Chapter 1

VATS Protocol

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Section 1 -- Introduction

As many as 1.5 million Americans are currently infected with HIV-1.[1] Although there have been significant advances in development of relatively effective anti-retroviral therapies, as well as prophylactic and therapeutic strategies for prevention and/or treatment of several of the major opportunistic infections striking HIV-1-positive patients, these measures are only temporizing. Current estimates for the median time from infection to progression to end-stage disease (AIDS) range from 10 to 12 years,[2,3] with survival following a diagnosis of AIDS averaging approximately two years.[4] Prolongation in survival of HIV-1-infected patients is resulting in emergence of different manifestations of HIV-1-induced immunosuppression, including an increase in HIV-1-associated lymphomas, chronic wasting syndromes, and CNS manifestations.[1] These trends presage increased utilization of health care resources, including transfusion support,[5,6] by HIV-1-infected persons over the next decade.

Virologically, the course of HIV infection represents progression through a series of dynamic phases.[7] High levels of HIV-1 are present in the peripheral blood in the infected person early in infection prior to seroconversion.[8,9] These levels fall dramatically with the development of neutralizing antibody and cell-mediated immunity.[7-9] During the subsequent period of clinical latency, the concentrations of circulating virus in plasma and infected peripheral blood mononuclear cells are relatively low and stable,[7,10-13] although viral replication in lymphoid tissues continues at a moderate-to-high level,[7] resulting in turnover of up to 10^8 viral particles per day.[14] This high turnover leads to rapid emergence of viral mutants that are resistant to immune and anti-viral control, and which may have enhanced virulence. Progression of disease correlates with increases in the concentration of circulating virus in plasma, as measured by end-point dilution cultures or quantitative RNA assays such as polymerase chain reaction (PCR) branched DNA (bDNA) analysis.[7,10,12,13]

There is considerable variability in the length of the clinical latent period of HIV infection, and there has been much work to elucidate cofactors which govern progression of disease.[2,3,7,15] There is extensive in vitro[16] and in vivo[17] evidence indicating that HIV-1 replication is highly dependent on the activation state of target lymphocytes. Whereas unstimulated lymphocytes infected with HIV-1 do not yield virus, substantial viral replication occurs after these cells are stimulated immunologically by antigens or mitogens. Recent studies of HIV-1 replication in vivo following a variety of immunological events, such as vaccinations,[18] IL-2 infusions,[18] and spontaneous herpes viral infections,[18] have demonstrated significant increases in plasma viremia, presumably secondary to immune activation.[19]

There is also substantial evidence demonstrating that transfusion of allogeneic blood (particularly fresh, non-leukoreduced blood) results in immune activation.[20-23] Data from animal models indicate that this immune activation can result in viral reactivation,[24] as well as immunomodulation leading to enhanced tumor recurrence and bacterial infection.[22,25] In an in vitro study,[26] allogeneic donor leukocytes have been shown to induce reactivation of HIV-1 infection from peripheral blood mononuclear cells from HIV-1-infected persons, followed by dissemination of HIV-1 to uninfected cells. No effect was seen when leukocyte reduced blood components (packed red blood cells or platelets) were used in co-cultivation studies. These studies suggest that uninfected donor lymphocytes present in the transfused blood may activate HIV-infected and uninfected lymphocytes in the recipient, triggering an increase in viral replication and accelerating clinical progression.[26,27]

Three retrospective studies have supported an adverse effect of transfusion on the course of HIV-1 infection.[28-31] Ward et al. found that the number of blood transfusions given at the time of HIV-1 infection correlated significantly with a shortened time to development of AIDS.[28] The second study showed that the number of transfusions given in early AIDS was associated with a shortened overall survival.[29,30] In the third report, focused on patients with CD4 counts less than 250, Sloand et al. demonstrated that the incidence of clinically significant CMV infections correlated positively with the number of transfusion received.[31] A problem in interpreting these retrospective studies is that, although the authors attempted to control for the stage of disease, transfusion could be a surrogate for poor prognosis. Recently, two small prospective studies were conducted to examine the short-term effects of transfusion on HIV viremia in HIV-1-infected persons. These studies showed only a modest rise in plasma p24 antigen and HIV-1 RNA levels (determined by quantitative RT-PCR and bDNA assays) several weeks following

transfusion, compared to control non-transfused patients or recipients of filter-leukoreduced blood.[32,33] Of note, the blood transfused in these pilot studies was sub-optimal for inducing immune activation in the recipient: in one study only long-term refrigerated blood (>16 day storage) was used,[33] while the other study used irradiated blood components.[32] Storage and irradiation are known to markedly reduce the immune cell function and immune modulating properties of transfusions.[20,23,25,34-36]

