exceeded five times the upper limit of normal and was 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 clarified and until the toxicity resolved to less than two and a half times the upper limit of normal.

b) For visual toxicity: Photopsia did not represent grounds for withdrawal. If a patient experienced loss of vision (blindness), it was unlikely that it is due to the study drug; however, the study drug was to be held until etiology was established.
c) For cutaneous toxicity: A skin rash did not constitute grounds for withdrawal unless there was skin necrosis or ulceration or generalized exfoliative dermatitis.
d) For neurologic toxicity: If hallucinations occurred, attempts were to 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 could 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 occurred, study medication was to be held, and the subject underwent further assessment by a cardiologist to evaluate the significance of the findings. If the PI and cardiologist concluded that it was not related to the study drug, study drug could be resumed as specified above. Otherwise, the patient was to be withdrawn from study treatment.
f) For renal insufficiency: The patient experienced serious renal impairment requiring hemodialysis and IV study drug.
g) For any other Grade III or IV toxicity according to the NCI CTCAE Version 3.0 which was not typically expected in the course of BMT and thought to be possibly related to study drug.

3. Systemic amphotericin B (or one of the lipid formulations) or caspofungin was given for more than 14 consecutive days.
4. The patient required terfenadine, astemizole, cisapride or sirolimus, maintenance phenytoin/anticonvulsant therapy, or any of the drugs prohibited at study entry.
5. The patient was an outpatient and required IV study drug for more than 14 consecutive days and could not tolerate oral study drug.
6. The patient's creatinine clearance declined to < 25 mL/min and persisted for more than 14 days and the patient required IV study drug and could not tolerate oral study drug.
7. The patient failed to engraft and required chemotherapy.
8. The patient relapsed and required chemotherapy.
9. The patient became pregnant.
10. The patient withdrew consent.

If a patient was prematurely withdrawn from study drug prior to Day 100 for reasons other than a presumptive, probable or proven fungal infection, fluconazole was allowed for fungal prophylaxis until Day 100. No other antifungal agents were allowed.

Definitions of toxicity

Thirty transplant-associated and known study drug toxicities were assessed by the NCI CTCAE in most cases except for those noted below. Neural toxicity was defined as convulsions. Cardiac toxicity was defined as grade III toxicity on the Bearman scale (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%) (Bearman SI, Appelbaum FR, Buckner CD, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. .J Clin Oncol. 1988;6:1562–8). Hepatic toxicity was defined as grade III toxicity on the Bearman scale (severe hepatic dysfunction with bilirubin > 20 mg/dL; or, hepatic encephalopathy; or,

ascites compromising respiratory function. Renal toxicity was defined as use of dialysis (Bearman grade III toxicity). The rates of neurologic, cardiac, hepatic, and renal toxicities were monitored up to 100 days post-transplant.

Galactomannan positivity: A positive GM assay was defined as two positives (index of 0.5 or greater) on the same specimen or two consecutive positives on different specimens.

Presumptive IFI: This category was added to the protocol on December 2005 for the reasons stated in the Methods. All cases subsequent to and prior to this protocol amendment were scored using this category in the consideration.

Risk Status: Patients were assessed as poor risk if they met any one of the following:

- CML in blast crisis,
- AML not in CR or > second CR
- ALL not in CR
- MDS – RAEBT
- CMML

All other eligible diagnoses were considered standard risk.

RESULTS

TABLE S1. CUMULATIVE INCIDENCE OF PROVEN/PROBABLY/PRESUMPTIVE IFI AT 100, 180, AND 365 DAYS

	N	At 100 Days Estimate (95% CI)	p-value	At 180 Days Estimate (95% CI)	p-value	At 1 Year Estimate (95% CI)	p-value
Fluconazole	295	9.5% (6.2%–12.8%)	0.10	11.2% (7.7%, 14.7%)	0.12	13.7% (9.8%, 17.6%)	0.59
Voriconazole	305	5.6% (3.1%–8.1%)		7.3% (4.4%, 10.2%)		12.7% (9.0%, 16.4%)	

TABLE S2. OVER-ALL AND RELAPSE-FREE SURVIVAL AT 100, 180, AND 365 DAYS

	N	Events	At 100 Days Estimate (95% CI)	At 6 Months Estimate (95% CI)	At 1 Year Estimate (95% CI)
Relapse-Free Survival					
Fluconazole	295	112	83.1% (78.3%, 86.9%)	74.9% (69.6%, 79.5%)	63.3% (57.4%, 68.6%)
Voriconazole	305	122	86.1% (81.7%, 89.6%)	73.9% (68.5%, 78.5%)	61.2% (55.5%, 66.5%)
Overall Survival					
Fluconazole	295	97	85.4% (80.9, 89.0)	80.0% (75.0%, 84.1%)	70.2% (64.6%, 75.1%)
Voriconazole	305	104	90.1% (86.2, 93.0)	81.2% (76.3%, 85.1%)	67.8% (62.1%, 72.8%)

