# TRIAL TO REDUCE ALLOIMMUNIZATION TO PLATELETS

# (TRAP)

## PROTOCOL

## 1/14/91

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# 1. INTRODUCTION

Between 1971 and 1980, red blood cell transfusions in the United States rose from 6.3 million to 9.9 million, an increase of 57%[1]. However, there was a concurrent 598 percent increase in the use of random donor platelet concentrates, from 0.41 million to 2.86 million annually. Although the use of red blood cells for transfusion has leveled or even decreased slightly in the past several years, the use of platelets has continued to increase at a rate of at least 5 to 10 percent each year. This dramatic and continued increase in the use of platelet concentrates is largely the result of the treatment of thrombocytopenic cancer patients. In addition, open heart surgery patients and others given massive transfusions also receive substantial platelet support. Nevertheless, it is those chronically transfused thrombocytopenic patients who frequently develop platelet alloimmunization that represent the major factor in the increase in platelet demand. For example, a recent survey in a large transfusion service indicated that 8 percent of the patients had received 35 percent of the random-donor pooled platelet concentrates. Although some alloimmunized patients can be supported by HLA-matched, apheresis-donor platelets, suitably matched donors are not available in sufficient numbers for every patient. Furthermore, some communities have limited resources for providing single-donor matched platelets. Thus, platelet transfusion programs that can prevent, or at least delay, platelet alloimmunization would be of substantial benefit.

Limited studies have suggested several approaches to reduce or prevent platelet alloimmunization[2]. These approaches are based upon theoretic considerations, as well as on data from limited clinical trials. The first consideration involves reducing the number of foreign antigens to which a recipient is exposed. If HLA antigens be the predominant immunogens, providing only HLA-matched apheresis-donor platelets should prevent or reduce platelet alloimmunization[3,4]. However, because of the complexity of the HLA system, it has not seemed feasible to provide adequate numbers of HLA-matched donors for even a small percentage of chronically thrombocytopenic patients. It is possible, though unproven, that some HLA antigens are more important in platelet immunogenicity than others[5]. This could reduce the complexity, analagous to the matching of red blood cells for transfusions by ABO and Rh(D) types. Alternatively, some investigators have used random, single-donor, apheresis platelets. Two studies have demonstrated a reduced frequency of alloimmunization in recipients of single-donor, apheresis platelets compared to individuals receiving pooled random-donor platelet concentrates[6,7]; however, a third study has shown a benefit only if the single-donor platelets were also leukocyte-poor[8]. In addition, studies in a dog platelet transfusion model have suggested that single-donor, apheresis platelets may delay, but not prevent, platelet alloimmunization[9].

Another approach involves providing leukocyte-poor blood products. Leukocytes possess both Class I and Class II HLA antigens, while platelets have only Class I[9]. The immune recognition process is activated when presented with cells carrying both classes of HLA antigens[9,10]. Thus, the question has been

raised whether leukocyte-poor blood products would be less immunogenic. Some human, as well as animal, transfusion studies have demonstrated an apparent value of leukocyte-poor platelet products in preventing platelet alloimmunization[8,11–15]. Others have failed to confirm the efficacy of this approach[16,17]. Several of the reported studies were not comparable, either in their design or in the technical aspects of white cell removal. Furthermore, it was not determined if different levels of leukocytes in the transfused material influenced the results; the degree of reduction of leukocytes necessary to make a product truly "leukocyte-poor" could be an important variable. Thus, the question remains unresolved whether blood collection operations can develop a practical system of providing sufficiently leukocyte-free platelet preparations to prevent alloimmunization or even if this approach is useful.

In addition to these two approaches, recent data suggest that a major route of recipient alloimmunization occurs through the transfusion of antigen-presenting cells (APCs)[18,19]. Dendritic cells, a subpopulation of leukocytes, are considered to be the major APCs[20]. Thus, techniques either to remove donor dendritic cells from the transfused material[21], or to inactivate them[22], may be successful in preventing alloimmunization. Although the recipient's own dendritic cells can also recognize, process, and present donor antigens to the recipient's antibody-forming cells, this process is considered to be much less efficient than when the antigen is presented by donor cells. Of substantial interest has been the observation that ultraviolet (UV) irradiation of blood or tissue may inactivate donor APCs[19]. UV-irradiation of donor platelets prevented platelet alloimmunization for 11 of 12 recipients in a dog transfusion model[23]. Furthermore, such UV irradiation did not impair the function of human platelets, at least by in vitro measurements[24].

The objective of this trial is to determine the best, clinically useful procedure to prevent or minimize platelet alloimmunization as a cause of refractoriness to platelet transfusion. Patients will be randomized to one of three treatment arms or to a control arm. Comparisons will be made between each treatment arm and the control arm and among treatment arms.

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## 2. OVERVIEW OF TRIAL DESIGN

Objectives

To identify the optimal method to minimize or prevent platelet alloimmunization in patients undergoing therapy for AML .

Eligibility

Patients with AML
Age $\geq$ 15 years
Informed consent
No prior treatment for leukemia (other than hydroxyurea within one week, cerebral irradiation, or cytapheresis).
No prior transfusions for hematopoietic disorder (myelodysplasia, P. vera, CML, etc.) > 2 months ago and no more than 10 donor exposures between 2 weeks and 2 months
No prior chemotherapy within the past 2 years and no prior radiation therapy for any reason, except radiation restricted to local area
Chemotherapy regimen:
    Must NOT include corticosteroids as part of the anti-leukemia regimen
    Must administer Daunorubicin $\geq$ 90 mg/m$^2$, Mitoxantrone $\geq$ 30 mg/m$^2$, Idarubicin $\geq$ 30 mg/m$^2$, or Ara-C $\geq$ 700 mg/m$^2$ (total dose)