Latent CMV infection may also be triggered to reactivate by transfusion, and this may be associated with contaminating allogeneic leukocytes in the transfused blood.[24,37] Leukocyte-reduction of blood has been established to decrease the risk of transmitting CMV from seropositive donors to CMV-seronegative recipients.[38] However, only one small pilot study involving cardiac transplant patients has examined the effect of leukoreduction on CMV reactivation and disease in seropositive individuals; that study showed a reduction in clinically significant CMV manifestations in recipients of leukoreduced blood vs non-leukoreduced blood.[39] Although primary and reactivation CMV infections are usually asymptomatic in immunocompetent persons, they can be life-threatening for immunosuppressed patients. Indeed, CMV is the most common serious viral infection in AIDS patients and is associated with retinitis, colitis, esophagitis, hepatitis, encephalitis, and pneumonia. Nearly 100% of homosexual men and injection drug users with HIV-1 infection have serological evidence of CMV infection, and more than half shed CMV in the urine or semen. As noted above, the retrospective study by Sloand, et al.[31] found a significant increase in CMV-related morbidity and mortality in HIV-1 infected persons following transfusion. Although the direct effect of allogeneic transfusions on CMV viremia in HIV-infected patients has not been studied in detail, preliminary data generated for this study show a several-fold increase in CMV DNA levels in leukocytes during the week post-transfusion in several HIV-positive recipients.[33]

Transfusion-associated graft-versus-host disease (TA-GvHD) occurs in immunocompromised persons who are transfused with blood containing viable allogeneic lymphocytes, i.e. non-irradiated blood stored at 4^{o}C less than 2 weeks.[40-42] TA-GvHD has been described in individuals with bone marrow transplants, congenital immune deficiency states, and in some cancer patients. Recently TA-GvHD has been reported in immunocompetent individuals who receive blood from family members with closely related HLA type, or from donors who, by chance, share an HLA haplotype with the recipient.[41,42] Data also suggest that donor lymphocytes proliferate transiently in immunocompetent recipients, apparently in an abortive GvHD reaction.[23] Adequate gamma-irradiation of blood products eliminates this risk. The American Association of Blood Banks recommends irradiation of all blood transfused to individuals with these underlying diseases, as well as to persons receiving blood from closely related persons. Surprisingly, TA-GvHD has not been reported in AIDS patients.[43,44] This may be because cases of TA-GvHD in AIDS patients go unrecognized because many of the clinical manifestations of GvHD regularly occur in late stage HIV-1 infection. Alternative explanations for the absence of TA-GvHD in AIDS patients include rapid infection and destruction of proliferating alloreactive donor lymphocytes by HIV,[43] or failure of the HIV-infected patient's immune system to contribute effector cells to the GvHD immunopathological process.[44,45] No studies have been reported to date that directly examined persistence of donor leukocytes in HIV-infected transfusion recipients.

Current transfusion practice for HIV-1-positive patients varies widely and is highly controversial.[5,6] Although theoretical arguments for allowing autologous donations by HIV-1-positive patients (when possible) are compelling,[46] concerns over possible transfusion error as well as the infectious exposure of blood bank and hospital personnel has led FDA to discourage this practice. Recent studies showing that erythropoietin (EPO) can reduce blood utilization in HIV-1-infected patients are promising.[47] However, these studies indicate that EPO is most effective in mild anemias and rarely eliminates the need for all allogeneic blood in HIV-1-positive patients; EPO is also expensive. Consequently, transfusion of allogeneic blood components will probably continue to be the major modality for managing HIV-1-associated anemia and thrombocytopenia for the foreseeable future.[5,6]

Unfortunately, there is currently a lack of consensus on how best to manage allogeneic transfusion support of HIV-1-infected patients. In a recent AABB survey of institutions with large HIV-patient populations, 36% of hospitals routinely use leukoreduced blood for all HIV-positive patients, 28% routinely irradiate all blood, and 20% use CMV-negative blood for all HIV-positive patients irrespective of recipient CMV status. Although there are no known adverse effects from the use of CMV negative blood components, CMV negative donors are limited in number and, depending on the region, vary from 50% to less than 10% of the population[38]. The use of filters for leukocyte reduction appears to be relatively safe although rare reactions to the filters, characterized by severe hypotension and respiratory distress, have been recorded[64]. The cost of such specialized component support is substantial, and will grow as more HIV-positive patients require transfusion. Clearly, data are needed to guide policy in this area.

