High Dose Granulocyte Transfusions for the Treatment of Infection in Neutropenia

The RING Study (Resolving Infection in Neutropenia with Granulocytes)

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Study Sponsor: National Heart, Lung, and Blood Institute

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Conducted by the Transfusion Medicine/Hemostasis Clinical Trials Network

CONCEPT SYNOPSIS AND STUDY SCHEMA

Indications Studied:
The study will evaluate neutropenic patients who have undergone dose-intensive chemotherapy or hematopoietic stem cell transplantation within the past 60 days, and have a proven or probable infection.

Herein, "subjects" refers to neutropenic patients as described above. "Donors" refers to community or family donors of G-CSF/dexamethasone-mobilized granulocytes.

Primary Objective
To evaluate whether subjects treated for neutropenia who receive G-CSF/dexamethasone-mobilized granulocyte transfusions in addition to organism-directed antimicrobial therapy will be more likely to survive to 42 days and achieve microbial response, compared to subjects who receive standard care of organism-directed antimicrobial therapy alone.

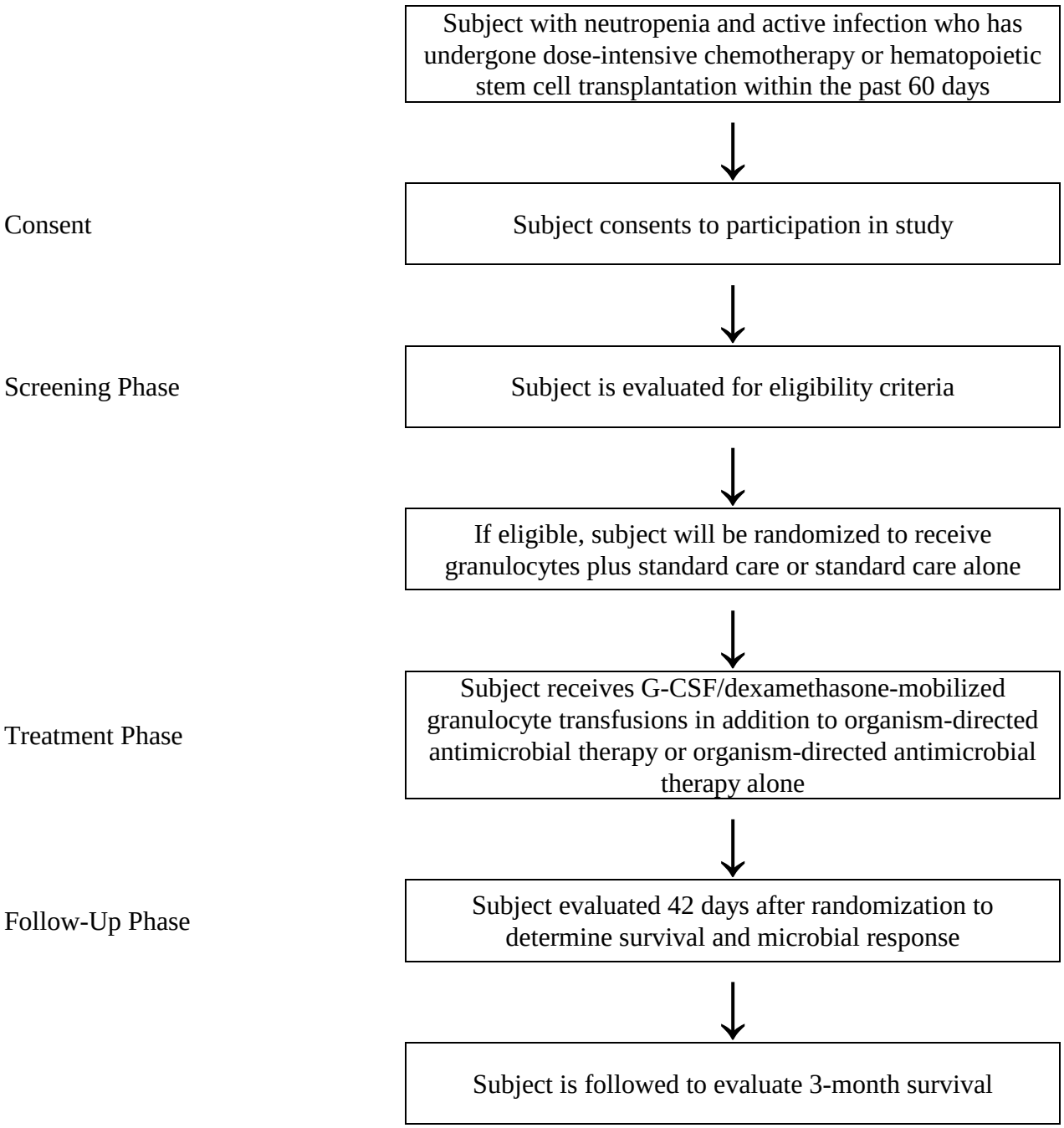
Secondary Objectives
1) To evaluate safety in subjects who receive standard care plus granulocyte transfusion compared to subjects who receive standard care alone.
2) To determine the efficacy of G-CSF/dexamethasone-mobilized granulocyte transfusions.
3) To evaluate 3-month survival of subjects who received G-CSF/dexamethasone-mobilized granulocyte transfusions and standard care compared to subjects who received standard care alone.

Population
1) Subjects who are neutropenic and have undergone dose-intensive chemotherapy or hematopoietic stem cell transplantation within the past 60 days.
2) Subjects must have a fungemia, bacteremia, invasive tissue bacterial infection, or proven/probable invasive tissue fungal infection confirmed by culture, histopathologic, or radiologic criteria.
3) Subjects must meet the eligibility criteria discussed in section 3.1 and 3.2.

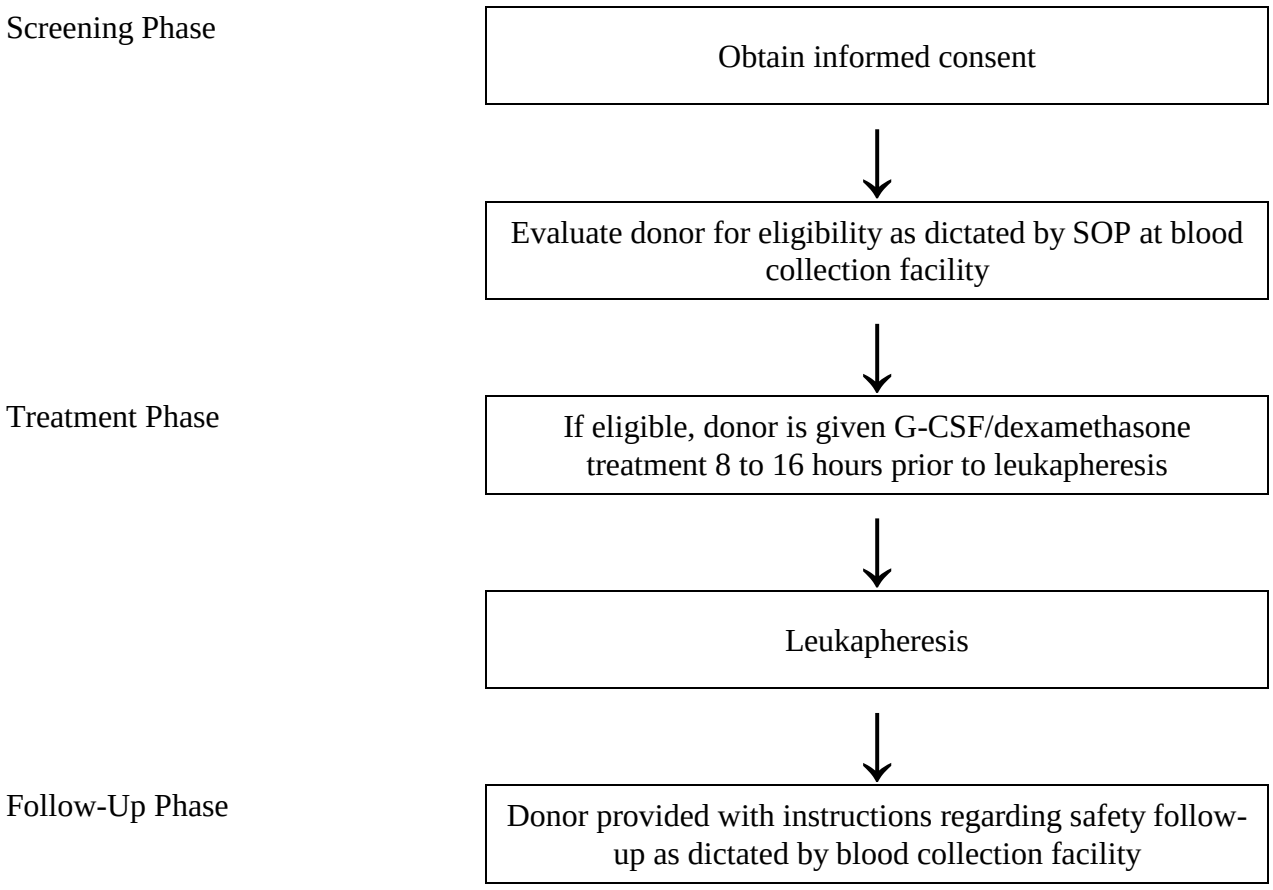
Study Design
This is a two arm, unblinded, Phase III, randomized clinical trial.

After evaluation for eligibility, subjects will be randomized to receive either G-CSF/ dexamethasone-mobilized granulocyte transfusion in addition to organism-directed antimicrobial therapy or organism-directed antimicrobial therapy alone. Subjects in the granulocyte arm will receive daily granulocyte transfusions for up to 42 days. Subjects in both arms will receive standard care antimicrobial therapy as defined in Appendix A. Subjects will receive study treatments and testing for 42 days after randomization and be followed for a total of 3 months to evaluate survival.

STUDY OVERVIEW - SUBJECTS



STUDY OVERVIEW - DONORS



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1 BACKGROUND AND SIGNIFICANCE

Infections in neutropenic subjects continue to cause substantial morbidity and mortality in the setting of aggressive chemotherapy and hematopoietic stem cell (HSC) transplantation. In particular, fungal infections are becoming an increasingly important cause of death in neutropenic cancer and HSC transplant subjects [1-3]. For example, among marrow transplant recipients treated at Fred Hutchinson Cancer Research Center (FHCRC) using fluconazole as antifungal prophylaxis and ceftazidime as antibacterial prophylaxis during 1992-1996, only 40% (23 of 58 subjects) who developed fungemia during neutropenia had clearance of the fungemia within 10 days of the first positive blood culture and survived to 4 weeks. Similarly, < 30% of subjects who developed invasive mold infections during neutropenia who were transplanted during 1992-1996 were alive at 12 weeks after the onset of infection.

The strongest predictor of progression and recovery from invasive infection in the cancer/stem cell transplant setting is the recovery of adequate neutrophils [4]. Therefore, any method that provides adequate numbers of functional neutrophils to infected subjects during the neutropenic period should be of benefit. Transfusion of granulocyte concentrates obtained without growth factor stimulation was shown to be of benefit for the treatment of gram-negative bacteremia outside the marrow transplant setting, with survival increasing from 36% for the control group to 75% for the 27 subjects who were transfused in one study [5]. Other trials have also shown a benefit [6, 7] or a partial benefit [8, 9] from transfusions. In spite of these results, however, granulocyte transfusion therapy fell out of favor, primarily because of unimpressive clinical results, most likely due to the fact that only relatively small doses of granulocytes could be provided. The current renewed interest in this form of therapy springs from the ability to deliver much greater numbers of cells by stimulating granulocyte donors with recombinant granulocyte colony-stimulating factor (G-CSF).