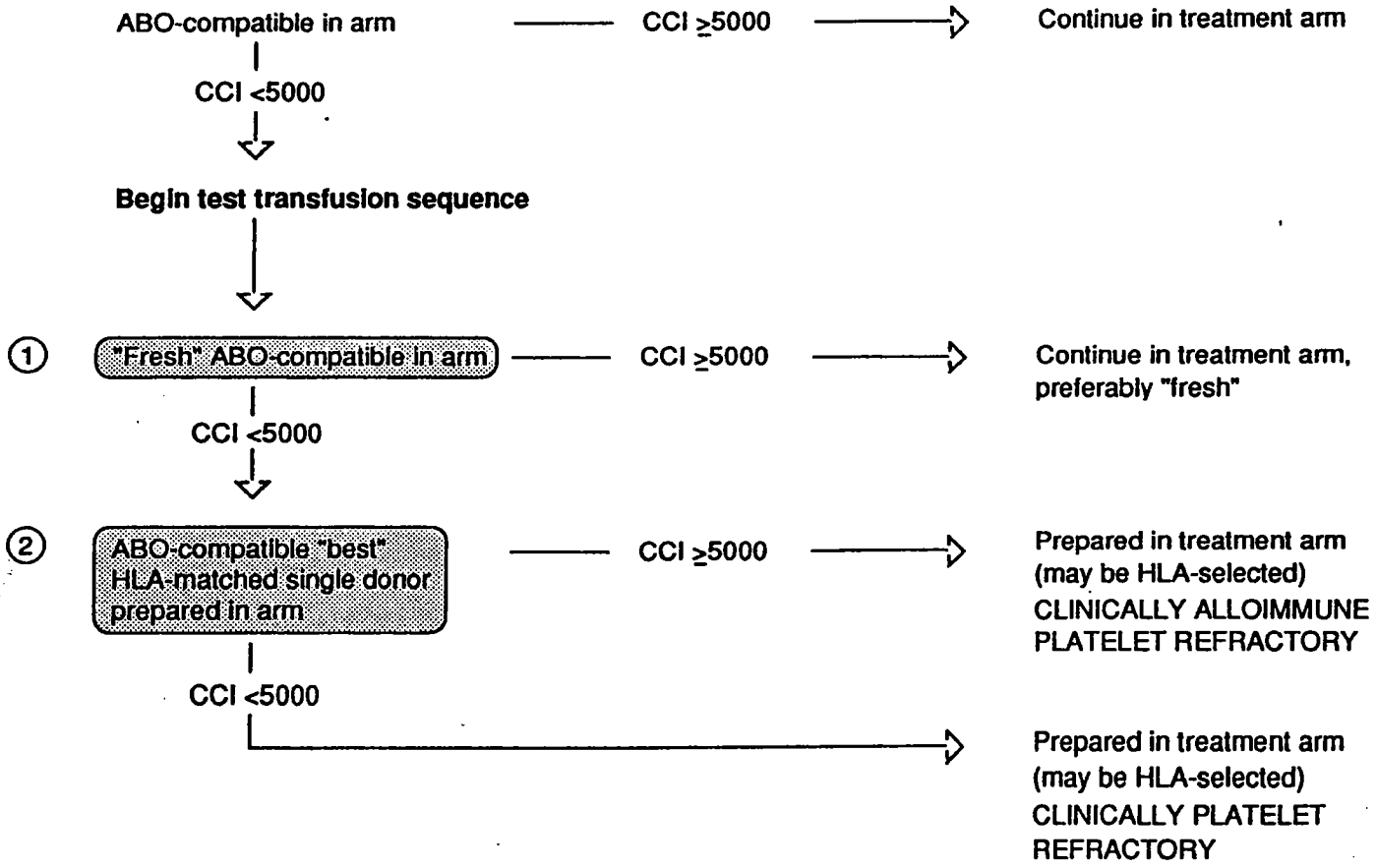
Data Collection

Platelet count within 60 minutes after every platelet transfusion
Measurement of antibody (weekly x 8, monthly x 1 year)
Platelet and WBC counts of transfusion product

Transfusion Therapy

RBC - All transfusions will be leukocyte depleted.
Platelets - Randomization to
    Arm 1 - Pooled random donor platelets
    Arm 2 - UV-B irradiated pooled random donor platelets
    Arm 3 - Leukocyte-poor filtered pooled random donor platelets
    Arm 4 - Leukocyte-poor filtered non-HLA-selected single donor platelets
If 2 successive platelet transfusions have corrected count increment (CCI) < 5000, initiate Test Transfusion Sequence.

## TEST TRANSFUSION SEQUENCE




Prepared in treatment arm: Prepared (UV-B irradiation, filtration, or neither) as in assigned treatment arm
Fresh: <48 hours from collection
Best HLA-matched: A, B1U, or B2U matched

## CORRECTED COUNT INCREMENT (CCI)

CCI: difference between pre- and post-transfusion count per $10^{11}$ platelets transfused per square meter of body surface area.

EXAMPLE of calculation of CCI:

1. $4 \times 10^{11}$ platelets administered to patient.
2. Patient has BSA of 2 $m^2$.
3. Patient pre-transfusion platelet count: 10,000/$\mu$L.
4. Patient post-transfusion platelet count: 50,000/$\mu$L.
5. $CCI = \frac{(50,000-10,000) \times 2}{4} = 20,000.$

The post-transfusion count must be obtained within 60 minutes after the transfusion.

For a test transfusion, the pre-transfusion count must be obtained within 60 minutes before initiating the transfusion. If not a test transfusion, the pre-transfusion count may be the morning count.

## 3. PATIENT ELIGIBILITY AND EXCLUSION CRITERIA

### A. General Trial Population

Patients who have been admitted for ablative treatment for acute myelogenous leukemia are eligible for the trial. In most cases patients will be in a chemotherapy trial, however those patients who are not in a chemotherapy trial but are having ablative treatment are to be included.

The histologic diagnosis of acute myelogenous leukemia is based on FAB classification (M0-M7).

The following histologies are acceptable:

0. Undifferentiated acute myelocytic leukemia (M-0)
1. Acute myelocytic leukemia without maturation (M-1)
2. Acute myelocytic leukemia with maturation (M-2)
3. Acute promyelocytic leukemia (M-3)
4. Acute myelomonocytic leukemia (M-4)
5. Acute monocytic leukemia (M-5)
6. Acute erythroleukemia (M-6)
7. Acute megakaryocytic leukemia (M-7)

### B. Exclusion Criteria

Patients are ineligible if they meet any of the following criteria:

1. Patient's age is less than 15 years.

2. Patient is (will be) on no chemotherapy or on low dose chemotherapy defined to be less than 90 $mg/m^2$ Daunorubicin, 30 $mg/m^2$ Mitoxantrone, 30 $mg/m^2$ Idarubicin, or 700 $mg/m^2$ Ara-C (total dose).

3. Patient is (will be) on corticosteroids as part of the leukemia induction regimen.

4. Patient has received transfusions for prior hematopoietic disorder (myelodysplasia, myelofibrosis, polycythemia vera, chronic myelogenous leukemia, etc.): either (a) any transfusions more than two months ago or (b) transfusions totaling > 10 donor exposures between two weeks and two months ago.

5. Patient has had prior treatment for leukemia (other than hydroxyurea within one week, cerebral irradiation, or cytapheresis).

6. Patient has had prior chemotherapy within the past two years or radiation therapy for a malignancy or for any other reason, except for radiation restricted to a local area.

7. Patient (or guardian) refuses informed consent to participate.

8. Patient's physician refuses consent for patient participation.

C. Antibody Positive Patients

Patients who are lymphocytotoxic or platelet antibody positive at enrollment are not excluded from the trial. Antibody status will be determined by the Central Laboratory AFTER enrollment from the initial blood sample taken prior to the study-assigned transfusion. Antibody positive patients will continue in their assigned transfusion arm and will be followed as are other patients enrolled in the randomized trial. However, data from antibody positive patients will be excluded from analysis of the primary endpoint of the randomized trial.

D. Recruitment

All age-eligible patients at the enrolling hospital who have acute myelogenous leukemia should be considered for the trial. The Eligibility form will be completed for each of the patients considered. Further data will be collected only on patients who are eligible for the trial (including patients who are antibody positive at entry as described above).

E. Stratification for Randomization

Randomization is stratified by clinical center (7 centers) and by whether or not there is a history of pregnancy and/or transfusion more than two weeks before enrollment (14 strata).

# 4. RANDOMIZATION ARMS: BLOOD PRODUCTS

## A. Platelet Products

Eligible patients will be randomized to one of four platelet transfusion treatment arms:

1. Pooled random donor platelets (control).
2. UV-B irradiated pooled random donor platelets.
3. Leukocyte-poor filtered pooled random donor platelets.
4. Leukocyte-poor filtered apheresis non-HLA-selected single donor platelets.

Transfusions will be ABO compatible if possible. Gamma irradiation of blood products in all treatment arms will be done at local option.

1. Pooled random donor platelets (control).

The standard arm will consist of pooled random donor platelet concentrates. The random donor platelet concentrates will be prepared and stored by standard techniques and should yield at least $5.5 \times 10^{10}$ platelets/concentrate (FDA standards). However, with appropriate centrifugation techniques, it should be possible to consistently achieve an average of $7.0 \times 10^{10}$ platelets/concentrate. The white cell counts of routinely-prepared platelet concentrates range between $0.7 \pm 0.5$ to $1.41 \pm 1.27$ white cells $\times 10^8$/platelet concentrate, depending on the method of preparation. The red cell contamination of most platelet concentrates is very low, with hematocrits of less than 1%.

The standard dose of pooled random donor platelets for the trial will be 6 units. However, it is possible that this standard platelet dose will need to be increased at local option for clinical reasons.

Platelet concentrates are currently licensed for up to 5 days of platelet storage, and in normal volunteers, autologous radiolabeled platelet viability studies demonstrate platelet recoveries in the range of $38\% \pm 7$ to $48\% \pm 6$ and survivals of $5.1 \pm 1.4$ days to $6.3 \pm 1.3$ days after 5 days of storage, compared to fresh recoveries of $59\% \pm 4$ and survivals of $8.1 \pm 0.2$ days.

In addition, stored platelet concentrate transfusion studies in thrombocytopenic patients have generally demonstrated acceptable post-transfusion platelet increments and control of bleeding.

2. UV-B irradiated pooled random donor platelets.

The standard dose of pooled random donor platelets for the trial will be 6 units. However, it is possible that this platelet dose will need to be increased at local option for clinical reasons. (See above for a

discussion of pooled random donor platelets.) If the volume of platelets exceeds the desired range of 250 to 420 ml, a second steri-cell bag will be required, and the platelet dose should be divided equally between the two bags.

The pooled random donor platelet concentrates will be UV-B irradiated with a Haemonetics UV-B irradiation machine.

Ultraviolet (UV) light (200-400 nm) alters the biologic function of blood cells that is, in part, dependent on the light's wavelength. UV-A (320-400 nm) has the least activity, UV-C (200-280 nm) is highly effective, and UV-B (280-320 nm) appears to be most relevant biologically.