TABLE S3. OCCURRENCE OF IFI RELATIVE TO KEY TRANSPLANT EVENTS

IFI Category	Day 0–180		Day 0–365	
	FLU	VORI	FLU	VORI
IFI after relapse/progression† (N)	1	3	2	8
IFI before engraftment (N)	12	8	12	8
IFI who had failure to engraft (N)	2	0	2	1
IFI after aGVHD (Grades 2–4) (N)	7	7	11	14
IFI while on study drug (up to d100) (N)	19	10	19	10
IFI after premature withdrawal of study drug (N)	10	8	11	16
IFI after start of prophylaxis other than study drug (N)	6	4	8	11
IFI after empiric therapy (N)	12	6	13	12

† – Relapse/Progression defined as first treatment for relapse/progression at follow-up.

Survival after IA Infection

Survival after onset of Aspergillosis was 47.1% vs. 58.8%, (p =0.56) at 6 weeks; 26.2% vs. 45.8% (p=0.30) at 12 weeks, for the fluconazole and voriconazole arms, respectively.

TABLE S4. GALACTOMANNAN RESULTS BY TREATMENT ARM

	Fluconazole	Voriconazole	Total
GM+*	43 (14.6%)	35 (11.5%)	78 (13.0%)
GM−	252 (85.4%)	270 (88.5%)	522 (87.0%)
Total	295	305	600

*although there were 82 GM positives, 4 were excluded due to concomitant piperacillin/tazobactam administration, without other documentation of IFI, and were deemed false positives.

TOXICITIES

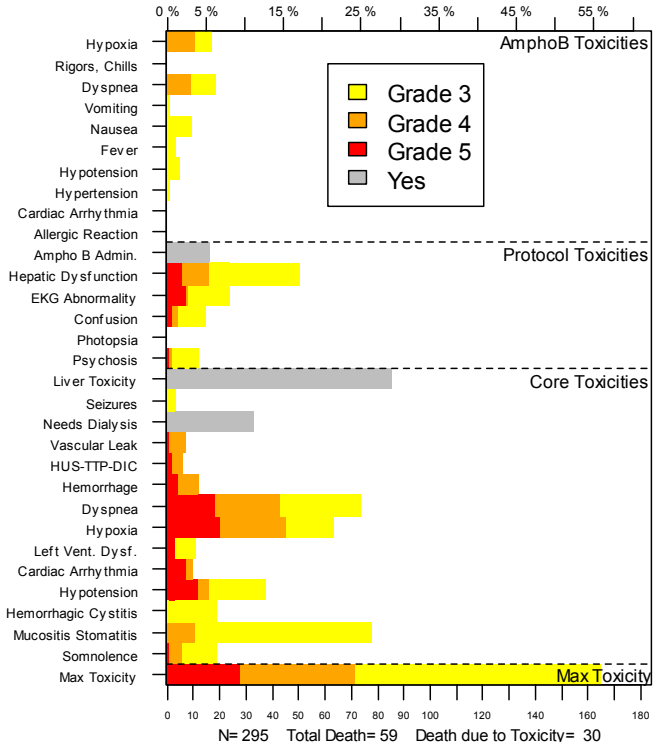

Figure S1. Toxicity frequency: maximum toxicity day 0–180, fluconazole

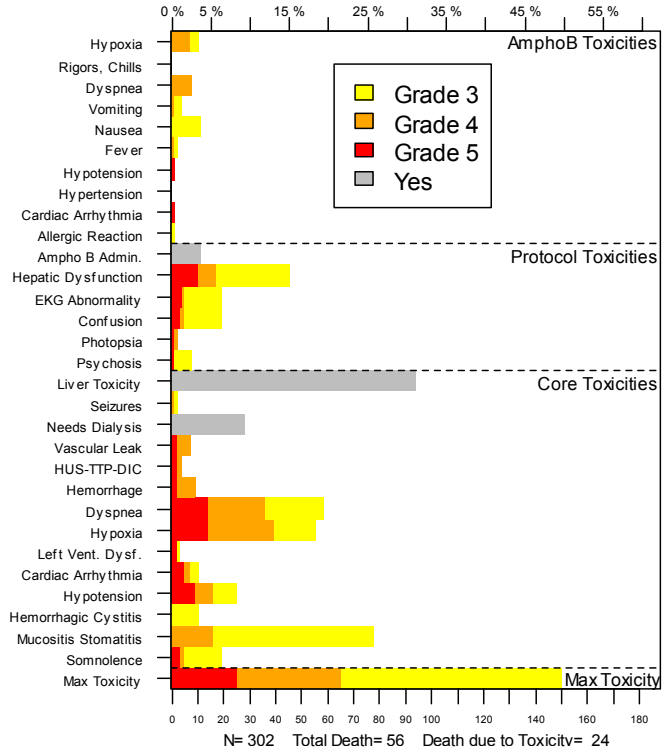

Figure S2. Toxicity frequency: maximum toxicity day 0–180, voriconazole

Note: "Hepatic Dysfunction", "EKG Abnormality" and "Somnolence" used the Bearman Toxicity Grading Scale. The grades shown in the plot = Bearman Grading Scale +1, as illustrated in the following table. This is only for demonstration purpose, to keep consistency as CTCAE Grading Scale, in which grade 5 is fatal toxicity.