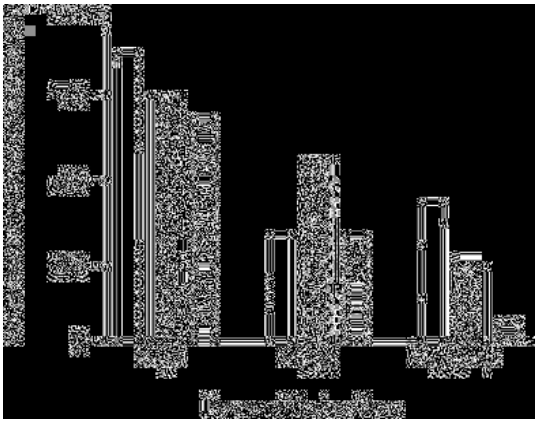
The use of G-CSF to mobilize granulocytes from donors for transfusion to severely neutropenic subjects was first reported in 1993 [10, 11]. The study of Bensinger et al [10] followed a series of careful investigations of the effects of G-CSF on neutrophil kinetics and function in normal subjects [11-14]. In the Bensinger study, treatment of donors with G-CSF permitted collection of 2 to 6 times as many cells as were collected previously from donors treated with corticosteroids. Perhaps more importantly, this study demonstrated that neutrophils collected with G-CSF remained in the circulation much longer than had ever been noted before. The mean neutrophil count at 24 hours post-transfusion was nearly 1 x 10^9 cells/L [10].

In a further effort to optimize the collection of granulocytes, Liles et al compared G-CSF (300 or 600 μg) with or without dexamethasone (8 mg P.O.) versus dexamethasone alone, with measurement of the CBC at 0, 6, 12, and 24 hours [15]. These studies showed that the blood neutrophil count of normal subjects could be elevated as much as ten-fold at 12 hours after G-CSF (600 μg) and dexamethasone (8 mg), and suggested that the optimum time for leukapheresis was 12 hours after this combination. Dexamethasone significantly increased the maximal ANC induced by either low dose (300 μg) or high-dose (600 μg) G-CSF [15]. In a second study, the response to 450 μg G-CSF plus dexamethasone (8 mg) was equivalent to 600 μg G-CSF plus dexamethasone (8mg) [16]. Based on these results, the kinetics and function of neutrophils

collected by leukapheresis 12 hours after single doses of G-CSF (600 μg) and dexamethasone (8 mg) were determined [17]. This study showed that approximately 8.0 x 10^{10} neutrophils could now be routinely collected, and the recovered neutrophils circulated with a significantly prolonged half-life when re-infused into donors. The functional properties for these cells were normal or near normal.

A phase I/II trial of G-CSF and dexamethasone-mobilized granulocyte transfusions was conducted for subjects with documented fungal and/or bacterial infections [18]. Data for the first 165 transfusions (1 to 17 transfusions per subject, mean 8.6 transfusions per subject) showed that transfusion of approximately 8.0 x 10^{10} neutrophils increased blood neutrophil counts to normal (i.e., greater than 1.5 x 10^{9}/L) in almost all subjects. Thereafter, continued transfusion maintained counts in the normal range in recipients who were previously severely neutropenic. The study also demonstrated that these cells had the capacity to migrate to the oral cavity (demonstrated with oral washes with neutrophil counting). Clearance of fungemia was also documented. Careful monitoring of these subjects showed that hypoxia (as reflected by regular monitoring of oxygen saturation), fever, chills, and other adverse events attributable to the transfusions occurred very infrequently. Most importantly, this trial showed that it was feasible to use community donors as a source for granulocytes for supportive care in this setting. Infection resolved in 8/11 subjects with invasive bacterial infections or candidemia; none of the eight subjects with invasive mold infection, however, survived 30 days to document clearance of infection. Of note, this trial was conducted prior to the availability of antifungal agents that are more effective and less toxic than conventional amphotericin B, including the third generation triazole voriconazole [19]. It is possible that the administration of more effective therapy would allow the incremental benefit of granulocyte therapy to be demonstrated, but this hypothesis remains to be proven.

Figure 1: Percentage of progressive or fatal infection at day 30 after diagnosis, by infection type



Key
White bars = unrelated granulocyte donors
Checked bars = related granulocyte donors
Striped bars = control subjects
All comparisons NS with exception of bacterial infections (p=0.04). From Hubel et al [20].

More recent studies of related and unrelated granulocyte transfusions have shown that recipients of unrelated granulocytes have a shorter time from diagnosis to first transfusion when compared to recipients of granulocytes from relatives, which might theoretically lead to improved outcomes [20]. Although some subjects with mold infection who received unrelated or related granulocytes did survive in this study, their survival was no greater when compared with a matched cohort that received antifungal therapy alone (Figure 1). These negative results must be interpreted with caution. This study was not a prospective randomized study, the "controls" were partly historical, and the subjects selected to receive granulocyte transfusions are likely to have had more severe illness. Regardless, these data do

not provide clear evidence that granulocyte transfusions are clinically effective. Rather, the data indicate that effectiveness must be proven in a trial where severity of illness is controlled via the randomization process.

No recipient of granulocyte transfusions in the study of Price et al developed anti-neutrophil antibodies [18]. During the course of the study, a positive lymphocytotoxic crossmatch with the neutrophil donor was detected in 8 of the 19 (42%) subjects. The presence of leukocyte antibodies had no effect on the post-transfusion neutrophil increment or the buccal neutrophil responses, nor did they affect the incidence of transfusion-related side effects such as chills, fever or hypoxemia [18]. Adkins and colleagues, however, found that subjects with a positive lymphotoxicity screening assay who received prophylactic granulocyte transfusions from related donors had delayed neutrophil engraftment, more febrile days, and greater platelet transfusion requirements [21].

Recent studies have addressed the feasibility of multicenter granulocyte transfusion trials. In a multicenter trial that was coordinated by the National Marrow Donor Program, five centers in the US successfully provided daily granulocyte transfusions obtained from predominantly unrelated community donors for 40 subjects with serious infections during neutropenia [22]. Of the 351 days that granulocytes were required by protocol, 329 transfusions were administered (94% success in administration). Survival with complete or partial responses at 4 weeks after enrollment in this contemporary series varied by infection type [9/24(38%) for invasive mold infection, 2/5 (40%) for bacteremia/candidemia, 6/10 (60%) for severe bacterial infection]. Adverse events were also frequent in this seriously ill population (median 2 AEs/subject, range 0-11), though only two serious adverse events (two cases of transfusion-associated lung injury that resolved with discontinuation of granulocyte support) were deemed related to the granulocyte therapy. These data provide further evidence that granulocyte transfusion therapy is feasible. Clinical efficacy, however, appears to vary considerably according to the population studied, again highlighting the need for prospective, randomized studies.

Several additional studies have also shown that G-CSF administration to normal donors is well tolerated and results in the collection of large numbers of apparently normally functioning granulocytes and that these cells circulate when administered to neutropenic subjects [23-26]. Apparent clinical efficacy in these uncontrolled studies has been mixed [23-25, 27].

The safety of short-term administration of G-CSF (+/- corticosteroids) to normal donors has been studied extensively; both in the setting of stimulation of granulocyte donors and for the collection of peripheral blood stem cells. Stroncek et al [28] and Anderlini et al [29] reported on the effects of five days of G-CSF administration in 142 donors. Symptoms were seen in 90-98% of donors, consisting of bone aching, headache, myalgia, fatigue, and nausea in 80%, 40-70%, 25%, 15%, and 10% of subjects, respectively. These symptoms were mild to moderate in most donors and were effectively treated with antipyretics and anti-inflammatory agents. Symptoms resolved within a few days after the last dose of G-CSF, and the donor's leukocyte count returned to normal within 7-10 days. Adverse effects seen in donors given only one dose of G-CSF are similar to those given multiple doses, but the incidence is less, the symptoms less intense, and the duration shorter. In a study of 82 community donors stimulated once with 600ug G-CSF and 8

mg dexamethasone, 41%, 30%, and 30% experienced mild to moderate bone pain, headache, and insomnia, respectively. Twenty eight percent of these donors experienced no side effects, and 98% indicated a willingness to undergo future G-CSF stimulation for leukapheresis [18]. Two studies have shown that spleen size increases in donors given G-CSF for five days and returns to normal after the drug is discontinued [30,31]. Rare side effects of G-CSF treatment of normal donors, almost always seen only after multiple doses, have included exacerbation of underlying inflammatory or immunologic disorders, cardiac events in donors with underlying coronary artery disease, crisis and even death in subjects with sickle cell disorders, and splenic rupture. Long term side effects have not been reported. The National Marrow Donor Program has in place a comprehensive long-term follow-up program for all donors given G-CSF for peripheral blood stem cell collection, to date consisting of over 3000 donors. Because of this extensive body of knowledge and the large ongoing study being conducted by the National Marrow Donor Program, it is not the intention of the proposed investigation to study the donor effects of G-CSF or dexamethasone.

Thus several investigators have shown that G-CSF (+/- corticosteroid) stimulation of normal donors is well tolerated, although side effects do exist, and results in the ability to collect large numbers of apparently functional cells. The transfusion of these large numbers of granulocytes is in general well tolerated by subjects, although serious side effects can occur, particularly pulmonary reactions. These reactions do not seem to be any more common that those seen with the administration of granulocytes obtained without donor G-CSF stimulation. The major question to be answered is whether the transfusion of large doses of granulocytes is clinically effective in eradication of infection and/or prolonging subject survival. Evidence for efficacy to date is only anecdotal or based on small uncontrolled series. Some of these series have suggested efficacy, impressing some clinicians that the therapy is useful; others have been less impressive. The current situation is thus one of clinical equipoise. It is not clear whether this rather expensive therapy would be advantageous, disadvantageous, or neutral, given that clinical efficacy is uncertain and there are known possible adverse effects for both donor and subject. The proposed Phase III randomized controlled study is designed to determine whether transfusion of granulocytes to subjects with documented bacterial or fungal infection during neutropenia is associated with significant improvement in clinical outcome.

2 OBJECTIVES

2.1 Primary Objective

The primary endpoint of this study for subjects includes survival to 42 days after randomization AND microbial response, which will depend on infection type at enrollment. The response will be defined as a negative blood cultures test at 42 days after randomization for subjects with fungemia (candidemia or fusariosis) or bacteremia, and the improvement of signs and symptoms of disease (complete or partial response) at 42 days after randomization for subjects having invasive tissue mold, yeast or bacterial infections.

Achievement of the primary endpoint in neutropenic subjects who receive G-CSF/dexamethasone-mobilized granulocyte transfusions in addition to organism-directed antimicrobial therapy will be determined and compared with control subjects who receive standard antimicrobial therapy alone. Antimicrobial therapy is broadly defined as within the standard of care for a particular infection, but recommended antimicrobials for various infection types are listed in Appendix A.

2.2 Secondary Objectives

1. Safety among recipients of granulocytes vs. standard care (where appropriate), including the occurrence of the following endpoints:
   - Alloimmunization, defined as the appearance of anti-HLA or anti-neutrophil antibodies
   - Serious granulocyte transfusion reactions, including febrile, allergic and pulmonary reactions (transfusion arm only)
   - Graft vs. host disease among recipients of allogeneic stem cell transplantation
   - Overall incidence of adverse effects
   - Incidence of discontinuation of transfusions due to toxicity or intolerance (transfusion arm only)

2. Efficacy, determined by:
   - Outcome within each infection subgroup (invasive mold, invasive bacterial, etc) according to randomization arm
   - Time to resolve fever
   - Time to negative test for fungal antigenemia (e.g. galactomannan antigenemia among subjects with invasive aspergillosis)
   - Time to negative blood culture for subjects with positive blood culture at baseline.

3. Long-term survival. Though overall survival often primarily reflects disease status (rather than response to anti-infective therapy), we will follow all randomized subjects until 3 months after randomization for comparison of long-term survival.

2.3 Donor Objectives

1. Donor safety
2. Donor availability
3. Evaluation of granulocyte yield

3 STUDY POPULATION

3.1 Subject Inclusion Criteria

1. Subjects who have undergone dose-intensive chemotherapy or hematopoietic stem cell transplantation within 60 days prior to randomization.

2. Age: All ages
3. Subjects who have neutropenia (ANC < 500/ mm^3), and are expected to remain neutropenic for at least 5 days after randomization.
4. Subjects must have one of the following, as defined in Appendix A:
   - fungemia;
   - bacteremia;
   - invasive tissue bacterial infection;
   - proven invasive tissue fungal infection; or,
   - probable invasive tissue fungal infection.

   Note: patients with criteria meeting only the definition for a possible invasive fungal infection are not eligible to participate in the RING Study.