Lymphocytes irradiated with UV-B or UV-C lose ability to stimulate allogeneic cells in mixed lymphocyte culture (MLC). Rat dendritic cells subjected to UV-B irradiation do not stimulate allogeneic cells in MLC, and rat islet of Langerhans cells irradiated with UV light have prolonged allograft survival when transplanted across major histocompatability barriers. Interestingly, islet of Langerhans allografts have prolonged survival in nonimmunocompromised adult rats pretreated with blood irradiated with ultraviolet light. In a canine transfusion model, only one of 12 dogs that received UV-C exposed platelet concentrates became alloimmunized compared to an expected rate of 86-95%. In a canine marrow transplantation model, dogs transfused with UV-C exposed blood from the intended marrow donor uniformly achieved engraftment and behaved like untransfused dogs. Using the same model in an attempt to prevent graft-versus-host disease, marrow cells were exposed to UV-C or UV-B light. After exposure to UV-C, marrow T lymphocyte and hematopoietic precursor cell function was inactivated compared to T lymphocyte inactivation and sparing of hematopoietic precursor cell function following exposure to UV-B. In in vitro studies using platelet concentrates obtained from humans, UV-B irradiation abrogated lymphocyte response or stimulation in MLC. Platelet number and aggregation response to agonists was unchanged when compared to untreated platelets. These data were consistent with previous reports that showed UV irradiation of peripheral blood lymphocytes blocks the ability of these cells to stimulate or respond in MLC.

The mechanism by which UV light affects alloreactivity is unknown. Antigen-presenting cells, especially dendritic cells, are involved critically in the immunization process. Lymphocytes form tight clusters with dendritic cells before blast transformation and proliferation. Dendritic cells subjected to UV irradiation do not form clusters. Dogs transfused with UV-exposed blood to which small numbers of normal dendritic cells were added rejected bone marrow grafts; whereas dogs given UV-exposed blood (to which monocytes, but not dendritic cells, were added) had engraftment in four of five animals.

The biologically mediated effects of UV light require consideration of the immunogenic potential of such exposure. This includes: (1) selection of the appropriate wavelength (B vs C), (2) selection of the appropriate dose that abrogates immunologic responsiveness while sparing platelet function,

(3) investigation to determine whether platelet concentrates maintain viability during storage following UV irradiation, (4) choosing a plastic container that permits UV light penetration, and (5) determining whether alloreactivity recurs following storage of UV irradiated cells.

3. Leukocyte-poor filtered pooled random donor platelets.

Leukocyte-poor blood products may be prepared using a variety of techniques. (See above for a discussion of pooled random donor platelets.) Leukocyte-poor platelets may be prepared by centrifugation or filtration techniques. With new generation filters the efficiency and ease of white cell removal has improved, while minimizing unwanted reduction in cell viability and quantity. The effectiveness of these filters depends upon carefully following the manufacturer's instructions.

The pooled random donor platelet concentrates will be made leukocyte-poor by filtration with a Pall PL-100 filter. Filtration should reduce the white cell count to $< 5 \times 10^6$.

The standard dose of pooled random donor platelets for the trial will be 6 units, which can be filtered by one PL-100 filter. However, it is possible that this platelet dose will need to be increased at local option for clinical reasons. The Pl-100 may be used for up to 10 units of pooled random donor platelet concentrates. If more than 10 units of pooled random donor platelet concentrates are required, a second filter and pooling bag should be used, with the platelet dose divided equally before filtration between the two filters.

4. Leukocyte-poor filtered apheresis non-HLA-selected single donor platelets.

In contrast to pooled random donor platelet products which are obtained by combining the platelet concentrates from six whole blood donations, apheresis platelets are derived from single donors using cell separators. According to current standards, apheresis platelets must contain at least 3.0 to 3.5 $\times 10^{11}$ platelets per collection, or the equivalent of approximately six to seven units of pooled random donor platelet concentrates (FDA and AABB standards, respectively). For the trial a dose of $4.2 \times 10^{11}$ apheresis platelets will be considered equivalent to 6 units of pooled random donor platelet and will be the standard apheresis platelet dose. However, it is possible that this standard dose will need to be increased at local option for clinical reasons. It is also left to local option to choose to administer an entire donor collection of apheresis platelets to the patient.

The Cobe Spectra apheresis machine will be used for the trial.

The apheresis platelets will be made leukocyte-poor by filtration with a Pall PL-100 filter. Filtration should reduce the white cell count to $< 5 \times 10^6$. A greater apheresis platelet dose than the standard $4.2 \times 10^{11}$ dose may be filtered through one PL-100 filter, as this is designed to

accommodate the equivalent of 6-10 platelet concentrates. However, if studies demonstrate that white cell depletion is above the accepted level with large donor collections, either two filters should be used, with the apheresis platelets divided between the filters, or a smaller amount of platelets should be used.

B. Red Blood Cell Products

All red blood cell transfusion products are to be made leukocyte-poor by filtration with a Pall BPF4B filter. Filtration should reduce the white cell count to $< 5 \times 10^6$.

One BPF4B filter should be used for each unit of red blood cells transfused.

C. Rationale for Selection

The incidence of platelet alloimmunization in chronically transfused thrombocytopenic patients ranges between 8% to 100%[1]. The variability in the reported immunization rates is likely related to differences in blood products transfused, administration schedules, and/or the criteria used for defining platelet alloimmunization. There have been several strategies applied to the prevention of platelet alloimmunization that can be generally categorized as (1) reduce the exposure to donor antigens; (2) provide leukocyte-poor blood products; (3) inactivate or remove antigen-presenting cells from the transfused blood products; or (4) immunosuppress the transfused recipient.

1. Reduce exposure to donor antigens.

Limit the number of transfusions. A fundamental hypothesis has been that limiting the number of platelet transfusions will reduce the incidence of platelet alloimmunization[1]. Although some studies have suggested that there is a dose-response relationship between the number of transfusions given and the incidence of alloimmunization, other investigators have demonstrated that only a few transfusions may result in immunization[2]. A likely confounding variable in these studies is the failure to control for the effects of time on immunization rates. Usually 2-3 weeks are required for primary immunization, and whether the patient receives few or many transfusions during this time, the final outcome is often alloimmunization.

Therefore, although no specific attempt will be made to control the number of transfusions that each patient receives, prophylactic platelet transfusions may be provided at a platelet count of 20,000/$\mu$l or less. In addition, the standard dose of pooled random donor platelets for the trial will be 6 units. A dose of $4.2 \times 10^{11}$ apheresis platelets will be considered equivalent to 6 units of pooled random donor platelets. (However, it is possible that the standard platelet dose will need to be increased at local option for clinical reasons.) Thus, there will be some attempt made to standardize the total dose of platelets each patient

receives during the trial.

Limit the number of donors. Another technique of limiting the exposure to platelets, besides reducing the transfusion frequency, is to give single donor apheresis platelets. This strategy eliminates the multiple donor antigens a patient is exposed to during pooled random donor platelet transfusion therapy.

As HLA-antigens are known to be the major platelet immunigens, theoretically the best platelet support would be HLA-matched single donor platelet transfusions. However, when HLA-matched versus non-matched single donor apheresis platelet transfusion support was randomly compared in 33 cancer patients, there was no difference in number or severity of bleeding episodes, in number of platelet or rbc transfusions given per thrombocytopenic episode, in post-transfusion platelet increments, nor in antibody development among the two groups of patients[3]. Furthermore, the complexity of the HLA-antigen system makes it extremely difficult to provide the multiple HLA-matched donors required by most chronically thrombocytopenic patients. Thus, as there was no difference in the platelet support provided by the two types of apheresis donors in the reported study, and as single random donors are easier to provide, the only remaining question is whether single random apheresis platelet transfusions are less immunogenic than pooled random donor platelet therapy. In two studies[4,5], the incidence of platelet alloimmunization in recipients of single random donor apheresis platelet transfusions was reduced, compared to that found in patients receiving pooled random donor platelet transfusions. However, the patients in these two European studies received very few platelet transfusions, in contrast to the more liberal platelet support characteristically provided in the United States. In contrast to these two studies, observations by another group of investigators[6] showed a rate of platelet alloimmunization in recipients of single donor apheresis platelets that did not differ from historic controls who received pooled random donor platelet concentrates. In addition, studies in a dog transfusion model suggested that single donor platelet transfusions, compared to pooled random donor platelet transfusions, could delay, but not prevent, platelet alloimmunization[7]. For the purposes of this trial, because of the difficulties outlined in obtaining HLA-matched donors, and because there is no evidence that HLA-matching is better than single donor transfusions, single random donor apheresis platelets that have been made leukocyte-poor will be used as one treatment arm. (See below for rationale for leukocyte depletion.)