The present study is designed to determine if transfusion of allogeneic blood to HIV-1-infected persons leads to immune activation and consequent induction of HIV-1 and/or CMV replication, and whether this adversely affects clinical prognosis. The study will determine the role of donor leukocytes in producing this activation by examining the effect of removing leukocytes by filtration on viral induction and clinical progression. Finally, the study will examine the long-term persistence of circulating donor lymphocytes after transfusion to determine if occult GvHD may be a significant occurrence in AIDS patients.

The study will be a multicenter randomized clinical trial including approximately 640 HIV-1-infected patients. Patients scheduled for transfusion will be entered into the study at the time of their first transfusion and randomized to one of two arms: unmanipulated (i.e., non-leukoreduced and non-irradiated) red blood cells or filter-leukoreduced red blood cells. Patients will receive all blood components treated in this way for a period of one to three years. The effect of repeated transfusions, if needed, will also be assessed. Blood samples will be taken from the individuals before transfusion and at varying intervals after transfusion. These samples will be studied using the polymerase chain reaction and branched DNA technology and, in selected cases quantitative cultures, to determine if activation of HIV-1 or CMV occurs following transfusion.[48-53] Serial samples will also be tested for a number of immunological markers to measure immune activation.[54-56] Lymphocyte survival kinetics will be examined in a subset of patients (i.e., female recipients of male blood) by allele-specific PCR assays.[23] Tests will also be performed on specimens collected at intervals when the patient is not being transfused to determine the stability of

values at baseline. The individuals will be examined clinically on a regular basis and monitored for incidence and recurrence of opportunistic diseases and overall survival.

Section 2 -- Objectives

2.1. Primary Objectives

In patients with HIV requiring RBC transfusion, to compare leukocyte reduced RBC versus non-leukoreduced RBC transfusions with respect to:

2.1.1. Overall Survival

2.1.2 Change in HIV viremia at first transfusion, as measured by RNA PCR, from pre-transfusion level to the level one-week post transfusion.

2.2. Secondary Objectives

In Patients with HIV requiring RBC transfusion, to compare leukocyte reduced RBC versus non-leukoreduced RBC transfusions with respect to:

2.2.1. Time to death or first new serious HIV-related complication.

Endpoints include selected new AIDS defining conditions and serious bacterial infections. The criteria for selection were that the condition has a median survival of less than one year or an acute mortality of >5%. Additionally, in most cases only definitively diagnosed conditions were selected.

These diseases include: disseminated fungal infections (cryptococcus, coccicidiomycosis, histoplasmosis), invasive cervical carcinoma, cryptosporidium, CMV disease, visceral Kaposi's sarcoma, lymphoma, progressive multifocal leukoencephalopathy (PML), disseminated *Mycobacterium* avium complex or atypical mycobacterium, tuberculosis, *Pneumocystis carinii* pneumonia, toxoplasmosis and bacterial infections of a normally sterile site (bloodstream, CSF, etc.). See Section 7.5 for details.

2.2.2. Time to new CMV end organ disease, in patients with no prior CMV disease. Incidence of new CMV end organ disease or progression of existing CMV disease, in patients with prior CMV disease.

2.2.3. Among patients with detectable CMV viremia, change in quantitative CMV from pre-transfusion level to one-week post transfusion level. Among patients with non-detectable CMV viremia, percent positive after transfusion, and CMV level in those who do become positive.

2.2.4. Slope of HIV & CMV viral load over time, excluding 28-day periods after initiation of each transfusion episode. This endpoint will measure the long-term effect on viral load, excluding the immediate post-transfusion periods.

2.2.5. Incidence of individual HIV-related complications and infections described in Section 7.5.

2.2.6. Change in cytokine and lymphocyte activation marker levels from baseline to one week: TNF, TNF receptor, IL-6, β_2M.

2.2.7. Change in lymphocyte markers, including CD4, CD8 and activated subsets (CD8+CD38+, CD8+HLA-Dr+).

2.2.8. Functional status (Karnofsky) and quality of life.

2.2.9. Persistence of donor lymphocytes in the recipient at one and three months after initial transfusion.

2.2.10. Area under the curve (AUC) of HIV and CMV viral load over 3-months post transfusion.

2.2.11. Other measures of change in viral load, such as change from baseline to mean level over weeks

1 - 4, change from baseline to individual time points, and area under the curve adjusted for baseline level.