Once determined to be eligible for the study, subjects must be randomized within 24 hours. Subjects should receive the first granulocyte transfusion (if in the treatment arm) as soon as possible after randomization; every effort should be made to provide the first transfusion within 48 hours after randomization.

3.2 Subject Exclusion Criteria

1. Any subject unlikely to survive five days
2. Subjects previously enrolled in this study

3.3 Donor Inclusion Criteria

1. Donor screening. All donors will meet the standard blood donor criteria established by the participating local blood center, American Association of Blood Banks (AABB) and FDA regulations.
2. Though long-term toxicity is not expected with the frequent administration of G-CSF, dexamethasone, or hydroxyethyl starch, community donors will be restricted to a maximum of one donation every three days and no more than 8 donations per year in order to limit cumulative exposure to these agents. Family donors will be able to donate at any interval according to local blood bank criteria, with approval from a blood bank physician. Family donors will be limited to 8 donations per year.

Donors will be selected from each participating center's pool of volunteer community blood donors and/or from among the subject's friends or relatives. Infectious disease testing will be done per local blood bank policy and procedures. A product may be administered before infectious disease testing results are known per local blood bank policy. Donor and intended recipient will be red cell compatible. Donors will not be pre-selected on the basis of HLA or granulocyte type. If patient is CMV-negative, only donors who are CMV-negative will be used. CMV serology of the donor will be tested within 30 days prior to the granulocyte donation. Donations from CMV-positive donors will not be transfused to CMV-negative recipients.

3.4 Donor Exclusion Criteria

1. Personal or family history of severe sickle cell disease or variant (unless donor has tested negative). Testing for the presence of Hemoglobin S is not required.
2. Positive infectious disease test as dictated by blood collection center's SOP
3. Current uncontrolled hypertension
4. Diabetes mellitus
5. Active peptic ulcer disease
6. Pregnant or breast-feeding
7. Currently taking lithium therapy
8. History of autoimmune disease
9. History of coronary disease
10. History of deep vein thrombosis or venous thromboembolism
11. History of iritis or episcleritis.

4 TRIAL ENROLLMENT

4.1 Recruitment/Screening

Subjects will be recruited through the Transfusion Medicine/Hemostasis Clinical Trials Network and the Blood and Marrow Transplant Network sites. Potentially eligible subjects will be identified by the subject's physician. Screening logs will be maintained at each site to track the enrollment status of all patients considered for participation. Each subject will be counseled regarding center-specific options for the treatment of life-threatening infections in neutropenia; these would include (but are not limited to) granulocyte transfusions and other investigational pharmacologic agents. Subjects and donors must meet the eligibility criteria described in 3.1 through 3.4.

Donors will be counseled regarding the risks and hazards of the granulocyte harvest procedure.

Consent for both donor and recipient will be obtained using forms approved by the local institutional IRB.

4.2 Stratification and Randomization

Treatment group will be allocated using randomly permuted blocks within strata [32]. Subjects will be stratified according to risk status (high risk: stem cell transplantation or relapsed leukemia vs. other), and type of infection (invasive mold infection vs. other) in order to ensure balance for these important factors. Treatment allocation will also be balanced within each clinical center using dynamic balancing [32].

Subjects will be randomized with equal allocation to two treatment groups:

1. Standard antimicrobial therapy alone

2. Standard antimicrobial therapy plus daily G-CSF/dexamethasone-mobilized granulocyte transfusion therapy

In order to facilitate the administration of study granulocytes at the time of infection in the neutropenic period following transplantation and/or chemotherapy, consent may be obtained prior to transplantation.

5 INTERVENTION

5.1 Infection Documentation

Subjects will have infection documented before enrollment, confirmed as fungemia, bacteremia, invasive tissue bacterial infection, or proven/probable invasive tissue fungal infection by culture, histopathologic, and radiologic criteria as defined in Appendix A.

5.2 Granulocyte Procurement Procedure

G-CSF and dexamethasone administration – G-CSF, 480µg subcutaneously, and dexamethasone, 8mg orally, will be administered 8-16 hours prior to each granulocyte donation, as close to 12 hours prior to donation as is practically possible.

Venous access – peripheral venous access will be used for donors.

Granulocyte collection – granulocytes will be collected by continuous-flow centrifugation. This procedure separates the granulocytes continuously on the basis of differential sedimentation in a centrifuge. Blood will be processed using high molecular weight hydroxyethyl starch (6% Hetastarch, 1/12 starch/blood ratio). For recipients weighing at least 30 kg, seven to ten liters of blood will be processed. Recipients weighing less than 30 kg will receive a proportionally reduced granulocyte dose. Dose reduction may be accomplished by either proportionally reducing the amount of donor blood processed or by transfusion of a proportion of the collected cells. For example, if a recipient weighs 15 kg, either:

- 3.5 to 5 L of blood will be processed and the entire collection will be transfused; OR
- 7 to 10 L of blood will be processed and half of the collection will be transfused.

Granulocyte yield – the number of granulocytes in each granulocyte unit will be determined, with the goal of $\geq 4 \times 10^{10}$ per collection (or proportionately less for subjects under 30 kg).

Gamma irradiation – all granulocyte units will be exposed to 2,500 cGy of irradiation to the central plane of the bag with a minimum of 1,500 cGy to any other portion of the bag prior to infusion.

Time to transfusion – all granulocyte units must be available for transfusion as soon as possible. Every attempt will be made to begin transfusion within 6 hours from the completion of collection. A 6-24 hour interval between collection and transfusion is less desirable, but acceptable. Greater than 24-hour interval between collection and transfusion will be considered a protocol violation. All units will be stored at room temperature (20-24°C) without constant agitation.

Cost recovery – the cost of granulocyte procurement, including the cost of the leukapheresis itself as well as the cost of activities required by the study in relation to granulocyte procurement, will be charged to the subject on a cost-recovery basis.

5.3 Administration of Granulocytes

The entire granulocyte product will be infused through a standard blood administration set with a standard blood filter (150 to 280 microns). Leukoreduction filters are not to be used for granulocyte administration. Subjects under 30 kg will receive a maximum of 20 mL/kg. Transfusions will be infused over 1-2 hours as soon as possible (preferably within 6 hours but no longer than 24 hours) after collection. No other blood products will be given during the granulocyte transfusion. Transfusions will be staggered from the administration of any amphotericin B infusion by a minimum of 2 hours. Over a subject's transfusion course, granulocytes will be provided daily if at all possible. Days without granulocyte infusions will be permitted. Responses and subsequent relapses with the same organism will be considered variations of the same infection and success judged on the status at 42 days after randomization.

If granulocyte transfusions have been discontinued under Section 5.5 (below) and infection with the same organism recurs or worsens, granulocyte transfusion should be reinstituted if neutrophil recovery has not occurred. If infection with a different organism occurs after discontinuation of granulocyte transfusions and neutrophil recovery has not occurred, transfusions may be restarted at the discretion of the subject's physician.

Subjects in the control arm should not receive granulocyte transfusions in the 42 days after randomization; if they do receive granulocytes, it will be considered a protocol violation, but they will be included in the primary intent-to-treat analysis as a control subject. They will be excluded in secondary analysis of the primary outcome (see Section 8.2).

5.4 Antimicrobial and Adjunctive Measures during the Study Period

Antimicrobial therapy is broadly defined as therapy within the standard of care for a particular infection. Recommended therapy for specific infections such as invasive mold or candidal disease is noted in Appendix A. Antimicrobial strategies should be consistent within a given institution. Central line removal is recommended for subjects with 2 or more positive blood cultures of *Candida spp*. or *Fusarium spp*. Growth factors (G-CSF) may be administered at the discretion of the attending physician, but should be discontinued if the ANC exceeds 2500/μL for two consecutive days. Data on growth factor use in both treatment groups will be collected by the study. Investigational antimicrobial agents are permitted during the course of the study period, but must be recorded as such.

5.5 Discontinuation of Granulocyte Therapy

Granulocyte transfusions will be given every day to those randomized to the granulocyte arm (when possible), until one of the following conditions is met:

Recovery from neutropenia – if two successive next-morning ANCs rise to ≥ 3000 cells/μL, the subsequent (third) day's granulocyte transfusion will be held to determine whether recovery from neutropenia is occurring; the prospective granulocyte donor will thus not be primed the previous evening. Recovery will be defined as the first of two consecutive days with an ANC > 1000 cells/μL without granulocyte support.

Life-threatening toxicity – in the case of life-threatening toxicity, granulocytes will be discontinued immediately and will not be restarted. Such toxicity is expected to occur most often as a serious pulmonary reaction.

Resolution or improvement of underlying infection – at the discretion of the treating physician, but only if the subject has received at least five granulocyte transfusions over a minimum of seven days, granulocyte transfusions may be discontinued prior to recovery from neutropenia and in the absence of toxicity if complete or partial response has occurred. In this circumstance, transfusions should be re-instituted if infection with the same organism recurs or worsens, provided that ANC recovery has not yet occurred.

6 MEASUREMENT AND ACTIVITIES

6.1 Schedule of Subject Measurement

Subjects will be followed until 3 months after randomization or until death, whichever comes first. Subjects will be studied during only one episode of neutropenia.

Following is a list of the data that will be collected while the subject is on this study. Table 1 outlines the schedule of measurement.

All subjects, prior to randomization (baseline):

1. Inclusion/Exclusion criteria
   - Infection diagnosis documentation and treatment (refer to Appendix A)
   - Duration of pre-existing neutropenia
   - Documentation of dose-intensive chemotherapy or hematopoietic stem cell transplantation (must be within 60 days prior to enrollment)
   - Likeliness of 5 days survival
   - Likeliness of remaining neutropenic for 5 days.
2. Demographic and medical history
   - Weight
   - Height
   - Date of birth
   - Gender
   - Ethnic origin
   - Race
3. Signs and symptoms documentation
4. Laboratory
   - CBC with differential

All subjects, within 24 hours after randomization, but before granulocyte transfusion (if any):

1. Collections for Central Laboratory
   - Serum for HLA and granulocyte-specific antibodies
   - Serum for fungal antigens (only applicable for subjects with a proven or probable Aspergillus infection as the study qualifying infection)

All subjects, daily while hospitalized up to day 42:

1. Assessment
   - Maximum temperature

All subjects, daily until three days after engraftment takes place and then weekly until day 42:

1. Laboratory
   - Morning CBC with differential

Subjects with bloodstream infection, daily until two consecutive negative tests:

1. Laboratory
   - Blood culture

Each day of granulocyte transfusion:

1. Laboratory
   - CBC and differential within 4 hours prior to transfusion
   - CBC and differential 30 minutes to 2 hours post transfusion
2. Medication

- Medications given specifically in preparation for pending transfusion

3. Assessment
   - Vital signs within 15 minutes prior to the start of the transfusion
   - Vital signs within 15 minutes after the start of the transfusion
   - Vital signs once, 45 minutes to 1 hour and 15 minutes after the end of the transfusion
   - Vital signs as needed subsequently
   - Oxygen saturation 15 minutes prior to the start of the transfusion
   - Oxygen saturation 15 minutes after the start of the transfusion
   - Oxygen saturation once, 45 minutes to 1 hour and 15 minutes after the end of the transfusion
   - Oxygen saturation as needed subsequently

All subjects, 14 and 42 days after randomization (± 2 days)

1. Collections for Central Laboratory
   - Serum for HLA and granulocyte-specific antibodies

All subjects, 14, 28, and 42 days after randomization (± 2 days)

1. Signs and symptoms documentation

Subjects with confirmed or suspected study qualifying infection of aspergillosis, 7, 14, and 42 days after randomization (± 2 days)

1. Collections for Central Laboratory
   - Serum for fungal antigens

All subjects, as clinically indicated until 42 days after randomization

1. Serial studies documenting invasive infection
2. G-CSF administration
3. Treatments for infection

All subjects, once, 40 to 45 days after randomization

1. Studies documenting invasive infection

Patients with blood stream infections, once, 40 to 45 days after randomization

1. Laboratory
   - Blood culture

All subjects, 3 months after randomization

1. Vital Status

Table 1: Subject measurements

	Prior to randomization	Within 24 hrs after randomization, but before granulocyte transfusion (if any)	Within 24 hrs after randomization, but before granulocyte transfusion (if any), (only applicable for subjects with a proven or probable Aspergillus infection)	Within 48 hrs after randomization	Daily while hospitalized up to day 42	Daily until three days after engraftment, then weekly until day 42	Daily until two consecutive negative tests	Each day of granulocyte transfusion	Day 14, 42 (± 2 days)	Day 14, 28, 42 (± 2 days)	Day 7, 14, 42 (± 2 days)	As clinically indicated until day 42	Once, days 40 to 45	3 months after randomization
All subjects														
Consent	X													
Demographics	X													
Medical history	X													
Maximum daily temperature					X									
Signs and symptoms assessment		X								X				
Serum for HLA and granulocyte-specific antibodies		X							X					
Serum for fungal antigen			X								X			
Studies documenting invasive infection												X	X	
CBC with differential	X													
Morning CBC with differential						X								
Blood culture, if bloodstream infection							X						X	
Vital Status														X
G-CSF use												X		
Treatments for infection												X		
For each granulocyte transfusion														
1st Granulocyte transfusion (GT)				X										
CBC and differential within 4 hours pre-GT								X						
CBC and differential 30 min to 2 hours post-GT								X						
Medications given specifically in preparation for pending transfusion								X						
Vital signs w/i 15 minutes prior to the start of GT								X						
Vital signs w/i 15 minutes after the start of GT								X						
Vital signs 1 hour ± 15 min after the end of GT								X						
Vital signs as needed thereafter								X						
O_2 Saturation 15 minutes prior to the start of GT								X						
O_2 Saturation 15 minutes after the start of GT								X						
O_2 Saturation 1 hour ± 15 min after the end of GT								X						
O_2 Saturation as needed thereafter								X						

Schedule of Donor-Related Activities

Donors will be followed until immediately after donation. Donors will be studied during each donation.