2. Provide leukocyte-poor blood products.

It has been well-documented that alloantigen recognition requires the expression of both Class I and Class II HLA-antigens on the surface of the transfused cells[8,9]. As platelets, in contrast to white blood cells, express only Class I but not Class II HLA-antigens and red cells may express only low levels of Class I HLA-antigens, the question of whether leukocyte-poor blood products would prevent platelet alloimmunization has been explored. Studies in rodents[8,10] clearly demonstrated that leukocyte-

depleted platelets were not associated with alloantibody development. However, there may be species differences, as in both a dog and a baboon transfusion model; alloimmunization was not prevented by leukocyte depletion in small numbers of transfused recipients[11]. In some of these animal systems $10^6$ leukocytes per transfusion were well-tolerated; in others immunization regularly occurred with leukocyte counts of $10^5$ to $10^6$. Part of this discrepancy may be due to the method of documenting the alloimmunization and to the number and timing of the injections given. In some studies alloantibody detection was required, while in others failure of transfused platelets to circulate, i.e., platelet refractoriness, was required. In addition, some studies gave only a limited number of transfusions[10], while others[11] gave multiple transfusions.

In addition, human leukocyte-poor transfusion trials in the prevention of platelet alloimmunization have generally demonstrated a lower rate of immunization with leukocyte depletion with some exceptions[12-16]. These conflicting results are probably related to variable leukocyte contamination of the "standard" and "leukocyte-poor" transfusion products, inconsistent criteria used to classify patients as alloimmunized, small patient numbers, and different chemotherapy regimens used to treat patients while they were being transfused. Although the concept of being able to prevent platelet alloimmunization with leukocyte-poor blood products is probably correct, current technology may not be able to consistently provide products that are adequately leukocyte-depleted.

For the purposes of this trial, the therapeutic efficacy of leukocyte-depleted pooled random donor platelets, as well as leukocyte-depleted single random donor platelets, will be investigated. The apheresis machine used to prepare the platelet concentrate will be that device which gives the lowest initial leukocyte contamination of the apheresis product. Preparation of the routine platelet concentrates will be standardized to give a defined range of leukocyte contamination. In addition, both the pooled random donor platelets and the apheresis platelets will then be subjected to a leukocyte filtration device that yields the lowest residual white cell contamination while maintaining adequate numbers of platelets.

3. Inactivate or remove antigen-presenting cells (APC's).

Recent studies have suggested that a major route of alloimmunization occurs via APC's[17,18] . Donor APC's interact with T-helper cells of the transfused recipient to induce the recipient's plasma cells to produce alloantibodies. Therefore, techniques of either inactivating or removing APC's would be expected to impair the immune recognition of foreign antigens. UV-irradiation[19,20] has been documented to interfere with the function of APC's by any one of several possible mechanisms: (1) preventing the APC's from synthesizing and/or releasing immunoregulatory substances, such as interleukins that turn on T-helper cells; (2) interfering with a receptor on the surface of the APC's, thereby impairing the cell's ability to participate in the immune recognition process; or (3) inducing the formation of suppressor cells that reduce antibody production[19-21]. However, UV-irradiation does not change the expression of

either HLA-Class I or II antigens on the cell surface, as demonstrated by quantitative lymphocyte HLA-antigen typing experiments pre- and post-UV irradiation[22]. UV-irradiation prevents lymphocytes from either stimulating or responding in MLC; it is the failure to stimulate in MLC that is considered the relevant measurement to use for selecting the appropriate dose of UV-irradiation to inactivate the lymphocytes that contaminate the platelet product. In a dog transfusion model UV-irradiation was demonstrated to prevent refractoriness to platelet transfusions in 11 of 12 recipients (92%)[23].

Thus, another treatment arm will be pooled random donor platelet concentrates that have been UV-B irradiated.

4. Immunosuppress the recipient.

In patients with acute leukemia receiving chemotherapy the immune system is presumed to be suppressed. Paricularly during the marrow aplastic phase following treatment, most patients become immunoincompetent[24]. Furthermore, there is some evidence that large tumor burdens are themselves immunosuppressive[25]. If therapy is successful in reducing the tumor load, immunocompetence is often restored.

As with any antigenic stimulus, the immune response is related to such factors as the particular antigen presented, when it is given in relationship to the chemotherapy, how much chemotherapy is given, and the immunosuppressive potential of the treatment programs[24-27]. Most studies, using a variety of antigenic stimuli, have demonstrated only a quantitative, rather than a qualitative, defect in both cell-mediated and humoral immunity pre-, during, and post-treatment in leukemic patients undergoing chemotherapy. Certainly when careful observations are made, the majority of transfused leukemics develop alloantibodies[1].

In order to try to control for the effects of chemotherapy on the immune status of the leukemia patients entered into this prevention of platelet alloimmunization trial, participants will be limited to those receiving high dose chemotherapy.

5. Summary

In summary, in order to evaluate adequately the immunogenic effects of only the various platelet products, all red cell transfusions will be made leukocyte-poor to eliminate, as much as possible, transfused red cells as an antigenic stimulus. The control arm will be unirradiated pooled random donor platelet concentrates, and there will be three treatment arms that utilize modified blood products: leukocyte-poor, pooled random donor platelets; leukocyte-poor non-HLA-selected single donor apheresis platelets; and UV-B pooled random donor platelets.

The alternative of a single donor control arm was thoroughly discussed. However, the decision to use a pooled random donor control arm was based on the following considerations: (1) Even though some blood centers are

now moving toward the use of all single donor blood products, and others will do so as time goes on, for the forseeable future a substantial number of blood centers will continue to use pooled random donor blood products; thus, the results of the trial can be readily applied to current practice for these centers. (2) If the results of trial favor the UV-B irradiated pooled random donor treatment arm or the leukocyte-poor filtered pooled random donor treatment arms over the pooled random donor control arm, these results can be extended to single donor blood products as well; thus, the results of the trial can also be applied to the current practice of blood centers using only single donor blood products. (3) A pooled random donor versus single donor comparison can be made between the two leukocyte-poor filtered products.

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# 5. PATIENT MANAGEMENT

## A. Informed Consent

Once the patient is determined to be eligible for the trial and prior to randomization into one of the treatment arms, appropriate informed consent will be obtained from the patient (or the patient's guardian). The informed consent form must have prior approval of the Institutional Review Board of the local institution.

## B. Enrollment and Randomization

Enrollment will be by entering data from the Eligibility form on the clinical center's computer. This form will document the patient's eligibility for the trial. At the completion of data entry, the randomization assignment will be made by the computer. Age-eligible patients who are screened for the trial but found to be otherwise ineligible and those who are eligible but refuse consent will be recorded on the Eligibility form in order to maintain a record of the population from which trial participants are drawn.

Some patients who appear eligible initially will be found to have positive antibody status (either lymphocytotoxic or platelet) when baseline blood samples are analyzed by the central laboratory. These patients will be maintained on their assigned treatment arm, to the degree possible, and trial data will be collected on them. They will not, however, be included in the primary analysis of the trial.

## C. Blinding

All TRAP investigators, the patient, the patient's private physician, and staff clinicians and nurses treating the patient, will be blinded to the treatment arm assigned. This will be accomplished by blinding the platelet product by placing all trial products in a standard pooling bag for administration by the transfusion service. Details of labeling of the blood product will be left to local option, as long as the randomly assigned treatment of the product is not identified. The blood center will maintain records linking the patient's name to the TRAP identification number, randomization assignment, and preparation of the blood products, so that the blind can be broken, if necessary.

TRAP coordinators will not be blinded to the treatment arm assigned. They will be responsible for monitoring the blood center's preparation of the blood product.

## D. Transfusion Therapy

A prophylactic platelet transfusion may be given for platelet counts of ≤ 20,000/μl. However, a transfusion at a higher platelet count may be required if the patient has significant bleeding. (If the patient is having a surgical procedure, a prophylactic platelet transfusion may be given for platelet counts of up to 50,000/μl.) The standard dose of pooled random donor platelets for the trial will be 6 units. For the trial a dose of $4.2 \times 10^{11}$ apheresis platelets will be considered equivalent to 6 units of pooled random donor platelets. However, it is possible that the standard platelet dose of 6 units of pooled random donor or $4.2 \times 10^{11}$ apheresis platelets will need to be increased at local option for clinical reasons.