2.3. Other analyses will explore the associations between laboratory-based biological end points and clinical outcomes.

Section 3 -- Patient Selection Criteria

3.1 Inclusion Criteria

3.1.1. HIV infected; must be documented or presumed at entry. (See section 8.1.2.2.)

3.1.2. Symptomatic anemia (as defined by the treating physician) with planned RBC product for a transfusion that can wait 4 hours.

3.1.3. The reason for the initial transfusion event must be non-surgical (e.g., no surgery requiring general anesthesia in the prior 2 weeks and the current transfusion is not in preparation for an upcoming surgery).

3.1.4. Age ≥14 years.

3.1.5. Patients must have evidence of current or past CMV infection. Patients must have documented CMV end organ disease or have a positive CMV culture, antigen assay, antibody assay or diagnostic histology prior to enrollment.

3.1.6 Karnofsky score ≥40 (See Appendix II).

3.1.7 Expected survival > 1 month.

3.1.8 Expected to be available for follow-up for at least one month.

3.1.9 Informed consent obtained from patient or guardian.

3.2 Exclusion Criteria

3.2.1. Prior transfusion history including red blood cells, whole blood, platelets or other blood components or transplantation of tissues/organs with potential for viral transmission (Factor concentrates, cryoprecipitate, plasma).

3.2.2. Intravenous immunoglobulin within 6 weeks prior to entry.

3.2.3. Initiation of a new antiretroviral therapy, systemic immunomodulator (e.g., IL-2, Interferons) or GM-CSF within 2 weeks prior to entry.

3.2.4. Thrombotic thrombocytopenic purpura (TTP).

3.2.5. Renal failure requiring any form of dialysis.

3.2.6. Patients who have medical conditions which, in the opinion of the study staff, will interfere with study compliance.

3.2.7. Patients requesting directed donations.

3.2.8 Patient's physician objects to in laboratory leukocyte filtration.

Section 4 -- Registration & Randomization

4.1. Treatment Groups

Patients will be allocated randomly to one of two treatment groups: Leukocyte Reduced Red Blood Cells or Non-Leukoreduced Red Blood Cells . Except as specified in section 5.3, all RBC transfusions required while on-study (see section 7.2 for definition of on-study) should be according to the randomized treatment group.

4.2. Stratification Factors

Randomization will be stratified by CD4 or total lymphocyte count and by CMV disease. A dynamic balancing method[57] will also be used to ensure treatment balance within each institution.

4.2.1. CD4 or Total Lymphocyte Count. For purposes of this stratification factor, CD4 count history should be based on medical record or physician report. If these are not available, CD4 count may be based on patient self-report if the clinical coordinator judges it to be reliable. The two levels of this stratification factor are:

4.2.1.1. CD4 count <50/μL at any time in the past. In cases where CD4 count was never known to be <50/μL and there is no history of CD4 ≥ 50 within the past month, total lymphocyte count < 1000/μL within one week prior to study entry.

4.2.1.2. Not meeting criteria 4.2.1.1 (i.e., available documentation or reports suggest CD4 ≥50/μL, within the past month, or in absence of this information, total lymphocyte count ≥ 1000/μL within one week prior to study entry).

4.2.2. CMV Disease. (All patients must have evidence of current or past CMV infection.) The two levels of this stratification factor are:

4.2.2.1. Current or previous diagnosis of CMV end organ disease (excluding viremia alone; see sections 7.3 and 7.4).

4.2.2.2. No current or previous diagnosis of CMV end organ disease.

4.3. Patients are registered and randomized with the following procedure.

4.3.1. Verify eligibility criteria (see sections 3 and 8.1), ascertain stratification factors, (sections 4.2 and 8.1.3) and obtain signed informed consent. Clinic Randomization Form is completed. Eligible patients are assigned the next sequential patient ID number.

4.3.2. Call the coordinating center (NERI) at (617) 923-1062 from a touch tone phone. The caller will be asked questions to ascertain clinical site, patient ID number, eligibility, and stratification factors. The caller will receive a coded treatment assignment in the form of a five digit number.

4.3.3. If phone is busy, wait five minutes and try again. If after three tries the phone is still busy, or if phone call does not go through, open the next sequential sealed envelope. The envelope will contain a coded treatment assignment in the form of a five digit number. Call the VATS Data Manager at (617) 923-7747, extension 417 to report the problem. Go to step 4.3.4.

4.3.4. Each site will have a set of labels with potential VATS ID numbers for that site. Two labels with the assigned VATS patient ID should be used. One goes on the Clinic Randomization Form and other goes on the Blood Bank Randomization Form. The coded treatment assignment should be written on the Blood Bank Randomization Form.