Following is a list of donor data to be collected. Table 2 outlines the schedule of donor-related activities.

Prior to donor enrollment in trial

1. Medical history
   - Family history of sickle cell disease or variant
   - Donor history of sickle cell disease or variant
   - Blood pressure
   - Diabetes mellitus history
   - Peptic ulcer disease history
   - Lithium therapy history
   - Autoimmune disease history
   - Coronary disease history
   - Deep vein thrombosis or venous thromboembolism history
   - Iritis or episcleritis history
2. Laboratory
   - As required by SOP of blood center

12 ± 4 hours prior to leukapheresis

1. G-CSF and dexamethasone administration
2. Data collection
   - Date and time of G-CSF and dexamethasone administration

Prior to leukapheresis

1. Laboratory
   - CBC with differential
2. Data collection
   - Start date and time of leukapheresis

Post leukapheresis

1. Laboratory
   - Product volume
   - Product CBC and differential
2. Data collection
   - Stop date and time of leukapheresis
   - Adverse Events recorded as reported to local blood center
   - Donor will be given instructions regarding safety follow-up

Table 2: Donor-related activity schedule

	Prior to enrollment	12 ± 4 hours pre-leukapheresis	Immediately pre-leukapheresis	Immediately post-leukapheresis
Consent	X			
Medical history	X			
Laboratory per local SOP	X			
G-CSF/dexamethasone administration		X		
CBC with differential			X	
Start and stop date and time of leukapheresis			X	X
Instructions on possible adverse effects				X
Product volume				X
Product CBC with differential				X

6.2 Measurement Procedures

Protocol-specified procedures will depend on infection type at enrollment, as noted below:

Bloodstream infections – blood cultures will be obtained at a minimum once every 24 hours during the study period (regardless of body temperature) for all subjects entered on study due to fungemia or bacteremia until two consecutive blood cultures remain negative after 72 hours of incubation, in order to determine time to negative test for bloodstream infection.

Invasive tissue fungal or bacterial infections – for subjects with tissue infections, repeat site-directed tissue biopsies or diagnostic procedure such as BAL will be performed only as clinically indicated. For radiographically determined infections (typhlitis, pneumonitis, necrotizing fasciitis, etc), follow-up radiography (e.g., CT scan or other appropriate radiographic technique) will be performed as clinically indicated during the study and again at 40-45 days after randomization to determine response.

Vital signs - case report forms will be completed to capture maximum daily temperature for all subjects. Vital signs will be also be recorded within 15 minutes prior to the start of each granulocyte transfusion, 15 minutes after the start of transfusion, and at 1 hour after transfusion of granulocytes (or more frequently as clinically indicated).

Oxygen saturation – granulocyte recipients will be monitored with pulse oximetry within 15 minutes prior to the start of the granulocyte transfusion, 15 minutes

after the start of transfusion, and at 1 hour after transfusion of granulocytes, or more frequently as clinically indicated. A 10% decline in O_2 saturation for 5 minutes during the granulocyte infusion will be grounds for suspending the infusion, which can be resumed at 1/2 the rate when the O_2 saturation rises to baseline levels or > 90%. A decline of > 15% in O_2 saturation within 4 hours of the start of the transfusion that cannot be ascribed to causes other than the transfusions will be defined as pulmonary toxicity, and will be grounds for discontinuation of all further transfusions in that subject. Because the co-administration of amphotericin B products and granulocytes may be associated with pulmonary reactions, the administration of amphotericin products will be separated from the transfusions by a minimum of 2 hours.

6.3 Specimen Collecting Procedures

Hematology – all subjects will have daily CBC and differential until three days after engraftment and then at least weekly for the remainder of the 42-day study period. Engraftment is defined for the control subjects as the first of two consecutive days when the subject's absolute neutrophil count exceeds 1000 cells/μL. For subjects in the treatment arm, engraftment is defined as the first of two consecutive days with an ANC > 1000 cells/μL without granulocyte support.

Subjects receiving granulocytes will have CBC and differentials determined for every day of transfusion: pre-infusion, 30 minutes to 2 hours after the infusion.

Antibodies – subjects in both arms of the study will have 5 mL of blood collected at randomization (baseline) and at 14 and 42 days after randomization. From each of these samples, serum (2 mL) will be frozen. With the exception of the collection at baseline, samples may be collected up to 2 days before and until 2 days after these time points. For example, the sample for 14 days after randomization may be drawn from 12 to 16 days after randomization. Testing for HLA antibodies and granulocyte-specific antibodies will be performed on these samples retrospectively.

Serum for fungal antigens – all subjects in both arms of the study with confirmed or suspected study qualifying infection of aspergillosis will have 5 mL of blood collected in an EDTA tube at baseline, and at days 7, 14, and 42 after randomization (± 2 days). From each of these samples, serum will be obtained for testing for fungal antigenemia (e.g. galactomannan antigen). The amount of serum obtained shall be the minimum required for the test. A central lab will test all baseline samples for the presence of circulating fungal antigenemia. Those subjects with fungal antigenemia present at baseline will have their remaining samples tested to determine the time to clearance. The non-baseline samples for subjects that do not have fungal antigenemia present at baseline will be destroyed.

6.4 Adjudication Procedures

A blinded adjudication panel will be convened at the interim efficacy analysis and at the conclusion of the trial. The panel performing the study adjudication, consisting of three infectious disease specialists and one radiologist, will not be informed of the subject's treatment group. Adjudication will address the following three items:

a. Whether the subject was actually eligible for the study
b. The appropriateness of the antimicrobial therapy administered, including any surgical therapy
c. Whether or not there was a response

Adjudication findings will be recorded on the appropriate case report form. These decisions will be based on clinical summaries, laboratory results, cultures, reports of imaging studies (X-rays, CT, MRI), and data from the standard case report forms.

7 ADVERSE EVENT CRITERIA AND REPORTING

Reporting of all adverse events will follow the standard TMH CTN procedures described in the TMH Manual of Procedures (MOP), Chapter 6: Guidelines for Reporting Adverse Events. Reporting requirements are calibrated to the seriousness of the event and the perceived relationship to the study drug/device/treatment(s) (granulocyte transfusion, G-CSF, dexamethasone or granulocyte collection procedure). For this study the reporting requirements will be based on the type and severity of adverse event.

The TMH CTN will be using the descriptive terminology developed by the National Cancer Institute for use in reporting adverse events: Common Terminology Criteria for Adverse Events (CTC) version 3.0 dated December 12, 2003. The CTC includes a grading (severity) scale for each adverse event term. Grades were developed using the following guidelines:

Grade 0 - No adverse event or within normal limits
Grade 1 - Mild adverse event
Grade 2 - Moderate adverse event
Grade 3 - Severe adverse event
Grade 4 - Life threatening or disabling adverse event
Grade 5 - Death related to adverse event

In general, investigators should report adverse events as diseases or syndromes whenever possible, instead of reporting individual component symptoms, signs, laboratory abnormalities, and sequelae.

7.1 Definitions

Adverse Event (AE) – any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether or not it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite).

Life-threatening Adverse Event – any adverse event that, in the opinion of the investigator, places the neutropenic subject or the granulocyte donor at immediate risk of death.

Serious Adverse Event (SAE) – any adverse event that results in any of the following outcomes: death, a life-threatening event, inpatient hospitalization or prolongation of existing hospitalization, congenital anomaly/birth defect, and/or a persistent or significant disability/incapacity.

Unexpected Adverse Event – any adverse event attributed to be possibly, probably, or definitively related to one of the following: granulocyte transfusion, G-CSF, dexamethasone, or the granulocyte collection procedure; the specificity or severity of which is NOT listed in the study protocol, product inserts, or informed consent document.

Attribution – the determination of whether an adverse event is related to a medical treatment procedure.

Attribution categories:

1. *Definite* – the adverse event is clearly related to the study drug/device/treatment(s).
2. *Probable* – the adverse event is likely related to the study drug/device/treatment(s). The adverse effect is not likely to be caused by the subject's underlying medical condition or other concomitant therapy, and the nature of the adverse event or the temporal relationship between the onset of the adverse event and study drug/device/treatment(s) administration lead the investigator to believe that there is a reasonable chance of causal relationship.
3. *Possible* – the adverse event may be related to the study drug/device/treatment(s). The adverse event could be attributed to the subject's underlying medical condition or other concomitant therapy, but the nature of the adverse event or the temporal relationship between the onset of the adverse event and study drug/device/treatment(s) administration lead the investigator to believe that there could be a causal relationship.
4. *Unlikely* – the adverse event is doubtfully related to the study drug/device/treatment(s).
5. *Unrelated* – the adverse event is clearly NOT related to the study drug/device/treatment(s). The adverse event is most plausibly explained by the subject's underlying medical condition or other concomitant therapy.

7.2 Adverse Events in Subjects

Information about the following adverse events that occur in subjects will be collected:

1) Serious adverse events, regardless of attributed relationship to granulocyte transfusion
2) Any unexpected adverse event (all grades), attributed as possibly, probably or definitely related to granulocyte transfusion
3) Granulocyte Transfusion Events

7.2.1 Expedited Reporting of Adverse Events in Subjects

Serious adverse events and all unexpected adverse events will be reported to the Data Coordinating Center (DCC) within 24 hours of occurrence by phone or email. Such adverse events will be recorded on a Serious/Unexpected Adverse Event form and faxed to the DCC within 48 hours of the occurrence. A summary of the event will be reported within 10 days of occurrence. Data on serious and unexpected adverse events in subjects will be collected through end of study.

7.2.2 Regular Reporting of Adverse Events in Subjects

Adverse events of all grades that occur during the transfusion of granulocytes or within six hours after the end of a granulocyte transfusion will be monitored by the DCC for all subjects who receive granulocyte transfusions, using data reported on the Granulocyte Transfusion Event form. These events include: allergic reaction/hypersensitivity, sinus bradycardia, sinus tachycardia, hypertension, hypotension, dyspnea, hypoxia, wheezing, cough, hemolysis, fever, infection, and rigors/chills. Sites will data-enter Granulocyte Transfusion Event forms within two weeks of the occurrence of the adverse event. Serious granulocyte transfusion events and unexpected granulocyte transfusion events are also reported within 24 hours as described in Section 7.2.1.

7.2.3 Monitoring of Serious and Unexpected Adverse Events in Subjects

Serious adverse events and all unexpected adverse events must be reported to the Data Coordinating Center (DCC) in an expedited manner, irrespective of the attribution of the event to the study drug/device/procedure/treatment.