A prophylactic transfusion of red blood cells may be given at a hematocrit of ≤ 25. However, the patient's clinical condition may require a transfusion at a higher hematocrit count.

Patients should remain on their assigned treatment arm for all transfusions through 8 weeks from enrollment (even if a second course of chemotherapy is required during this period). The assigned transfusions should only be discontinued in the event of

1. Severe adverse reactions to the platelet transfusions
2. Granulocyte transfusion
3. Withdrawal of informed consent
4. Death

There should be no change in platelet transfusion therapy for patients who are antibody positive if the patient is not platelet refractory. Blood centers will attempt to provide the assigned transfusions if at all possible. If an assigned transfusion cannot be provided, the blood center should provide a transfusion product which is as close as possible to the assigned product. In particular, the product should be prepared as assigned (UV-irradiated or filtered) if possible. The patient should be returned to the assigned transfusions as soon as possible.

## E. Clinical Platelet Refractoriness

A patient is platelet refractory if the platelet corrected count increment (CCI) is < 5000, i.e., the difference between the pre- and post-transfusion platelet counts per $10^{11}$ platelets transfused per square meter of body surface area is < 5000. (An example of calculation of the CCI: if $4 \times 10^{11}$ platelets were administered to a patient with a BSA of 2 $m^2$ and the platelet count rose to 50,000 from a pre-count of 10,000, the CCI = $\frac{(50,000-10,000)2}{4}$ = 20,000.)

The post-transfusion count should be obtained within 60 minutes after completion of the transfusion. The pre-transfusion platelet count may be the morning count unless the transfusion is a test transfusion (see below). For test transfusions, the pre-transfusion count must be taken within 60 minutes of the transfusion.

The patient must be refractory to two consecutive ABO-compatible transfusions in the assigned transfusion treatment arm before the test transfusions begin.

First test transfusion - fresh platelets. If the patient is platelet refractory (CCI < 5000) to two consecutive ABO-compatible transfusions in the assigned treatment arm, he/she should be transfused with fresh random platelets prepared according to the patient's original treatment assignment. The fresh platelets should be as fresh as possible but must be transfused within 48 hours of collection. ABO compatibility is not required; however if a fresh transfusion is not ABO-compatible and fails, another fresh transfusion which is ABO-compatible is required to evaluate refractoriness. Patients who are not refractory to the fresh transfusion should continue on the assigned transfusion treatment arm, preferably with fresh platelets. Patients who are refractory to the "fresh" ABO-compatible platelet transfusion in the assigned treatment arm will be tested with an HLA-selected transfusion.

Second test transfusion - HLA-selected single donor platelets. Patients who are refractory (CCI < 5000) to the first test transfusion in the sequence should be transfused with the best available fresh HLA-selected single donor platelets, prepared as in the patient's originally assigned treatment arm. A "best" HLA matched transfusion is defined to be fully matched (an A match) or to have at most two blanks in the donor's HLA type with no incompatible antigens (B1U, B2U). ABO compatibility is not required; however if an HLA-selected transfusion is not ABO-compatible and fails, another HLA-selected transfusion which is ABO-compatible is required to evaluate refractoriness. The closest possible match should be used until a "best" match can be found. If an HLA-selected transfusion is successful, the patient is defined to be clinically alloimmune platelet refractory. The patient is defined to be clinically platelet refractory only if a "best" HLA-matched transfusion is not successful.

In both cases, further platelet transfusions should be prepared as the patient's originally assigned treatment arm prescribes; platelets may be HLA-selected, as available.

# 6. ENDPOINTS

## A. Primary Endpoint

The primary outcome measure is the time to first alloimmune refractory episode within the first 8 weeks after the first transfusion, either platelet or red blood cell, in the trial. For the primary endpoint a patient must be both clinically platelet refractory and antibody positive (lymphocytotoxic, platelet reactive, or platelet specific).

Clinically platelet refractory is defined as either one of the following:

Clinically platelet refractory is defined as refractory (the corrected count increment, i.e., the difference between the pre- and post-transfusion platelet counts per $10^{11}$ platelets transfused per square meter of body surface area, is < 5000) to (1) a fresh platelet transfusion prepared by the assigned treatment and (2) a "best" HLA-matched transfusion prepared by the assigned treatment.

Clinically alloimmune platelet refractory is defined as (1) refractory (corrected count increment, i.e., the difference between the pre- and post-transfusion platelet counts per $10^{11}$ platelets transfused per square meter of body surface area, is < 5000) to a fresh platelet transfusion prepared by the assigned treatment and (2) responsive to an HLA-selected transfusion prepared by the assigned treatment.

Antibody positive is defined as any one of the following:

A positive lymphocytotoxic antibody is defined as at least one cell with greater than 60% kill, or two or more cells with greater than 40% kill, confirmed by repeat testing on the same sample.

A positive platelet antibody sample is defined as a reproducible antibody reaction with platelets as the test cell.

A positive platelet specific antibody sample is reproducibly platelet antibody reactive with a non-HLA platelet surface antigen.

## B. Secondary Endpoints

The time to event (within 8 weeks) with regard to platelet refractoriness or alloantibody status alone will be secondary endpoints:

1. Clinical platelet refractoriness (platelet increment < 5000 per $10^{11}$ platelets transfused per square meter of body surface area) by 8 weeks.

2. Clinical alloimmune platelet refractoriness (platelet increment < 5000 per $10^{11}$ platelets transfused per square meter of body surface area)

by 8 weeks.

3. Lymphocytotoxic antibody status of the blood sample by 8 weeks. A positive antibody is defined as at least one cell with greater than 60% kill, or two or more cells with greater than 40% kill, confirmed by repeat testing.

4. Platelet reactive antibody status of the blood sample by 8 weeks. A positive reaction is defined as a reproducibly positive antibody reaction with platelets as the test cell.

5. Platelet specific antibody status of the blood sample by 8 weeks. A positive platelet specific antibody sample is reproducibly platelet antibody reactive with a non-HLA platelet surface antigen.

C. Other Outcome Measures

Other possible outcome measures include the following:

1. CMV status. Comparison will be made between CMV antibody negative patients who receive leukocyte-poor products versus those who receive standard products for CMV seroconversion. The last serum sample within 8 weeks will be compared to the baseline sample.

2. Leukemia status. Dates of response, relapse, death will be collected at each 6-month follow-up.

3. Antibody development and loss as determined from the weekly and monthly serum samples.

4. Type of antibody identified: lymphocytotoxic, platelet reactive, or platelet specific.

5. Bleeding events defined as follows:
    a. Any central nervous system hemorrhage
    b. Hematuria with clots
    c. Bright red blood, melena, or tarry stools per rectum
    d. Pulmonary hemorrhage (hemoptysis) followed by radiographic changes in the lungs
    e. Retroperitoneal hemorrhage

6. Number of red blood cell and platelet transfusions required during and after the initial 8-week period.

7. Severe adverse reactions to a transfusion. Severe reactions are characterized by the following reactions during or within one hour after transfusion:
    a. Increase in temperature greater than 3 degrees Fahrenheit (2 degrees centigrade)
    b. Chills with rigors

c. Extensive urticarial eruption
d. Moderate to severe pulmonary symptoms (dyspnea, bronchospasm, or cyanosis)
e. Anaphylaxis

8. Death.

# 7. DATA COLLECTION

## A. Baseline

The following data will be collected at baseline:

1. Information necessary to document eligibility and to stratify at randomization: age, prior history of hematopoietic disorder, leukemia classification, chemotherapy program, prior transfusion status, prior pregnancy status.

2. Baseline serum/plasma sample.

3. Baseline patient data.

## B. Acute Treatment Phase

During the first 8 weeks after the first transfusion (either platelet or red blood cell) in the trial the following data will be collected:

1. Weekly serum/plasma samples (even for patients who become platelet refractory or who suffer adverse reactions).

2. Data on response to each platelet transfusion, including adverse reaction data.

3. Data on each red blood cell transfusion.

4. Summary data on the 8-week outcome.

5. Serum sample at death.

In the event of a granulocyte transfusion or a bone marrow transplant during this period, no further data and no further serum samples will be collected.