4.3.5. The original Blood Bank Randomization Form is taken to the blood bank. (A copy should go to the study file). The blood bank will have the key to determine to which treatment group the patient has been assigned.

4.3.6. Blood bank personnel will circle the assigned treatment assignment on the Blood Bank Randomization Form and prepare the blood units (See Section 5.1.) IT IS IMPORTANT TO KEEP THE TREATMENT ASSIGNMENT BLINDED TO OTHER STUDY STAFF AND TO THE PATIENT. The blood bank will keep a copy of the Blood Bank Randomization Form (again, not available to other staff) and refer to it in the event of any subsequent transfusions. (Ordinarily, all subsequent transfusions will be prepared in the same way as the original transfusion. See section 5.3).

4.3.7. The Blood Bank will mail the original Blood Bank Randomization Form to NERI within 24 hours, using the envelope provided.

4.3.8 NERI will send a confirmation of registration to the clinical coordinator.

Section 5 -- Product Preparation, Administration & Blinding

5.1 Study Components

For the purposes of this protocol, the following terms will be used interchangeably and all refer to a blood component rendered leukocyte reduced as specified: leukocyte reduced, leukoreduced, leukodepleted, or leukocyte filtered.

5.1.1. Red cell leukoreduction will occur prestorage, within 72 hours of collection.

5.1.2. Leukoreduction filter type will not be specified but will be recorded. Study sites will obtain from their suppliers blood filtered to contain no more than 5×10^6 leukocytes. Quality assurance data will be obtained from suppliers.

5.1.3. No units will be more than 14 days in age. Within the inventory of 14 day old or less units, the oldest unit will be chosen for issue. If no units 14 days or less are in the inventory, the blood bank should make every effort to obtain a unit(s) of appropriate age. The age of all units will be recorded. Following transfusion of every 20 units at a site, NERI will evaluate for bias of age between the two arms.

5.1.4. All platelet components will be leukocyte reduced unless emergent transfusion is indicated in which case every effort will be made to provide a leukocyte depleted component. Apheresis platelets are preferred, however the freshest available platelet components are to be issued.

5.1.5. A whole blood segment will be obtained from the original collection bag. A second sample of donor blood will also be obtained from the study bag of packed red blood cells at the time of issue. For platelet transfusions, a single sample at the time of issue will be retained (from each apheresis unit or pool). (See Laboratory Procedures section of the VATS Study Manual for storage and shipment information.)

5.1.6. Components will not be required to be CMV screened prospectively.

5.1.7. In compliance with AABB standards and federal regulations, all red blood cells will be ABO group compatible. Within these standards and for the purpose of this study all red blood cell units issued will be either group O or A. Red blood cell components will not be irradiated, except as per section 5.1.10.

5.1.8. Leukocyte filtration of all blood components will be performed at the blood center or transfusion service unless emergency transfusion requires bedside filtration per section 5.1.4.

5.1.9. The weight of each component will be recorded by transfusion service staff at the time of issue.

5.1.10 Irradiated study components will be allowed if, after enrollment, a patient develops a condition (e.g., lymphoma) requiring irradiated components. The patient should continue to receive blinded study components, either leukoreduced or non-leukoreduced, except that irradiation of components will not be prohibited.

5.2 Blinding

5.2.1. All investigators, including the clinical coordinator, will be blinded.

5.2.2. All patient care personnel and the patient will be blinded.

5.2.3. Only the blood bank technical staff will know the study arm. Clinical coordinators will make a phone call to NERI and receive a coded treatment assignment number. The blood bank technical staff will have a list of translations for the coded treatment assignments. See section 4.1.

5.2.4. All study components will have a "generic" study component label. Transfer into a study bag will be performed at the time of issue. Records as to leukoreduction of components will be kept at the blood center or transfusion center.

5.2.5. Transfusion medicine physicians will remain blinded at the time of investigation of transfusion reactions (see Sections 7.6, 7.7).

5.2.6. A designated transfusion coordinator at each center, other than the investigators and clinical coordinator, will review records to ensure protocol compliance.

5.3 Discontinuation of Blinded Study Components.
There are 4 possible reasons for discontinuation of study products:

a. Patients who meet transfusion reaction criteria for leukoreduction

The criteria in Sections 7.6 and 7.7 will guide the transfusion medicine physician in evaluation of transfusion reactions for the purpose of removing a patient from study components and placing him/her on leukocyte reduced blood components. At the time of evaluation of a transfusion reaction, the transfusion medicine physician will remain blinded. If the patient meets criteria as defined in section 7.7. and signs and symptoms are uncontrolled by premedication with antipyretics, antihistamines or meperidine, the transfusion medicine physician may elect to place the patient on all leukocyte reduced components without unblinding of prior components. However, subsequent components will not be blinded. If, despite providing the patient with leukoreduced blood components, reactions persist, the transfusion medicine physician will be unblinded in order to determine if reactions may have been due to leukocyte filters.

b. Patient's M.D. requests leukoreduced components.