Serious adverse events and all unexpected adverse events will be promptly reviewed by the Medical Monitor at, or associated with, the DCC. The Medical Monitor has medical expertise relevant to the study protocol and may request the subject's treatment assignment when reviewing the adverse event.

The Medical Monitor or DCC representative is responsible for notifying the NHLBI Project Officer of all serious and unexpected adverse events, and of any concerns regarding the frequency or type of adverse event(s) on a study or study treatment arm. The NHLBI Project Officer will be notified immediately if an event is determined to be possibly, probably, or

definitively related to a granulocyte transfusion; additionally, information about the adverse event will be included in the monthly summary report. If an event is determined to be unrelated or unlikely related to a granulocyte transfusion, it will be included in the monthly summary report (see Figure 2).

The NHLBI Project Officer (or designee) is responsible for reviewing the adverse event materials to determine if the materials are complete. If there are any concerns regarding the type or frequency of the event, the NHLBI Project Officer will request that the DSMB Executive Secretary notify the DSMB Chair. The DSMB Chair will review the adverse event materials, determine if the information is complete, determine if additional DSMB review is required, and make recommendations to the NHLBI concerning continuation of the study. Full documentation of the procedures will be available at the DCC.

The DCC will prepare monthly summary reports of all serious and unexpected adverse events for the NHLBI Project Officer.

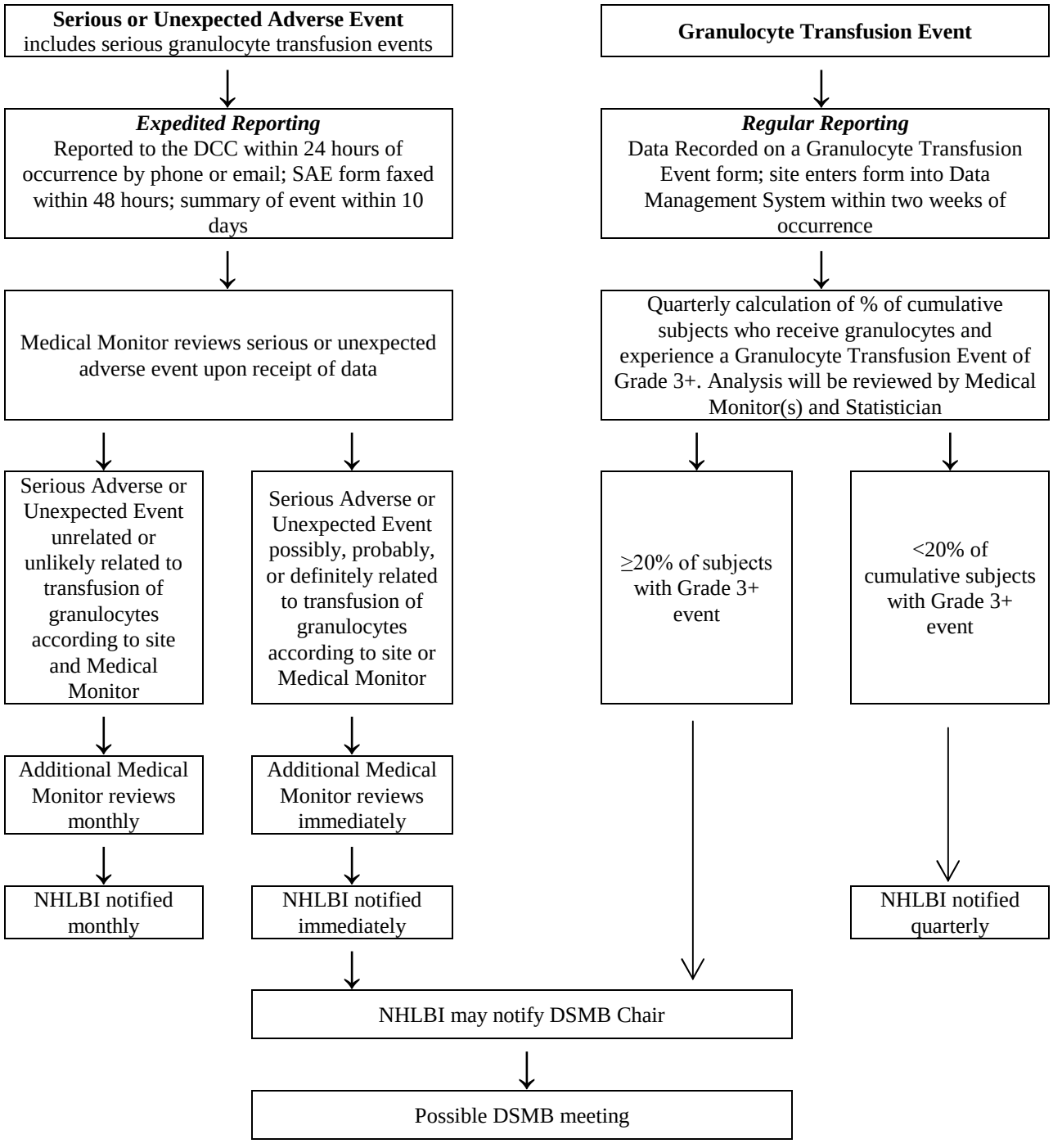
7.2.4 Monitoring of Adverse Events Associated with Granulocyte Transfusions

Adverse events of all severity levels that occur during the transfusion of granulocytes or within six hours after the end of a granulocyte transfusion (e.g. events related to allergic reaction/hypersensitivity, sinus bradycardia, sinus tachycardia, hypertension, hypotension, dyspnea, hypoxia, wheezing, cough, hemolysis, fever, infection, and rigors/chills) will be reported on Granulocyte Transfusion Event forms, which will be data-entered within two weeks of the event's occurrence. Serious and unexpected granulocyte transfusion events are also reported within 24 hours, and monitored as described in Section 7.2.1.

The cumulative frequency of granulocyte transfusion events of Grade 3 or higher will be determined each quarter by the DCC. The DCC Protocol Statistician will calculate the percentage of subjects with at least one granulocyte transfusion event of Grade 3 or higher among those subjects who receive granulocyte transfusions. The Protocol Statistician and Medical Monitors will meet to review and discuss the results of this analysis. Granulocyte transfusion events of Grade 3 or higher are expected to be rare. In this subject population, approximately 5% of subjects receiving granulocytes would be expected to have at least one such event. If 20% of subjects receiving granulocytes experience a Grade 3 or higher transfusion event, the DCC will notify the NHLBI Project Officer, who will request that the DSMB Executive Secretary contact the DSMB chair (see Figure 2). This monitoring plan is designed to identify a possible

excess of transfusion events; it is not intended to serve as a formal stopping rule for this study.

Figure 2: Adverse Event Monitoring Plan for Subjects



7.3 Adverse Events in Donors

Information about serious adverse events, regardless of relationship to G-CSF, dexamethasone, or granulocyte collected procedure, that occur in donors will be collected. All known serious adverse events that occur from immediately after G-CSF and dexamethasone administration to one week after administration will be reported.

Figure 3 summarizes the donor monitoring in this study.

7.3.1 Expedited Reporting of Adverse Events in Donors

Serious adverse events will be reported to the DCC within 24 hours of occurrence by phone or email. Such adverse events will be recorded on a Serious/Unexpected Adverse Event form and faxed to the DCC within 48 hours of the occurrence. A summary of the event will be reported within 10 days of occurrence.

7.3.2 Monitoring of Serious Adverse Events in Donors

Serious adverse events must be reported to the Data Coordinating Center (DCC) in an expedited manner, irrespective of the attribution of the event to the study drug/device/procedure/treatment.

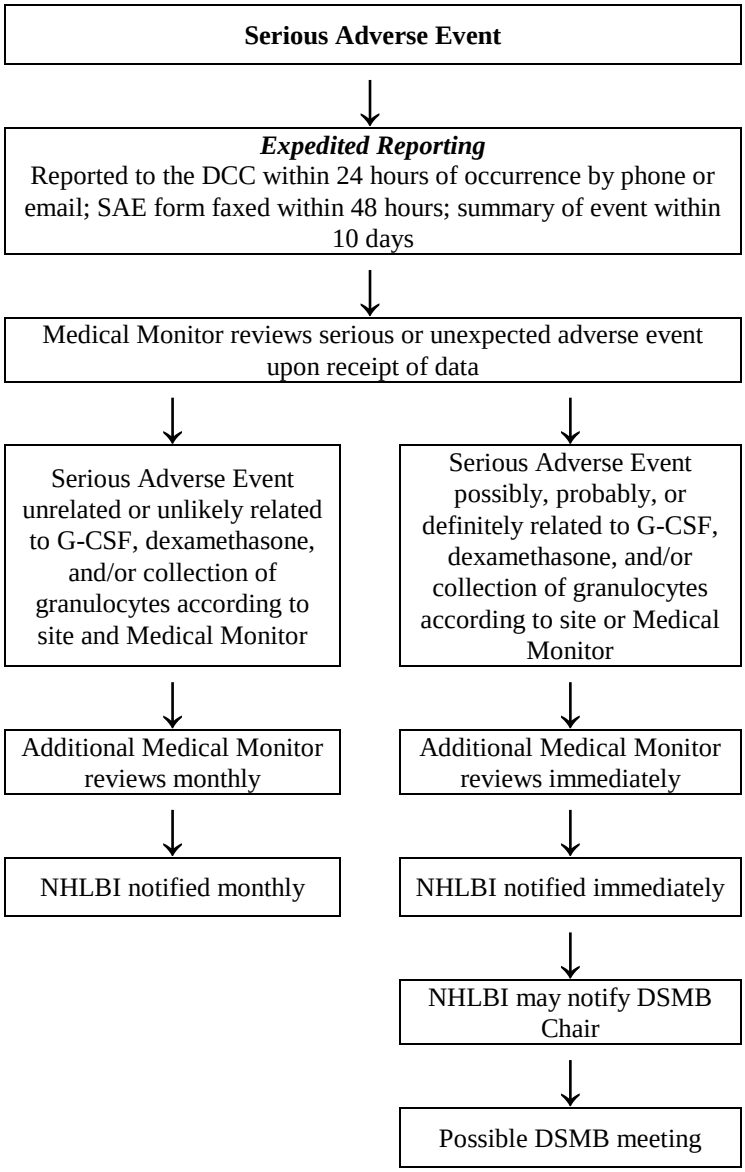
Serious adverse events will be promptly reviewed by the Medical Monitor at, or associated with, the DCC. The Medical Monitor has medical expertise relevant to the study protocol.

The Medical Monitor or DCC representative is responsible for notifying the NHLBI Project Officer of all serious adverse events, and of any concerns regarding the frequency or type of adverse event(s) on a study. The NHLBI Project Officer will be notified immediately if an event is determined to be possibly, probably, or definitively related to a granulocyte donation; additionally, information about the adverse event will be included in the monthly summary report. If an event is determined to be unrelated or unlikely related to a granulocyte donation, it will be included in the monthly summary report (see Figure 3).

The NHLBI Project Officer (or designee) is responsible for reviewing the adverse event materials to determine if the materials are complete. If there are any concerns regarding the type or frequency of the event, the NHLBI Project Officer will request that the DSMB Executive Secretary notify the DSMB Chair. The DSMB Chair will review the adverse event materials, determine if the information is complete, determine if additional DSMB review is required, and make recommendations to the NHLBI concerning continuation of the study. Full documentation of the procedures will be available at the DCC. In the event of a donor death, study accrual will be suspended immediately, pending review by the DSMB.

The DCC will prepare monthly summary reports of all serious adverse events for the NHLBI Project Officer.

Figure 3: Adverse Event Monitoring Plan for Donors



7.4 Data Collection and Validation

Data will be collected and entered into a web-based data management system (DMS) at each site participating in the TMH Granulocyte Study, and transferred electronically to the Data Coordinating Center. The DMS is programmed to validate all data entry fields as the data are entered. Validations are question-by-question checks that give immediate feedback to help catch data entry errors, form completion errors, and out-of-range values. Reports of outstanding edits, generated upon completion of data entry, will enable continuous cleaning of data at each site.

The DCC will regularly monitor all data for consistency and correctness. If the DCC observes inconsistent data or patterns of protocol violations or missing data, site staff will be contacted immediately to address the finding.

Data Collection Instruments – study personnel at each site will enter data from source documents corresponding to a subject's visit onto case report forms. All information contained in the case report form will be entered into the study database.