## C. Follow-up

Follow-up will continue for each patient until the completion of data collection. The following data will be collected during follow-up.

1. Monthly serum samples to 1 year.

2. Transfusion history of platelet transfusions to 1 year.

3. Transfusion history of red blood cell transfusions to 1 year.

4. Leukemic status at 6-month intervals for the total duration of the

trial. For patients entered during the first year of the trial this will be approximately three additional years (after the first year) of follow-up of disease status.

5. Mortality and other event (bone marrow transplant, etc.) data for the duration of the trial.

In the event of a granulocyte transfusion or a bone marrow transplant during the first year, no further data and no further serum samples will be collected.

# 8. PATIENT SAFETY AND CONFIDENTIALITY

## A. Introduction

Patient safety is of primary concern in the trial, and confidentiality of all patient information is assured by all participating centers.

## B. Risks of Procedures

During the course of venipunctures used for blood sampling, blood may escape from the vein into the surrounding tissues, possibly producing a bruise associated with swelling and tenderness. In addition, if sterility has not been maintained during the venipuncture, there may be a risk of developing an infection at the venipuncture site.

As with the transfusion of any blood product, a virus may be transmitted. All donor blood is routinely screened for the presence of hepatitis B, hepatitis C, ALT, core antibody to hepatitis B, the AIDS virus, the HTLV-1 virus, and, on occasion, for CMV. In spite of testing, infections with these or other viruses may rarely still result from the transfusion. In addition, because platelets are stored at room temperature, bacterial contamination may occur if there has been a break in the sterility of the system.

Acute adverse reactions to the transfusions are discussed below.

## C. Adverse Reactions and Removal from Assigned Arm

The decision to discontinue the assigned transfusions for a patient who has an adverse reaction will rest with the principal investigator, in consultation with the attending physician. It is suggested that the assigned transfusions should be discontinued if the patient experiences the new onset of <u>three or more episodes</u> of moderate to severe transfusion reactions. These are characterized by the following reactions during or within one hour after transfusion:

1. Increase in temperature greater than 3 degrees Fahrenheit (2 degrees centigrade).

2. Chills with rigors.

3. Extensive urticarial eruption.

4. Moderate to severe pulmonary symptoms (dyspnea, bronchospasm, or cyanosis). Only two episodes of bronchospasm are required.

Anaphylactic reactions are not expected. If one should occur, the reaction should be carefully and promptly evaluated, and discontinuation of the

assigned transfusions should be considered.

The Coordinating Center will periodically present to the Data and Safety Monitoring Board reports on possible adverse reactions to blood products. These reports include the kind and frequency of adverse reactions, numbers of patients withdrawn from the assigned treatment arm, the reasons for these withdrawals, and medications given to prevent reactions.

D. Breaking the Blind

Situations may arise when it is in the best interest of the patient to identify the assigned blood product treatment. Such instances should be rare, as most problems can be managed without knowing the assigned treatment. In the event that the blind must be broken, either the principal investigator, the clinical coordinator, or the local blood center can be contacted . The coordinator will have recorded the assigned treatment on the patient's Eligibility form, which will be kept in the patient's TRAP file, and the blood center will also have a record of the assigned blood product treatment.

E. Confidentiality

It must be stressed to all clinical personnel that confidentiality of patient information must be preserved. No unauthorized personnel should have access to patient records or results of interviews or tests. All record storage should be appropriately secured, in locked rooms and/or locked cabinets.

No data containing patient identifiers will be maintained in the Coordinating Center database. Individual patients will be identified in the database only by a code number.

# 9. QUALITY CONTROL

## A. Introduction

The quality of any study is based on the quality of the data collected, and a high degree of accuracy and completeness in data collection and recording is essential. Consistency of the data collected must also be a concern, particularly in a multicenter trial. Quality control is a concern of all units of the trial, with the clinical centers responsible for the careful gathering and recording of the data and the coordinating center responsible for monitoring the quality of the data gathered and for maintaining the database.

## B. Blood Products

The quality of the blood products will be monitored by the Central Cellular and Immunology Laboratory:

1. Initial counting proficiency testing. Prior to the start of the trial the Central Laboratory will establish the counting proficiency of the clinical centers to assure accurate platelet and white blood cell counts on blood products with normal concentrations, with low white counts, and with high platelet counts. The counting proficiency of a clinical center must be certified prior to enrollment of patients at the center.

2. Continuing counting proficiency testing. Throughout the trial the Central Laboratory will provide to the centers monthly samples of filtered and non-filtered blood components for platelet and white cell counting. Results of the proficiency testing will be reported, so that corrective measures can be taken at centers that fall below a pre-set standard of counting proficiency.

## C. Trial Data

Quality control of data collection begins with well designed data forms, and with a detailed and comprehensible manual of operations. The forms and the manual will be completed prior to patient enrollment and will be available during the initial training session. The manual will include a detailed description of each form, item by item, as well as information on all aspects of the trial protocol.

At the initial training session the details of both the trial protocol and data collection will be presented to clinical coordinators. All data forms will be presented in detail, and techniques of patient management will be discussed. Coordinators will be trained in data entry and transmission. Further training sessions will be prepared for later meetings as necessary.

Data entry at the centers will be by means of a programmed forms entry package with range, code, and logical checks, required data fields, and double entry verification. This method will assure that the major percentage of data errors will be caught at the center and can be corrected in a timely fashion.

On a regular basis the database will be surveyed to determine the completeness of data submission for each patient. A report will be sent to each center coordinator, summarizing forms which are past due and those which appear to be incorrectly or inconsistently coded. Every month a monitoring report will be distributed to principal investigators and coordinators, assessing center performance on patient enrollment and data submission.

Periodically, accuracy of data entry will be checked. A sample of data forms will be called from the centers and compared with data entered into the study database. Inconsistencies will be resolved and corrections made where necessary. Reports on the accuracy of data entry will be sent to the centers, and an effort will be made to identify and correct problem areas.

Interform data inconsistencies and data outliers will also be identified, and centers will be notified of questionable data which must be verified or corrected.

# 10. STATISTICAL ISSUES

## A. Estimated Sample Size

Patient enrollment began on January 14, 1991. A total of 570 patients will be enrolled equally into the three treatment arms and the control arm. It is estimated that 416 of these patients will complete the full 8 weeks of the trial. Withdrawals will occur due to granulocyte transfusions, severe adverse transfusion reactions, death, and withdrawal of consent.

## B. Alloimmunization Endpoints

The primary outcome measure is the time to first alloimmune refractory episode within the first 8 weeks after the first transfusion, either platelet or red blood cell, in the trial. For the primary endpoint a patient must be both clinically platelet refractory and antibody positive (lymphocytotoxic, platelet reactive, or platelet specific). The primary endpoint will be analyzed by survival analyses, in which patients in whom the assigned treatment is discontinued due to death, withdrawal of informed consent, or other reasons will be treated as censored observations.

The 8-week analysis with regard to platelet refractoriness and alloantibody status alone will be secondary endpoints. There are five of these endpoints:

1. Clinical platelet refractoriness (platelet increment < 5000 per $10^{11}$ platelets transfused per square meter of body surface area) to all test platelet transfusions by 8 weeks.

2. Clinical alloimmune platelet refractoriness (platelet increment < 5000 platelets transfused per $10^{11}$ per square meter of body surface area) to all test platelet transfusions except for the HLA-selected platelet transfusion by 8 weeks.

3. Lymphocytotoxic antibody status of the blood sample by 8 weeks. A positive antibody is defined as at least one cell with greater than 60% kill, or two or more cells with greater than 40% kill, which is confirmed by repeat testing of the sample. The 8-week sample is the last blood sample drawn between 6 and 8 weeks.

4. Platelet reactive antibody status of the blood sample by 8 weeks. A positive reaction is defined as a reproducibly positive antibody reaction with platelets as the test cell.

5. Platelet specific antibody status of the blood sample by 8 weeks. A positive platelet specific antibody sample is reproducibly platelet antibody reactive with a non-HLA platelet surface antigen.

These are distinct measures - an individual patient can be positive for one of these and negative for others. The refractoriness endpoints will be analyzed

by survival analysis methods as described for the primary endpoint.