This will be reviewed by the transfusion medicine physician and, if felt to be necessary, will be discussed with the Patient's M.D.

c. Withdrawal of consent to receive a study component.

d. Patients retrospectively found to be CMV sero-negative.

5.3.1. Once removed from study components and placed on leukoreduced components, data and specimen collection should continue as scheduled in the protocol.

5.3.2. All red cell units transfused to these participants will continue to be $\leq$ 14 days old, prestorage filtered, and issued from the study inventory. However it will not be necessary to transfer the unit to a study bag or affix a blinded study label at the time of issue.

Section 6 -- Concomitant Medications

6.1. This protocol will not affect routine clinical care or mandate a change in the use of any prophylactic or therapeutic medication (other than protocol specified preparation of blood components and provision of ophthalmologic examinations). However, within the first 2 weeks after the first 2 transfusion episodes (see section 7.1 for definition), enrollees and their care providers are strongly discouraged from adding new antiretrovirals, systemic immunomodulators, systemic corticosteroids, anti-virals or GM-CSF, because of their potential effect on the study's virologic measures.

Agents falling into these categories are:

Antiretrovirals/Systemic Immunomodulators/GM-CSF
ritonavir (Norvir)
nelfinavir (Viracept)
bis-Pom-PMEA
delavirdine mesylate
didanosine (ddI, Videx)
hydroxyurea (Hydrea)
interleukin 2
interferon alpha (Roferon, Wellferon, Intron A)
interferon beta (Betaseron)
interferon gamma (Actimmune)
lamivudine (3TC)
loviride
indinavir (Crixivan)
nevirapine
saquinavir (Invirase)
stavudine (d4T, Zerit)
thalidomide
zalcitabine (ddC, Hivid)
zidovudine (AZT, ZDV, Retrovir)
other antiretrovirals or immunomodulators
GM-CSF

Antivirals
acyclovir (ACV, Zovirax)
CMV monoclonal antibodies
cidofivir (HPMPC)
famciclovir (Famvir)
foscarnet (Foscavir)
ganciclovir (oral, IV, implant) (Cytovene)
valacyclovir (Valtrex)
other antivirals

Systemic Corticosteroids
prednisone
hydrocortisone
dexamethasone
other systemic corticosteroids

6.2 Routine use of corticosteroids as premedication for transfusions is strongly discouraged.

6.3. The use of accepted prophylaxis for opportunistic infections [e.g., PCP prophylaxis] is encouraged.

6.4. Use of erythropoetin (EPO) and G-CSF is allowed.

Section 7 -- Definitions and Endpoints

7.1. Transfusion Episode

A new transfusion episode begins whenever a RBC transfusion is given ≥ 7 calendar days after the most recent RBC unit was transfused. This 7 calendar day interval does not require that seven 24 hour periods have elapsed. For example, if the first transfusion consists of 1 or more units given on a Monday evening, the earliest that the next transfusion episode could begin would be the following Monday morning. If the first transfusion is on a Monday with another transfusion on Wednesday, the earliest that the next transfusion episode could begin would be the following Wednesday.

7.2 On Study

Once randomized , all patients will remain "On Study", unless they withdraw voluntarily, are lost-to follow-up, die or the study ends or is terminated early. If patients refuse blood draws or in-person visits but are willing to be followed by telephone, chart review, or contact with their care provider, then they will remain "On study". If a patient discontinues study components (see section 5.3), then they will remain "On study".

7.3. CMV end organ disease; Criteria for diagnosis:

7.3.1 Retinitis - diagnosis by ophthalmologist.

7.3.2 GI - diagnosis by histopathology with compatible clinical symptoms, signs or laboratory findings

7.3.3 CNS - compatible symptoms/signs with positive CMV CSF culture, PCR or other diagnostic tests.

7.3.4 Pulmonary - requires histopathologic confirmation by biopsy or autopsy.

7.3.5 Other involved organ (e.g., skin) - requires histopathologic confirmation or Ag or nucleic acid detection

7.4. CMV disease Progression; patients with prior CMV end organ disease

7.4.1. Retinitis - involvement of a new eye, or a new lesion in previously affected eye or progression requiring a change in anti-CMV therapy, or

7.4.2. Involvement of a new organ system (see section 7.3 above for definitions). For purposes of GI disease classification, the areas from the mouth to the proximal duodenum will be considered one organ. The area distal to the proximal duodenum is a second organ.