Confidentiality – each subject and donor is assigned a unique number to assure confidentiality. Any publication or presentation will refer to subjects and donors by this number and not by name. The medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents. Subject research files will be kept in a locked room.

Data Management – NERI will serve as the trial coordinator for this study. NERI will monitor timely entry of data into the study database. Access to all source documentation maintained by the Investigator, including correspondence and source data, will be available for monitoring and audit purposes.

Data archives – at all times, appropriate backup copies of the database and related software files will be maintained and the information will be appropriately protected from illegitimate access.

7.5 Expected Adverse Events in Subjects

Certain adverse events are common and expected in the setting of chemotherapy and/or HSC transplantation; however, these toxicities are generally not likely to be due to the administration of the granulocyte product and will not undergo reporting as an adverse event. Toxicities that will not undergo adverse event reporting include:

- Fatigue
- Fever
- Anorexia (as defined under "Gastrointestinal")
- Mucositis

- Hypokalemia
- Anemia, leukopenia, thrombocytopenia

Recipients of marrow or PBSC transplants incur risks from pre-transplant conditioning, the graft itself, and post-transplant therapies. Major risks following transplantation include:

- Damage of any major organ may occur as a result of cumulative toxicity from anti-neoplastic therapy, the conditioning regimen, drug toxicity, infection, or GVHD.
- Graft failure can result from genetic disparity between donor and recipient,
- GVHD can be either acute or chronic; both types predispose to infection.
- Life-threatening infections may develop in subjects with and without GVHD. These can be of a bacterial, viral, parasitic, or fungal nature.
- Relapse of the underlying disease may occur, especially in subjects with far advanced disease status at time of transplant.

All of these toxicities may be severe enough to result in death.

Subjects undergoing aggressive chemotherapy incur the following major risks:

- Damage of any major organ may occur as a result of cumulative toxicity from anti-neoplastic therapy, drug therapy, or infection.
- Life threatening infections may develop. These may be bacterial, viral, parasitic, or fungal.
- Relapse of the underlying disease may occur.

All of these toxicities may be severe enough to result in death.

Subjects receiving granulocyte transfusions incur the following major risks:

- Anaphylaxis
- Acute lung injury
- Transmission of infectious disease

All of these toxicities may be severe enough to result in death.

In addition to the adverse events listed above, Appendix C lists events that are considered expected in the setting of chemotherapy and/or HSC transplantation, and will not be reported. However, if any event meets the definition of a serious adverse event (see Section 7.1), it must be reported as specified in Section 7.2.1.

7.6 Expected Adverse Events in Donors

Donors undergoing G-CSF/dexamethasone stimulated leukapheresis procedures incur the following major (although extremely rare) risks:

- Splenic rupture
- Moderate/severe rash
- Persistent moderate/severe thrombocytopenia
- Severe aching, bone pain, etc that requires further medical attention
- Sickle crisis

- Thromboembolic event
- Coronary event
- Exacerbation of underlying inflammatory/autoimmune disorder

Several of these toxicities may be severe enough to result in death.

Risks Related To Dexamethasone – The side effects of dexamethasone may include insomnia, an increase in appetite, euphoria, depression, fluid accumulation, and weight gain. Corticosteroids have been reported to cause cataracts in the eyes when given to patients for treatment of disease. Whether this is a risk for healthy granulocyte donors is unknown. Two studies of granulocyte donors showed no definite increase in cataract risk, but the trends did suggest a possible relationship. Cataracts in these studies were tiny, did not impair vision, and were detected only by special eye examinations. Because there is no established risk of cataracts in granulocyte donors, no general recommendations can be made.

Risks related to hydroxyethyl starch – The side effects of hydroxyethyl starch may include allergic reaction, fluid accumulation, and weight gain.

7.7 Reporting and Management

Study participants will be receiving potentially toxic preparative therapy, and significant regimen-related toxicity is anticipated. Study Case Report Forms (CRFs) are designed to capture information on these adverse events. Likewise, substantial mortality is anticipated and will be captured via filing of appropriate CRFs. All unexpected adverse events will be reported for the duration of the study.

7.7.1 Interim Reporting

This section describes scheduled reports that will be sent to the study sites and the DSMB. Reporting requirements for events that will be monitored continuously (e.g. unexpected serious adverse events) are described above.

7.7.1.1 Monthly Reports to Sites

Reports of accrual information, outstanding queries, and protocol violations will be distributed to the sites monthly

7.7.1.2 Quarterly DSMB Reports

The DSMB will receive quarterly updates on:

- Site status;
- Accrual overall and by site;
- Study compliance issues; and
- The status of adverse event monitoring:
  - ♦ The cumulative percentage of subjects in the granulocyte arm with at least one Grade 3+ transfusion-related event, if the cumulative percentage is $\geq$20%;

- ♦ The types and frequencies of Grade 3+ transfusion-related events, if the cumulative percentage of subjects with such events is ≥20%;
- ♦ All reported adverse events in donors
- ♦ Whether the continuous review or monthly discussion raised issues that were reported to NHLBI as being of possible concern; and
- ♦ Any actions taken in response to those concerns

7.7.1.3 Semi-Annual DSMB Reports

The DSMB will meet every 6 months, either in-person or via teleconference. Reports will include:

- Baseline characteristics overall and by treatment arm;
- Serious adverse events overall and by treatment arm;
- Transfusion related events of all severity levels for subjects randomized to granulocyte transfusions;
- Site status;
- Accrual overall and by site; and
- Study compliance issues.

7.7.1.4 Other DSMB Reports

The DSMB will receive the results of a formal interim analysis of the primary endpoint after half the planned subjects have completed 42 days of follow-up (see Section 8.5.1).

The DSMB will receive the results of a formal interim analysis of death rates in the two arms each time the total number of deaths increases by 14 (see Section 8.5.2).

8 STATISTICAL CONSIDERATIONS

8.1 Baseline Characteristics

Demographic and other baseline characteristics of subjects in the two treatment arms will be described.

8.2 Analyses for the Primary Endpoint

The primary evaluation of the primary endpoint will follow the intention-to-treat principle. Therefore, when the data are analyzed, patients who are randomized to receive only antimicrobial therapy but receive some granulocytes will be included in the control group, while those who are randomized to the granulocyte group but who do not receive granulocytes or who receive less than the prescribed regimen of granulocytes will be included in the granulocyte group. The rate of primary endpoints in the two treatment groups will be compared using Fisher's exact test. This test was selected instead of the chi-square test to allow for the possibility that

the number of events at an interim analysis may be too small to justify the assumed large sample properties of the chi square test.

A secondary analysis will be restricted to those subjects who meet both of the following criteria: 1) survive 3 days after randomization and 2) are retrospectively determined by the adjudication panel to have been eligible for enrollment. In this analysis we will explore the relationship between the granulocyte dose delivered and survival.

A secondary, per-protocol, analysis will also be conducted that will include only those subjects who receive their assigned treatments as described in the protocol. This analysis will exclude

- Subjects who are randomized to only receive antimicrobial therapy but receive one or more granulocyte transfusions in the 42 days after randomization;
- Subjects who are randomized to receive granulocyte transfusions, but refuse to receive them. (Subjects who are randomized to receive granulocyte transfusions but do not receive them, or have them discontinued, for reasons specified in the protocol will be included, i.e. patients who experience recovery from neutropenia, life-threatening toxicity, resolution or improvement of infection, unavailability of granulocytes, or death before granulocyte transfusion can occur); and
- Subjects who did not receive appropriate organism-directed antimicrobial therapy, as determined by the adjudication committee. (See Section 6.5)

If there are baseline characteristics associated with the primary endpoint then another secondary analysis will be conducted using exact logistic regression to adjust for the differences in distribution of the baseline traits.

8.3 Analyses for Secondary Endpoints

8.3.1 Safety of granulocyte transfusions

8.3.1.1 Alloimmunization

Alloantibody profiles (anti-HLA and anti-neutrophil) will be obtained from each subject at randomization and after 14 and 42 days on study (± 2 days). Each subject will be scored for the emergence of new alloantibodies by comparing the profiles at the start and end of each of the two intervals defined by these time points. The scores will be treated as binary variables (presence/absence of new antibodies during an interval). A subject may develop new alloantibodies during multiple intervals, but the major issue of interest is whether the subject developed any new alloantibodies at any time during the follow-up period. Therefore, the analysis will focus on time to first new alloantibody. Treatment arms will be compared using the approach developed by Gray [33], which accounts for competing risks. In

this case, the competing risk is death. A routine for the cumulative incidence function and Gray's test is currently available in the R library. The approach developed by Hudgens et al. [35] will be employed to take account of the interval censoring inherent in the scheduled observations.

8.3.1.2 Serious transfusion reactions

This safety endpoint applies only to subjects who receive granulocyte transfusions. Serious transfusion reactions that are attributed to granulocyte transfusions will be tabulated and the frequency distribution of the number of serious transfusion reactions per subject will be derived. Analyses will be done to assess whether the reactions are randomly distributed among subjects and transfusions. This analysis will be adjusted for the number of transfusions each subject receives.

8.3.1.3 Graft vs. Host Disease

The incidence of GvHD will be compared between treatment arms using the cumulative incidence functions described earlier for alloimmunization. In this case, however, no adjustment for interval censoring will be needed. Graphs of cumulative incidence vs. time, which are similar in form to Kaplan-Meier plots but take account of the competing risk, will also be produced (see Marubini and Valsecchi [34]). The comparison will be limited to subjects with allogeneic stem cell transplantation. Follow-up for this endpoint will be censored at 42 days.

8.3.1.4 Adverse events

The type and number of serious adverse events in each arm will be tabulated, as will the type, number and severity of events related to granulocyte transfusions. The formal interim monitoring plan for safety, however, focuses only on mortality (see Section 8.5.2).

8.3.1.5 Discontinuation of transfusions due to toxicity or intolerance

This safety endpoint applies only to subjects who are randomized to receive granulocyte transfusions. The number of subjects for whom granulocyte transfusions must be discontinued because of toxicity or intolerance will be tabulated. The risk of discontinuation will be described by developing a Kaplan-Meier plot using the number of transfusions, rather than time on study, as the measure of exposure. Subjects will be censored at 42 days, at death, or when transfusion treatment is halted for reasons other than toxicity or intolerance, whichever occurs first.

8.3.2 Efficacy Outcomes

8.3.2.1 Outcome within infection subgroups

The rates of primary endpoints in the two treatment arms will be compared separately for subjects in each of the following subsets.

- All invasive mould infection
- Mould pneumonia
- Mould extrapulmonary
- Mould disseminated (i.e. 2 or more contiguous sites)
- Bacteremia
- Fungemia
- Bacterial tissue infection

Fisher's exact test will be used for the comparisons. These subsets not being mutually exclusive, treatment effects will not be compared among them.

8.3.2.2 Time to resolve fever

Analysis of time to resolution of fever, for subjects with fever at baseline, will follow the methods that will be used for GvHD (Section 8.3.1.3). Fever resolution in the two treatment arms will be compared using Gray's model [33] to account for the competing risk of death. An appropriate routine is currently available in the R library. Graphs of cumulative incidence vs. time will also be produced (Marubini and Valsecchi [34]). Follow-up for this endpoint will be censored at 42 days.

8.3.2.3 Time to clear antigenemia

Tests for antigenemia will be performed at baseline for all subjects with confirmed or suspected aspergillosis as the study qualifying infection. Subjects with fungal antigenemia present at baseline will then have their day 7, 14, and 42 samples tested as well. Time to clear circulating fungal antigenemia will be assessed in subjects with fungal antigenemia present at baseline (e.g. galactomannan antigenemia among subjects with invasive aspergillosis). Clearance times in the two treatment arms will be compared using cumulative incidence functions with adjustment for interval censoring as described above for development of alloantibodies.

8.3.2.4 Time to negative blood culture

Analysis of time to negative blood culture, for subjects with a positive blood culture at baseline, will follow the methods that will be used for GvHD (Section 8.3.1.3). Time to negative blood culture in the two treatment arms will be compared using Gray's model [33] to account for the competing risk of death. An appropriate routine is currently available in the R library. Graphs of cumulative incidence vs. time will also be produced (Marubini and Valsecchi [34]). Follow-up for this endpoint will be censored at 42 days.