For any of these definitions of alloimmunization, the time-to-event analysis of alloimmunization rates depends on the assumption that alloimmunization is independent of the censoring mechanisms, which are death, severe adverse reaction, withdrawal of informed consent, and loss to follow-up. The most concern is the independence of death, which is assumed to be the major source of censoring. The independence assumption for death seems reasonable since most patients can be supported by HLA-matched apheresis platelets and it is not usual for patients to die because they become alloimmunized. The independence assumptions for all sources of censoring will be examined by comparing the times to censoring for patients who are alloimmunized and not alloimmunized. In addition, the robustness of the test statistics which compare alloimmunization rates among the treatment arms to departures from independence will be examined. One way to do this would be to count a certain percentage of censored observations as being alloimmunized at the time of censoring.

C. Other Analyses

Baseline characteristics of patients in each of the randomized groups will be compared. Covariates will be examined by descriptive statistics, tables, and graphical methods and compared by chi-square tests, non-parametric tests, and analysis of variance. In other analyses, care will be taken to consider and adjust for covariates which are found to have important differences among the groups.

The time to alloimmunization will also be analyzed by Cox survival methods. The effect of various patient and treatment characteristics will be studied. The units of blood given before a patient becomes refractory will be compared using a discrete time survival analysis with units of blood being the units of time.

Other outcome measures include leukemia disease status. antibody profile over time, and transfusion requirements. Leukemia status as measured by survival and time to remission will be compared among the groups by the log rank statistic. Cox survival analysis will be used to model the effect of covariates. Antibody profiles will be assessed by measurements based on the periodic samples. Time to development of antibody and time from development to disappearance of the antibody are of interest. Cox survival analysis with time-dependent covariates will be used to model the effect of clinical characteristics, events, and chemotherapy treatments. Transfusion requirements will be compared by analysis of variance and regression methods. Non-parametric methods will be used as appropriate.

D. Power and Sample Size

Power for the study is based on the exponential maximum likelihood estimate. For the ith treatment, assume that time to the first refractory episode is

exponentially distributed with parameter $L_i$. Let $t_i$ be the total time on study and $d_i$ be the number of first refractory episodes observed for treatment i. The maximum likelihood estimate of $L_i$ is $d_i/t_i$ and its variance is approximately $L_i^2/E(d_i)$, where $E(d_i)$ is the expected number of first refractory episodes observed for treatment i(1). By the delta method, the variance of $\ln(d_i/t_i)$ is approximately $1/E(d_i)$. Thus the natural log of the hazard ratio HR for treatments i and j is asymptotically normally distributed with mean $\ln(L_i/L_j)$ and variance $1/E(d_i) + 1/E(d_j)$.

The power is

$$P( \ln HR > s_0 z_\alpha) = P( Z > (s_0 z_\alpha - \ln HR_A)/s_A ),$$

where HR is the hazard ratio, $s_0$ is the standard deviation under the null hypothesis, $s_A$ is the standard deviation under the alternative, $z_\alpha$ is the normal $1-\alpha$ quantile, and Z is a normal(0,1) random variable. Thus, the necessary quantities to be estimated for the power calculation are $L_i$ and $E(d_i)$ for each arm.

In order to calculate the expected number of first refractory episodes observed $E(d_i)$, assume the time of censoring W is exponentially distributed with parameter w (the same for all treatment arms). Let Y be the time to first refractory episode and T the observation period. (In this study T is 8 weeks for all patients.) Then

$$P(\text{first refractory episode is observed}) = P(Y < W, Y < T)$$

$$= \int_0^T L_i \exp(-L_i t) \exp(-wt)\, dt$$

$$= (L_i/(L_i+w))(1-\exp(-(L_i+w)T))$$

Patients are assigned to each of the four treatment arms with equal probability. If there are N patients, the expected number of first refractory episodes observed in the ith treatment group is

$$E(d_i) = (N/4)(L_i/(L_i+w))(1-\exp(-(L_i+w)T))$$

Since P(not yet refractory at time T) = $1 - \exp(-L_i T)$ under the exponential assumption, if $p_i$ is the expected proportion of refractory patients at time T for treatment i, $L_i$ can be estimated by $(-\ln(1-p_i))/T$. Similarly w is estimated by $(-\ln(1-p_w))/T$ where $p_w$ is the expected proportion of patients censored by time T.

For TRAP, it is assumed that over 3 years 568 patients will be randomly allocated equally to 4 treatment arms. The observation period is 8 weeks, and it is assumed that 40% of the patients will be censored by 8 weeks, primarily due to early mortality. Mortality is assumed to be independent of alloimmune refractoriness. The estimated proportion of alloimmune refractory patients by 8 weeks in the control group is .50. Using the formulas above, for a simple

comparison between the control group and one of the treatment arms, the power to detect a reduction in alloimmunization to .30 with a .05 level two-sided test is .92. The Bonferroni method can be used to adjust for the three possible comparisons of treatment and control. Using this method, the power in each two-group comparison is .84. If a reduction from a control rate of .40 to an alternative rate of .20 is considered, the power is .95 for the simple comparison and .90 corrected for 3 comparisons. If the censoring rate is less than 40%, power will be somewhat greater than these estimates.

## E. Sequential Monitoring

The trial will be sequentially monitored at the meetings of the Data and Safety Monitoring Board, which are to occur at 6 months intervals (6 meetings). The usual two-group sequential monitoring schemes are not appropriate for TRAP since TRAP is planned to have 4 arms - a control and 3 treatments. The trial will be monitored in two ways. First, if any treatment is worse than the control, that treatment will be dropped. For this purpose the log-rank statistics for each two-group comparison (treatment versus control) will be used and compared to an O'Brien-Fleming boundary for a one-sided test with level .02. (The .02 level is a Bonferroni correction for multiple comparisons.) A treatment that is dropped by this test would be unlikely to be the best of the three treatments. Dropping this apparent loser would increase the power for the other comparisons as future patients would be allocated among only three arms.

A second sequential monitoring of overall significant differences will be done for advisory purposes. For this comparison, the log-rank statistic comparing all four groups will be compared to the O'Brien-Fleming boundary for a two-sided test with level .05. A significant difference will be brought to the attention of the Data and Safety Monitoring Board for further consideration. Simultaneous confidence intervals for all contrasts of interest will be provided to the committee. If the difference occurs because all three treatments are better than the control, presumably the control will be dropped and enrollment into the three treatment arms continued. If one of the treatments is better than both the control and the other two treatments, the trial might be stopped all together. Since there are many possibilities for a difference among the four arms, this monitoring rule is, as stated, only advisory.

Conditional power calculations will also be used for the monitoring process.

# 11. ORGANIZATION

## A. Introduction

The participating units in this clinical trial include seven clinical centers (one with two trial sites), a coordinating center, a central laboratory, and the National Heart, Lung, and Blood Institute (NHLBI) Project Office. A chairman of the Steering Committee and a Data and Safety Monitoring Committee have also been appointed.

During the planning phase, the organization consists of the Planning Committee and one Subcommittee. Subsequently, the Planning Committee will be transformed into the Steering Committee, and new Subcommittees as needed. An Executive Committee has also been formed.

## B. Participating Units

### 1. Clinical Centers

Seven Clinical Centers have participated in developing the protocol and are responsible for enrolling and following patients in the study. Specific responsibilities of each center are as follows:

a. To collaborate in the development of the study protocol.

b. To recruit and randomize patients, according to the protocol.

c. To collect the trial data accurately and completely and to transmit the data to the coordinating center in a timely fashion.

d. To perform all laboratory and other procedures as specified in the protocol, including submitting, upon request, blood product samples for quality control testing.

e. To administer the transfusion products according to the protocol.

f. To maintain patient files and to interview and examine the patients periodically according to the protocol.

g. To cooperate with other centers in assuring the trial is properly conducted.

h. To assist in making appropriate protocol modifications.

i. To participate in the analysis and reporting of trial results.

To accomplish the above, each Clinical Center has a principal investigator (with possibly a co-principal investigator), co-

investigator(s), and a clinic coordinator.

The seven centers and their principal investigators are as follows:

University of Florida, Gainesville, FL - Kuo-Jang Kao, M.D.
Johns Hopkins University, Baltimore, MD - Hayden G. Braine, M.D.
University of Maryland, Baltimore, MD - Charles A. Schiffer, M.D.
University of Minnesota, Minneapolis, MN - Jeffrey J. McCullough, M.D.
Puget Sound Blood Center, Seattle, WA - Sherrill J. Slichter, M.D.
Blood Center of Southeastern Wisconsin, Milwaukee, WI - Janice McFarland, M.D. (originally Jay Menitove, M.D.)
University of Wisconsin, Madison, WI - Robert D. Woodson, M.D.