Bearman Scale	Grade Shown in the Plots
Grade 2	Grade 3
Grade 3	Grade 4
Grade 4	Grade 5

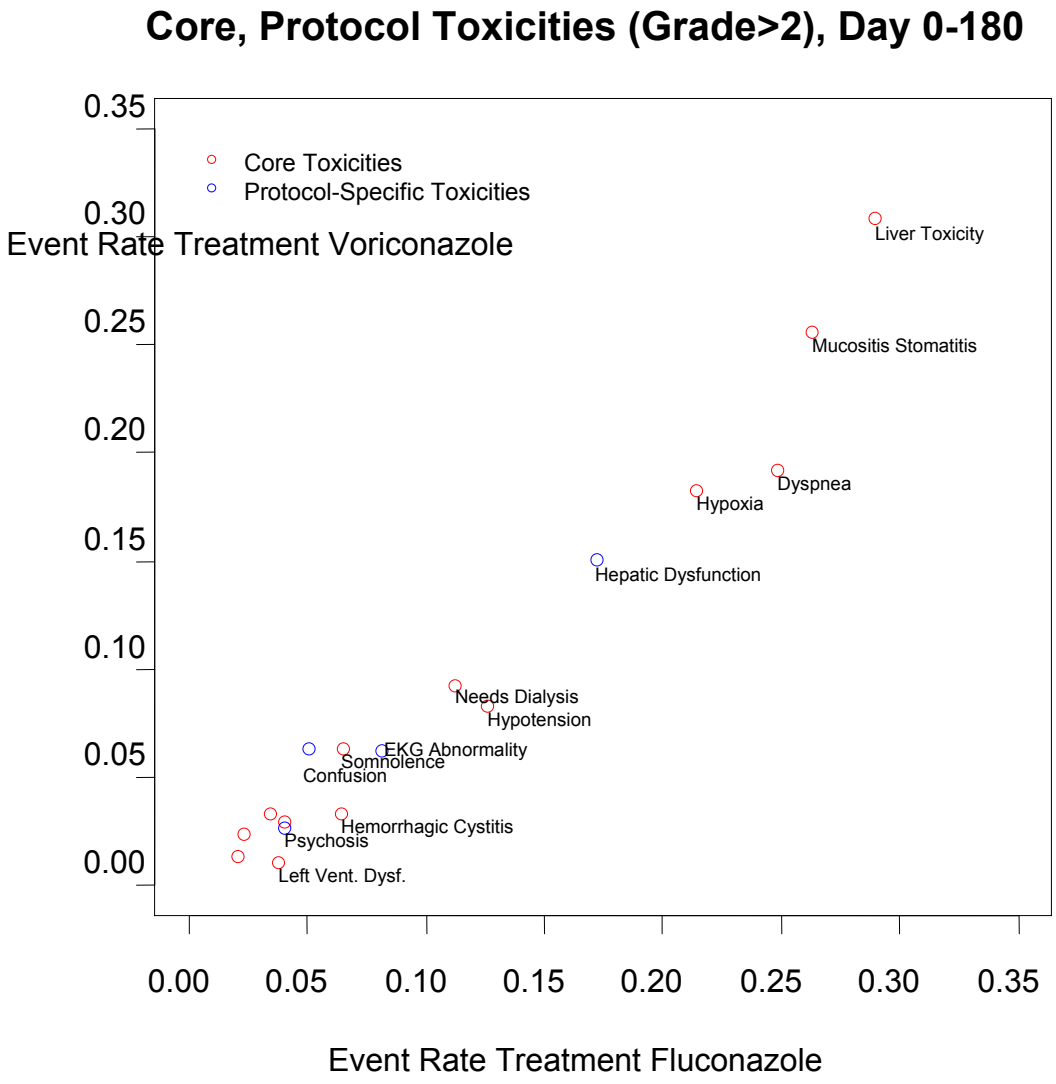

Figure S3. Core and protocol-specific toxicities (grade >2) day 0–180

Notes:

1 – Labels for toxicity type with rates in both arms less than 0.05 were omitted if labels overlay. The upper and lower dotted lines denote the ±5% deviation from the center line.

2 – Grade ≥ 2 toxicities are shown in the plot for "Hepatic Dysfunction," "EKG Abnormality" and "Somnolence."

3 – Collected toxicities included: photopsia, CNS toxicity, psychosis, seizure activity, confusion, EKG abnormalities, cardiac arrhythmia, hypotension, left ventricular systolic dysfunction, dialysis use, liver abnormalities, mucositis, stomatitis, hemorrhage, HUS/TTP/TMA, vascular leak syndrome, hypoxia, dyspnea.