8.3.3 Long term survival

Though overall survival often primarily reflects disease status (rather than response to anti-infective therapy), we will compare survival through 3 months after randomization using a Kaplan-Meier plot and a log-rank test.

8.3.4 Donor Endpoints

8.3.4.1 Donor safety

The types and number of adverse events reported by donors will be tabulated. Analyses will be done to assess whether serious adverse events are randomly distributed among donors and donations. If so, the risk per donation of each type of serious adverse event, and of any serious adverse event, will be estimated.

8.3.4.2 Donor availability

Donor availability may be an important consideration in judging the feasibility of a granulocyte transfusion program. Therefore, the proportion of scheduled transfusion days on which granulocytes were available, overall and at each treatment center, will be calculated. The proportion of scheduled transfusion days on which granulocytes were available will also be calculated for each subject and the frequency distribution of these proportions will be derived overall and at each treatment center.

8.3.4.3 Evaluation of granulocyte yield

Descriptive statistics will be calculated for the number of granulocytes collected per donation. If a full donation is not attempted because the intended recipient is under 30 kg, this will be taken into account. For example, if 5 liters of blood are processed rather than 10 liters, because the intended recipient weighs 15 kg, and the total yield of the donation is 4×10^{10}, then the yield will be considered to be 8×10^{10} for purposes of these analyses.

8.4 Sample Size and Power Calculations

Successful outcome for the aggregate control population in the current era (based upon Hubel et al [20] and Nichols et al [25]) is expected to be approximately 50%. Given the expense and potential toxicity of the approach, we have designated a 20% absolute difference in the rate of primary endpoints as the minimum that could be deemed clinically significant (70% success rate). We have designed this study with 80% power to detect this difference (alpha=0.05, two sided), which yields a sample size of 103 subjects per arm. To account for a small percent of misdiagnoses (estimated at 5%) and the effect of interim analysis on the Type I error, we have inflated the sample size to 118 subjects per arm.

8.5 Interim Analysis

8.5.1 Interim Analysis for Efficacy and Futility

Interim monitoring for both efficacy and futility will be employed in this

trial. If there is strong evidence at an interim analysis that subjects in one arm are much more likely to succeed than subjects in the other arm (using the primary outcome as the definition of success) it may be ethical to halt the trial early. Because of the cost and potentially serious adverse effects of granulocyte transfusions, it would also be difficult to justify continuing a trial if there was strong evidence at an interim analysis that the null hypothesis of no treatment effect is very unlikely to be rejected. Efficacy and futility are monitored simultaneously using a single test statistic at each monitoring point.

One interim analysis is planned after 118 subjects (50% accrual) have completed 42 days of follow-up. Lan-DeMets spending functions that approximate O'Brien-Fleming boundaries will be used. The critical p-values for this plan, assuming statistical power of 0.80 and a two-tailed Type I error rate of 0.05, are listed in Table 3. P_{1k} and P_{2k} denote the critical values for efficacy and futility respectively at each look. As is characteristic of O'Brien-Fleming boundaries, stronger evidence (more extreme p-values) are required at the earlier analysis than at the later analysis. At the final analysis, $P_{1K}=P_{2K}$ which means that one hypothesis or the other must be rejected.

Table 3: Critical p-values for rejecting the null and alternate hypotheses at two equally-spaced analyses

Analysis (k)	% of final sample size	P_{1k} (reject H_0)	P_{2k} (reject H_1)
1	50	0.0031	0.7181
2	100	0.0516	0.0516

Adding interim monitoring for efficacy and futility slightly inflates the maximum number of subjects the study must plan for. However, this interim monitoring plan reduces the expected number of subjects that will actually need to be enrolled, because the trial may halt after approximately 118 subjects have been enrolled. (Somewhat more than 118 subjects will probably be enrolled in the study before there are 118 subjects with primary outcome data collected, analyzed, and discussed by the DSMB.)

8.5.2 Interim Analyses for Safety

Expected and unexpected adverse events and serious adverse events will be monitored, with regular reports to NHLBI for consideration by the DSMB, as described in Section 7. Statistical monitoring for safety will be limited to comparisons of death rates through 42 days of follow-up in the two treatment arms. This interval was chosen so that the comparison would focus on the interval during which subjects randomized to granulocyte transfusion would be receiving treatments, on the assumption that any effect (positive or negative) of granulocyte transfusions on the risk of death would likely be realized during this interval. Given the severity of the underlying condition of the subjects in this trial and the expected high

mortality rate in the control arm, an excess of deaths in one treatment arm was considered the only safety concern that would lead to halting the trial. An excess of nonfatal serious adverse events in one arm may be acceptable if that arm also has a lower mortality rate.

The problem of developing a monitoring plan is complicated by the lack of information on which to base reasonable predictions of the magnitude and the direction of a difference in death rates between treatment arms. The infectious disease specialists who provided input to protocol development indicated that 30-40% of subjects in the control arm would be expected to die within 42 days. The death rate that should be expected in subjects randomized to granulocytes is unclear. Serious adverse reactions to granulocytes could result in an increase in the death rate. On the other hand, if granulocyte transfusions promote clearance of infections, then the death rate in the transfusion arm could be lower than the death rate in the control arm.

The following monitoring plan is proposed. Rather than comparing death rates at specific time points, analyses will take place after specified total numbers of deaths have occurred. An interim analysis will be performed after each increment of 14 total deaths in the study (e.g. after 14, 28, 42, etc. total deaths). Fisher's exact test will be employed to compare the proportions dying in the two arms through 42 days of follow-up. This test was chosen because the number of events at the earliest interim analyses is unlikely to be large enough to justify the assumptions underlying approaches, such as the chi-square test, that depend on large sample properties. Two-tailed tests will be used because the expected direction of the difference in death rates is unknown. A flat p-value boundary of 0.02 will be used at each test. NHLBI will be notified of the results of each analysis. If a p-value < 0.02 is found at any interim analysis, NHLBI will request that the DSMB discuss the possibility of halting the trial. This flat monitoring boundary was selected because the focus is on subject safety. Monitoring boundaries, such as the O'Brien-Fleming boundary, that require stronger evidence to halt a trial at earlier analyses than at later time points seem less appropriate when the endpoint is subject safety.

The operating characteristics of this design, and a number of other potential designs, were investigated in a series of simulations. Parameters investigated were

a) performing analyses after every 6 deaths, every 10 deaths, or every 14 deaths
b) using 0.01, 0.015, 0.02, 0.03, or .004 as the critical value
c) death rates in the control arm of 0.30, 0.40, or 0.50
d) between-arm differences in death rates of -0.20, -0.10, 0.00, 0.10, or 0.20

For each scenario, 5,000 replications were run.

The proposed design was chosen because it provides

- a Type I error rate close to 0.05 under a wide range of scenarios
- good power to detect an absolute difference in death rates of at least 20% (either at an interim analysis or at the end of the study)
- a high probability of halting early if the true death rates have an absolute difference of at least 20%.

There are three possible outcomes from the planned analyses of mortality. Each row of Table 4 describes a particular scenario (pair of true mortality rates). The last 3 columns in each row show the percentage of simulations which exhibited each of the three possible outcomes.

1. If the observed death rates in the two randomized treatment arms are very different as the study progresses, the criteria for considering an early halt to the trial could be met. The percentage of simulations where this occurred is shown in column 5 of Table 4.
2. If the criteria for early stopping are not met, there might still be a statistically significant difference in mortality rates at the final analysis. The percentage of simulations where this occurred is shown in column 6.
3. If the criteria for early stopping are not met, there might be no statistically significant difference in mortality rates at the final analysis. The percentage of simulations where this occurred is shown in column 7.

Table 4: Operating characteristics for proposed interim analysis of mortality, based on 5,000 replications of each scenario

Note that because of the two-sided testing only one set of simulations was used for each pair of death rates (for example, the row for 30% mortality in the control arm and 50% mortality in the granulocyte arm is derived from the same set of simulations as the row for 50% mortality in the control arm and 30% mortality in the granulocyte arm).

P(Death) in Arm 1	P(Death) in Arm 2	Estimated Type I Error Rate (declaring significant difference when no difference exists)	Estimated Power (declaring a significant difference when a difference exists)	% Halted Early	If not halted early	
					% Significant at End	% Non-significant at End
0.30	0.10	--	0.9422	86.70	7.52	5.78
	0.20	--	0.3146	26.82	4.64	68.54
	0.30	0.0418	--	3.86	0.32	95.82
	0.40	--	0.2670	23.54	3.16	73.30
	0.50	--	0.8048	75.90	4.58	19.52
0.40	0.20	--	0.8526	80.36	4.90	14.74
	0.30	--	0.2670	23.54	3.16	73.30
	0.40	0.0452	--	4.30	0.22	95.48
	0.50	--	0.2604	23.54	2.50	73.96
	0.60	--	0.8042	75.80	4.62	19.58
0.50	0.30	--	0.8048	75.90	4.58	19.52
	0.40	--	0.2604	23.54	2.50	73.96
	0.50	0.0530	--	4.92	0.38	94.70
	0.60	--	0.2582	23.68	2.14	74.18
	0.70	--	0.8150	76.32	5.18	18.50

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APPENDIX A: Definitions of infection and suggested treatments

I. Definitions of Infections

Table 1: Proven invasive fungal disease (if any of the criteria apply)

	Molds*	**Yeasts**	**Agents of endemic fungal disease§**
Microscopy – sterile material	Histopathologic, cytopathologic, or direct microscopic examination† of a needle aspiration or biopsy specimen showing hyphal or melanized yeast-like forms with evidence of associated tissue damage (either microscopically or as an infiltrate or lesion by imaging)	Histopathologic or cytopathologic examination† of a needle aspiration or biopsy specimen from a normally sterile site excluding mucous membranes showing yeast cells e.g. *Cryptococcus* species indicated by encapsulated budding yeasts, *Candida* species showing pseudohyphae or true hyphae ‡	if culture is sterile or not obtained, histopathologic or direct microscopic demonstration of appropriate morphologic forms is considered adequate for dimorphic fungi having a truly distinctive appearance
Culture – sterile material	Recovery of a Mold or 'black yeast' by culture from a sample obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL, cranial sinus cavity, and urine.	Recovery of a yeast by culture from a sample obtained by a sterile procedure (including a freshly (<24h) placed drain) from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process	Must be proven by recovery in culture from a specimen obtained from the affected site, and the host must at the same time have an illness consistent with a fungal infectious disease
Culture – blood	Blood culture that yields a Mold, e.g. *Fusarium* spp. in the context of a compatible infectious disease process	Blood culture that yields yeast (e.g. *Cryptococcus* species, *Candida* species), or yeast-like fungi (e.g. *Trichosporon* spp.)	Blood culture that yields an agent of endemic mycosis
Serology	Not applicable	**Disseminated cryptococcosis** cryptococcal antigen in CSF	**Histoplasmosis** diagnosis of disseminated disease can be established by means of a positive *Histoplasma* antigen EIA test on CSF, urine or serum, or by showing the presence of characteristic intracellular yeast forms in a peripheral blood smear or in bone marrow. **Coccidioidomycosis** demonstration of coccidioidal antibody in CSF, or a 2-dilution rise measured in two consecutive blood samples tested concurrently in the setting of an ongoing infectious disease process.

* if culture is available, append identification at genus or species level from culture.

† tissue and cells submitted for histopathologic or cytopathologic studies should be stained by Grocott-Gomori methenamine silver stain or by periodic acid Schiff stain to facilitate inspection of fungal structures. Where possible, wet mounts of specimens from foci related to invasive fungal infectious disease should be stained with a fluorescent dye (e.g., calcofluor or Blankophor ™)

‡ *Candida*, *Trichosporon* and yeast-like *Geotrichum* species and *Blastoschizomyces capitatus* may also form pseudohyphae or true hyphae

§ Histoplasmosis, blastomycosis, coccidioidomycosis, paracoccidioidomycosis, sporotrichosis and infection due to *Penicillium marneffei.* Onset within 3 months defines a primary pulmonary infection

Table 2 Criteria for defining probable invasive fungal disease (At lease one of each of the 3 elements must b present). Cases for which microbiologic criteria are absent are considered "Possible" and are not eligible for the RING Study.