2. Coordinating Center

The Coordinating Center has a major role in the design and implementation of the trial. Specific responsibilities of the Coordinating Center are as follows:

a. To provide scientific leadership and direction to the trial as a whole.

b. To attend to all management details of the trial.

c. To collaborate in the development of the study protocol.

d. To prepare the Protocol document and update it as needed.

e. To play a major role in the development, pretesting, and distribution of data forms.

f. To prepare the Manual of Operations and update it as needed.

g. To make a random assignment to a treatment group for each enrolled patient.

h. To receive, file, and analyze collaboratively trial data from all cooperating centers.

i. To check the completeness, accuracy, and timeliness of all data submitted to the Coordinating Center.

j. To prepare periodic reports recording the progress of the trial for the Clinical Centers, the Data and Safety Monitoring Committee, and the NHLBI Project Office. These reports include recruitment, adherence to procedures, and completion of forms.

k. To notify the Clinical Centers of problems with regard to adherence to the protocol and to keep the Project Office informed of major problems.

l. To analyze periodically the frequency of adverse reactions by treatment group and to report these data to the Data and Safety Monitoring Committee.

m. To assist the Clinical Centers in interpreting the protocol in unusual or ambiguous circumstances. This interpretation will be done by the medical consultant at the Coordinating Center in a manner best able to maintain the treatment blind for the appropriate persons, but not at the expense of patient safety.

n. To review patient management decisions for adherence to the protocol.

o. To participate in the analysis and reporting of trial results.

p. To assist in the preparation of other scientific reports of the trial.

q. To make visits to Clinical Centers with the Project Office staff, as necessary.

r. To analyze data from quality control testing done by the Central Laboratory.

s. To implement adequate security for any confidential trial data.

t. To help train Clinical Center personnel in the execution of the study protocol.

u. To prepare and maintain an address directory, including telephone numbers and FAX numbers.

v. To take, distribute, and maintain minutes of all trial meetings.

w. To transmit to the NHLBI Project Office tapes with all trial data, along with appropriate documentation, at the conclusion of the trial.

The Coordinating Center is located at the University of Washington, Seattle, WA. The director of the Coordinating Center is Kathryn Davis, Ph.D., and the Medical Consultant to the Coordinating Center is Sherrill Slichter, M.D.

3. Central Laboratory

Specific responsibilities of the Central Laboratory are as follows:

a. To perform lymphocytotoxic and platelet antibody testing on patient samples submitted by the clinical centers, in a timely fashion.

b. To develop and carry out a quality control program to assure accurate platelet and WBC counting at the clinical centers.

c. To perform MLC testing on UV-B irradiated platelet samples submitted by the clinical centers, in a timely fashion.

d. To develop and maintain a serum repository.

e. To record test results accurately and completely and to transmit the data to the Coordinating Center in a timely fashion.

f. To participate in the analysis and reporting of trial results.

4. National Heart, Lung, and Blood Institute Project Office

The Project Office is responsible for providing organizational, scientific, and statistical oversight and for participating in the design, conduct, and analysis of the trial. Project Office responsibilities are as follows:

a. To collaborate in protocol design, data analysis, and paper writing activities.

b. To maintain contact with trial investigators for the purpose of ensuring collection of high quality data. This may entail site visits. In cases of inadequate performance, the Project Office may consult with the Data and Safety Monitoring Committee to consider terminating participation of an individual center.

c. To organize and facilitate the functioning of the Data and Safety Monitoring Committee.

d. To implement major protocol changes (e.g., early cessation of the trial or of an individual treatment arm). The advice of the Data and Safety Monitoring Committee and Steering Committee will be sought.

e. To review manuscripts written for publication.

f. To be responsible for matters pertaining to NHLBI policy.

g. To be responsible for contractual and financial arrangements between NHLBI and participating Centers.

Charles Hollingsworth, D.P.H. (Project Officer), and George Nemo, Ph.D., are NHLBI program officials responsible for the trial.

5. Chairman of the Steering Committee

The Chairman of the Steering Committee is appointed by NHLBI and presides over meetings of the Planning Committee and the Steering

Committee.

Specific responsibilities of the Chairman of the Steering Committee are as follows:

a. To chair Planning Committee and Steering Committee meetings.

b. To lead all meetings of the Executive Committee.

c. To represent the clinical centers at the Data and Safety Monitoring Committee meetings.

d. To collaborate in the writing and reviewing of publications from the trial.

Kenneth Sell, M.D., Emory University, Atlanta, GA, is the Chairman of the Steering Committee.

6. Data and Safety Monitoring Committee

The Data and Safety Monitoring Committee acts in a senior advisory capacity to NHLBI throughout the duration of the trial. In addition, it periodically reviews trial results by treatment group and evaluates the treatments for beneficial and adverse effects.

The committee consists of a chairman and six additional voting members who are appointed by NHLBI for the duration of the trial. Additional committee members may be appointed by NHLBI. The chairman of the Steering Committee, the director and deputy director of the Coordinating Center, the medical advisor to the Coordinating Center, and the NHLBI program officials will participate as non-voting members. No voting member of the committee may participate in the trial as an investigator. The committee will meet twice yearly or more often, as necessary.

Specific responsibilities of the Data and Safety Monitoring Committee are as follows:

a. To review the Protocol initially and make recommendations to NHLBI.

b. To review subsequent changes to the Protocol and advise NHLBI.

c. To examine outcome and adverse reaction data by treatment group twice yearly.

d. To make recommendations to NHLBI on any proposed extension or early termination of the trial or treatment arm because of beneficial or adverse effects.

e. To assist NHLBI in resolving problems referred to it by the

Steering Committee.

f. To monitor the performance of the Clinical Centers, the Central Laboratory, and the Coordinating Center, and to advise NHLBI regarding discontinuation of any centers which perform unsatisfactorily.

The members of the Data and Safety Monitoring Committee are Harvey G. Klein, M.D. (chairman), Ronald G. Strauss, M.D., Margot Kruskall, M.D., Janice P. Dutcher, M.D., Janet Wittes, Ph.D., and Joseph C. Frantantoni, M.D.

C. Study Administration

1. Planning Committee

During the planning phase of the trial, the Planning Committee has responsibility for developing the study protocol and for initiating the development of the Manual of Operations and data forms.

The Planning Committee consists of principal and co-investigators from the Clinical Centers and Coordinating Center and the staff of the NHLBI Project Office. Each participating center has one vote on the Planning Committee. Kenneth Sell, M.D., the Chairman of the Steering Committee, serves as Chairman of the Planning Committee. The Planning Committee will be reconstituted into the Steering Committee once the protocol has been developed.

Subcommittee of the Planning Committee:

a. Data Forms: This subcommittee suggested data to be collected, at baseline, during the acute phase of the trial, and during follow-up.

2. Steering Committee

Subsequent to the planning phase, the Steering Committee will provide scientific direction for the trial at the operational level. It will consist of representatives from the Clinical Centers, the Central Laboratory, the Coordinating Center, and the NHLBI Project Office. Although more than one member of each center may attend meetings, each center has only one vote.

Specific functions of the Steering Committee are as follows:

a. To make scientific policy decisions.

b. To approve changes in the trial protocol. Major changes must also be approved by the Data and Safety Monitoring Committee and NHLBI.

c. To review the performance of the clinical and other centers with regard to quality control, patient recruitment, adherence to trial protocol, data forms completion, report generation, and special procedures.

d. To advise and assist the Coordinating Center and Central Laboratory on operational matters.

e. To review and approve ancillary studies.

f. To report major problems to the Data and Safety Monitoring Committee.

g. To collaborate in the writing and reviewing of publications from the trial.

The Steering Committee meets semi-annually. Additional meetings may be called by the Executive Committee if necessary.

3. Executive Committee

The Executive Committee consists of the chairman of the Steering Committee, the director of the Coordinating Center, the medical advisor to the Coordinating Center, and the NHLBI program officials.

Specific functions of the Executive Committee are as follows:

a. To make any decisions which might be required between Steering Committee meetings and to report these decisions to the Steering Committee. (If the issues are of major importance, a mail or telephone vote or a special meeting of the Steering Committee will be held.)

b. To recommend to the Steering Committee actions and policies for consideration.

c. To prepare the agenda for Steering Committee meetings.

d. To serve as ex-officio members of all other committees.

4. Subcommittees

To help the Steering Committee accomplish the task of managing the trial, subcommittees will be appointed as deemed necessary.