7.5. HIV-Related Complications.

HIV-related complications for the primary endpoint must be definitively diagnosed (except for toxoplasmosis and progressive multifocal leukoencephalopathy; see below) and were chosen to be conditions having a median survival of less than one year or an acute mortality of >5%.

The date of the event will be the date of the diagnostic study, with the exception of diagnoses based on autopsy findings which will be dated based on presenting symptoms/signs or laboratory findings.
Each event must be reviewed by a clinical physician at the site and must be readily available for central review. Documentation of the first event in each patient will be forwarded to NERI for central review by an endpoints committee.

The conditions and criteria for diagnosis are adapted from the CDC 1993 Criteria. [1]

HIV Related Condition	Diagnostic Criteria
Pneumocyctis carinii pneumonia (PCP)	Microscopy (histology or cytology)
Chronic intestinal cryptosporidiosis (>1 month duration)	
Visceral Kaposi's sarcoma	
Lymphoma (Burkitt's, immunoblastic, primary brain)	
Invasive cervical cancer	
Progressive multifocal leukoencephalopathy (PML)	Neurological syndrome consistent with PML and characteristic CNS radiographic study and either 1) Histological confirmation OR 2) PCR positive for JC virus on CSF.
Disseminated or extrapulmonary coccidiodomycosis	Microscopy (histology or cytology), culture, or detection of antigen in a specimen obtained directly from the tissues affected or a fluid from those tissues.
Extrapulmonary cryptococcosis	
Disseminated or extrapulmonary histoplasmosis	
CMV disease	See section 7.3 for diagnostic criteria
Disseminated or extrapulmonary *Mycobacterium avium* complex (MAC)	Positive blood culture or positive tissue culture
Disseminated or extrapulmonary *M. Kansasii*	
Disseminated or extrapulmonary other or unidentified species of *myco-Bacterium.*	
Mycobacterium Tuberculosis (TB)	Positive culture from any site or tissue
Toxoplasmosis of brain	The definition for presumptive diagnosis will be used. This includes: (a) Recent onset of focal neurologic abnormality consistent with intracranial disease or a reduced level of consciousness, AND (b) Evidence by brain imaging of a lesion having mass effect or radiographic enhancement with contrast, AND (c) Serum antibody to toxoplasmosis or successful response to therapy for toxoplasmosis

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HIV Related Condition	Diagnostic Criteria
Bacteremia	Laboratory-confirmed bloodstream infection must meet at least one of the following criteria: (a) One positive blood culture in the presence of systemic symptoms or a focal source of infection. OR (b) Isolation of the same bacterial pathogen from two or more positive blood cultures drawn at different sites or times. All positive blood cultures potentially meeting the above criteria should be reviewed by a clinical investigator at the site. For organisms commonly associated with blood culture contamination (e.g., diptheroids, coagulase-negative staphylococci *Bacillus*., *Proprionibacterium* spp., or micrococci), the blood culture(s) will <u>not</u> be included as bacteremia if the episode is not treated with antibiotics and the symptoms resolve or are found to be due to an unrelated cause.
Other bacterial infections	Isolation of bacterial pathogens from a normally sterile site, e.g., cerebrospinal fluid (CSF), pleural space, peritoneum
Catheter - related Bacteremia	a) Isolation of the same bacterial pathogen from a blood culture and a catheter tip or exit site culture in a patient with an indwelling or peripheral intravenous catheter and no other alternative site of infection. OR b) Isolation of a common skin contaminant (e.g., diptheroids, coagulase-negative staphylococci, *Bacillus* spp., *Proprionibacterium* spp., or micrococci) from two or more blood cultures drawn on separate occasions in a patient with an indwelling or peripheral intravenous catheter and no other alternative site of infection and the presence of one or more systemic symptoms or signs of infection. OR c) Isolation of a common skin contaminant (see above) from a single blood culture in a patient with an indwelling or peripheral intravenous catheter, no alternative site of infection, systemic signs/symptoms and in whom antibacterial therapy is instituted and continued for at least 72 hours with the patient demonstrating a favorable clinical response.

7.6. Reaction to red blood cell transfusions.

Signs or symptoms must occur during, or within 30 minutes following, a transfusion.

7.6.1. Definite Transfusion Reaction consists of a temperature rise $\geq 1\ C^{o}$ with no other explanation for fever and/or a history of recent recurrent fevers (no fever within 7 days).
OR
Chills and/or rigors with no other explanation for symptoms.