Host factors

Host factors are not synonymous with risk factors and are characteristics by which individuals predisposed to invasive fungal diseases can be recognized. They are intended primarily to apply to patients treated for malignant disease and to recipients of allogeneic hematopoietic stem cell and solid organ transplant. These host factors are also applicable to those receiving corticosteroids and other T-cell suppressants as well as those with primary immune deficiencies

1) Recent history of neutropenia (< 0.5 x 10^9/L {<500 neutrophils/mm^3} for >10 days) temporally related to the onset of fungal disease
2) Receipt of an allogeneic stem cell transplant
3) Prolonged use of corticosteroids (excluding patients with allergic bronchopulmonary aspergillosis) at an average minimum dose of 0.3 mg/kg/day prednisone equivalent for > 3 weeks
4) Treatment with other recognized T-cell immune suppressants such as cyclosporine, TNF-α blockers, specific monoclonal antibodies such as alemtuzumab, nucleoside analogues during the past 90 days
5) Inherited severe immunodeficiency (e.g., chronic granulomatous disease, severe combined immunodeficiency)

Clinical criteria*

Lower respiratory tract fungal disease	***Tracheobronchitis***	***Sinonasal infection***	***CNS infection***	***Disseminated candidiasis†***
The presence of one of the following "specific" imaging signs on CT: 1. Well defined nodule(s) with or without a halo sign 2. Wedge-shaped infiltrate 3. Air crescent sign 4. Cavity ***OR*** the presence of a new non-specific focal infiltrate ***PLUS*** at least one of the following: 1. Pleural rub 2. Pleural pain 3. Hemoptysis	Tracheobronchial ulceration, nodule, pseudomembrane, plaque or eschar seen on bronchoscopy	Imaging showing sinusitis ***PLUS*** at least one of the following: 1. Acute localized pain (including pain radiating to eye) 2. Nasal ulcer, black eschar 3. Extension from the paranasal sinus across bony barriers, including into the orbit	Focal lesions on imaging ***OR*** Meningeal enhancement on MRI or CT	At least one of the following: 1. Small, target-like abscesses (new nodular filling defects, bull's-eye lesions) in liver or spleen of a patient who has had candidemia within the previous 2 weeks 2. Progressive "cotton wool" exudates on ophthalmologic examination

* Must be consistent with the microbiological findings, if any, and must be temporally related to current episode.

† the presence of signs and symptoms consistent with sepsis syndrome indicates acute disseminated disease whereas their absence denotes chronic disseminated disease.

Table 2 continued

Microbiological Criteria	
Direct test - Cytology, direct microscopy or culture	**Mold**: sputum, BAL fluid, bronchial brush or sinus aspirate samples: the presence of fungal elements indicating a Mold **OR** recovery by culture of a Mold (e.g. *Aspergillus* spp., *Fusarium* spp., Zygomycetes, *Scedosporium* spp.) from sputum, BAL fluid or bronchial brush samples
	Candida*:* biopsy of skin ulcers or a swab of draining soft tissue lesions or fistulas : Detection of yeast by microscopy **AND** recovery of *Candida* species by culture of the lesion
Indirect tests (Detection of antigen, cell wall constituents or nucleic acid)	**Aspergillosis**: Galactomannan antigen detected in plasma, serum, BAL fluid or CSF, unless the subject has been treated with pipercillin/tazobactam within the last 48 hours (these drugs are associated with false positive galactomannan results).
	Mycosis other than cryptococcosis and zygomycoses Beta-D-glucan detected in serum Note: These tests are primarily applicable for aspergillosis and candidiasis and do not detect *Cryptococcus* species or Zygomycetes (e.g. *Rhizopus* spp., *Mucor* spp. *Absidia* spp.)

* Must be consistent with the microbiological findings, if any, and must be temporally related to current episode.
† the presence of signs and symptoms consistent with sepsis syndrome indicates acute disseminated disease whereas their absence denotes chronic disseminated disease.

II. **Definitions of Bacteremia**

Subjects with any positive isolate from bacterial blood cultures indicative of serious infection (e.g., gram negative bacteremia or S. aureus bacteremia; coagulase-negative staphylococcal bacteremias are excluded). Subjects must also be unresponsive to appropriate clinical and antimicrobial management for > 24 hours (i.e., ongoing pressor requirements or hemodynamic instability). Bacteremia in the absence of hemodynamic instability will not be grounds for inclusion in the study unless it has persisted for 72 hours in spite of appropriate antimicrobial therapy.

III. **Definition of Invasive Bacterial Tissue Infection**

Clinical signs and symptoms compatible with disease (sinusitis, pneumonia, intra-abdominal abscess) and radiographic evidence of disease and pure or predominant culture from sterile site biopsy or bronchoalveolar lavage (BAL). The positive culture is not required if the subject has a positive blood culture with an organism that is a plausible cause of the infection (e.g. isolation of Streptococcus pneumoniae from blood in a subject with pneumonia). Typhlitis (neutropenic enterocolitis) is defined as clinical signs and symptoms compatible with disease and typical radiographic evidence of disease with or without culture confirmation.

IV. **Suggested therapy for specific infections**

A. Invasive mold infections due to:
   1. Aspergillus species: voriconazole +/- caspofungin
   2. Zygomycetes (agents of "mucormycosis"): lipid formulation of amphotericin B (AmBisome or ABLC; 5mg/kg/day) is preferred first-line therapy. Posaconazole may be considered as salvage therapy.
   3. Fusarium species: voriconazole or lipid formulation of amphotericin B (AmBisome or ABLC; 5mg/kg/day)
   4. Scedosporium species: voriconazole

B. Candidemia or deep tissue invasive candidiasis: echinocandin (caspofungin, micafungin or anidulafungin), conventional amphotericin B ($\geq$ 0.6 mg/kg/day); lipid formation of amphotericin B (3 to 5 mg/kg/day). Removal of central venous catheter is advised.

APPENDIX B: Definitions of response to therapy

Response for ***tissue invasive bacterial/ fungal infections*** will be categorized as:

1. Complete response – survival to 42 days after randomization, plus
   A Resolution of all signs and symptoms of infection by clinical, laboratory and radiographic parameters, using the appropriate imaging technique
   AND
   B conversion of bacterial/fungal cultures to negative unless specimens cannot be obtained because an invasive procedure is contraindicated,

2. Partial response – survival to 42 days after randomization, plus
   A Clinically important improvement in disease, including a demonstrable improvement in radiological or other diagnostic imaging findings, but criteria for complete response are not satisfied

3. Failure – Any of the following:
   - death from any cause within 42 days after randomization
   - lack of improvement of infection at day 42 after randomization
   - progression of infection at day 42 after randomization

4. Indeterminate – insufficient information available to determine subject's response

Response ***for bacterial/fungal bloodstream infection*** will be categorized as:

1. Complete response – survival to 42 days after randomization, plus
   A Resolution of all signs and symptoms attributable to infection by clinical and laboratory parameters
   AND
   B conversion of bacterial/fungal cultures to negative by day 42 after randomization (e.g., negative for the organism for which the subject was enrolled; subjects with positive blood cultures for other organisms may still be coded as complete responders)

2. Failure – Failure to achieve complete response.

3. Indeterminate – insufficient information available to determine subject's response

APPENDIX C: CTC categories for expected adverse events in the setting of chemotherapy and/or HSC transplantation

Cardiovascular (General)
Edema
Hypertension
Hypotension

Constitutional
Rigors, chills
Weight gain associated with VOD

Dermatology/Skin
Hand-foot skin reaction
Pruritis
Rash/dermatitis associated with high-dose chemotherapy or BMT studies
Rash/dermatitis associated with GVHD for BMT studies

Gastrointestinal
Colitis
Dehydration
Diarrhea associated with GVHD for BMT studies
Dysphagia, esophagitis, odynophagia
Dysphagia-esophageal related to radiation
Dysphagia-pharyngeal related to radiation
Gastritis
Ileus
Pancreatitis
Stomatitis/pharyngitis for BMT studies
Typhlitis

Hemorrhage
Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia
Hematuria
Hemoptysis
Hematemesis
Melena/GI bleeding
Rectal bleeding/hematochezia
Vaginal bleeding
Epistaxis

Hepatic
Alkaline phosphatase elevation
Bilirubin elevation
Bililrubin elevation associated with GVHD for BMT studies

GGT elevation
SGOT (AST) elevation
SGPT (ALT) elevation

Infection/Febrile Neutropenia
Catheter-related infection
Febrile neutropenia
Infection with grade 3 or 4 neutropenia
Infection with unknown ANC
Infection without neutropenia

Metabolic/Laboratory
Acidosis
Alkalosis
Amylase elevation
Hypercalcemia
Hypercholesterolemia
Hyperglycemia
Hyperkalemia
Hypermagnesemia
Hypernatremia
Hypertriglyceridemia
Hyperuricemia
Hypocalcemia
Hypoglycemia
Hypokalemia
Hypomagnesemia
Hyponatremia
Hypophosphatemia
Lipase elevation

Musculoskeletal
Muscle weakness

Pain
Abdominal pain or cramping

Pulmonary
Hypoxia
Pleural effusion
Pneumonitis/pulmonary infiltrates
Voice changes/stridor/larynx

Renal/Genitourinary
Bladder spasms

Creatinine elevation
Dysuria
Renal failure
Urinary electrolyte wasting
Urinary frequency/urgency
Urinary retention

Sexual/Reproductive Function
Persistent amenorrhea

Syndromes (not included in previous category)
Tumor lysis syndrome

BMT Complex/Multicomponent Events
Graft versus host disease
Stem cell infusion complications
Veno-Occlusive Disease (VOD)

APPENDIX D: Script for Three-Month Telephone Follow-Up

INSTRUCTIONS TO INTERVIEWER

TEXT IN UPPERCASE CONTAINS INSTRUCTIONS FOR THE INTERVIEWER.
UNDERLINED TEXT INDICATES CALL-SPECIFIC INFORMATION.

TELEPHONE SCRIPT

Hello, my name is [your name] and I am calling from [name of your clinic]. You/[Name of subject] were/was enrolled in our RING Study. We last checked in with you/[Name of subject] back in [month] and I was calling today to see how things are going.

IF SUBJECT IS DECEASED: **I am very sorry to hear about his/her death. When did he/she die?**

IF SUBJECT IS ALIVE OR YOU'RE SPEAKING WITH THE SUBJECT: **I would like to thank you for your participation in the RING Study.**

Thank you for your time today.

CONFIDENTIALITY STATEMENT:

THE STATEMENT BELOW MUST BE READ TO ALL INTERVIEWEES:

Let me remind you that all information is strictly confidential and that [your name/name of subject] will not be used in any reports. If you have any questions or concerns about this study, you may call the Principal Investigator, [name of PI], at [PI telephone number]. If you have any questions about your rights as a research subject, you may call [IRB contact] of [name of site's] Institutional Review Board at [IRB telephone number].

ANSWERING MACHINE SCRIPT

IF LEAVING A MESSAGE ON AN ANSWERING MACHINE:

Hello, this is [your name] calling from [name of your clinic] regarding the RING Study. If you would like to contact me, I can be reached at [your phone number] between the hours of [time for interviewee to call back]. Thank you.

PROTOCOL SIGNATURE PAGE

I have read the foregoing protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study in accordance with the design and specific provisions outlined herein; deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment.

I will provide copies of the protocol and all pertinent information to all individuals for whom I am responsible who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the device and/or drug and the conduct of the study.

I will use only the informed consent form approved by the Data Coordinating Center and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board or Ethics Committee responsible for this study.

I also agree to report all information or data in accordance with the protocol and, in particular, I agree to report any serious adverse experiences as defined in Section 7 of this protocol.

I further agree that the NIH and the Transfusion Medicine/Hemostasis Clinical Trials Network Data Coordinating Center have access to any source documents from which case report form information may have been generated.

The below signed confirm herewith to have read and understood this trial protocol and/or amendment and appendices; furthermore, to accomplish this study in accordance with the protocol and Good Clinical Practice guidelines, as well as local regulations; and to accept respective revisions approved by authorized personnel of NIH and by competent authorities.

PRINTED OR TYPED NAME(S)	SIGNATURE	DATE
Principal Investigator(s)		
Principal Investigator(s)		