7.6.2. Possible Transfusion Reaction consists of a temperature rise $\geq 1\ C^{o}$ when other explanations for the fever exist,
OR
Chills and/or rigors, when an other explanation for symptoms exists.

7.7. Any of the following are criteria for considering leukoreduction:

1. 2 definite reactions
2. 1 definite and 2 possible reactions
3. 3 possible reactions
4. One or more of the following without other explanation of the symptoms occurring during or within 30 minutes following a transfusion:
    a. Hypotension as indicated by a 30% drop in systolic blood pressure or a recorded systolic blood pressure of less than 90mm Hg.
    b. Respiratory distress requiring intubation and/or supplemental O2 not due to volume overload.
    c. Chest pain of myocardial origin as defined by ECG changes and/or response to nitroglycerin.

7.7.1 The clinical coordinator will review all transfusion reports and those meeting the above criteria will be brought to the attention of the transfusion medicine physician. See section 5.3.a.

Section 8 --Clinical and Laboratory Evaluations

8.1 Screening and Pre-Entry

The screening and pre-entry period begins at the point a patient is identified as a potential study participant and ends at the point of central registration/randomization for eligible patients, or after review of eligibility requirements, for those ineligible. The following is a sequence of evaluations and tasks that need to be completed during the Screening and Pre-Entry period:

Screening:

8.1.1. Obtain written informed consent from participant or, when applicable, from participant's guardian.

8.1.2. Review patient's medical and medication history in relation to study inclusion and exclusion criteria. To be eligible for the study, patient must meet all inclusion criteria and not have a current (or previous when applicable) history that includes any of the specific study exclusions. To determine study eligibility, medication and medical histories may be obtained from patient self-report (if patient is deemed reliable), medical records, or through communication with the patient's primary physician, **with the following exceptions:**

8.1.2.1. Confirming CMV serostatus: If a history of CMV disease or a positive CMV serostatus cannot be reliably confirmed by these sources, CMV serology testing must be performed and results known PRIOR to randomization. In this case, only those who test positive for the CMV antibody will be eligible to enroll.

8.1.2.2. Obtain documentation of HIV-1 infection through one of the following positive tests: confirmed HIV-1 antibody, or HIV p24 antigen, HIV-1 culture, HIV-1 viral RNA, if available. If hard copy documentation is unavailable but HIV-1 infection is highly likely based upon available medical history, patients may be enrolled presumptively. HIV-1 EIA will be performed on all patients in the study by the central lab, using the baseline blood sample.

Pre-Entry**:**

8.1.3 Ascertain CD4 and CMV disease history/current status for treatment stratification purposes (see section 4.2). Patients cannot be randomized until this information is obtained. If a history of CD4 < 50 at any time in the past or CD4 ≥ 50 within the previous month is not available, and a recent absolute lymphocyte count (within 1 week) is not available, the enrollment CBC with platelets and WBC differential need to be drawn and sent STAT at this time.

8.1.4 Assess Karnofsky status.

8.1.5. Following instructions in section 4.3, assign the patient a study I.D. number, call NERI to register and randomize the patient, and forward the randomization number to the transfusion service.

Entry and Pre-Transfusion:

8.2 The patient is formally entered into the trial upon registration and randomization. The following may be obtained at screening, pre-entry or entry, but must be performed prior to the enrollment transfusion:

8.2.1. Obtain pre-transfusion blood draw for VATS' central lab as follows: 7 ml EDTA (purple top) tube, 7 ml ACD (yellow top) tube and 7 ml red top tube. This blood draw should be within 72 hours prior to transfusion and has a higher priority than the blood draw for local laboratory (see section 8.2.2).

8.2.2. Abstract results of CBC with differential

and platelet count obtained within 72 hours of entry, or draw and send pre-transfusion.

8.3 The following may be obtained at screening, pre-entry, or at anytime during the enrollment (entry) visit unless otherwise specified:

8.3.1. Obtain history of all AIDS-defining conditions and record site and date of any previous CMV end organ disease.

8.3.2. Assess approximate total duration of any prior use of licensed or experimental anti-retrovirals.

8.3.3. Medication history within 30 days of entry. If uncertain obtain further data from provider or records.

8.3.3.1. Start/Stop dates for antiretrovirals, systemic immunomodulators (including cortico-steroids and thalidomide), GM-CSF, EPO, antivirals, and any blinded study antiviral or antiretroviral medications (see section 6.1). No doses will be obtained.

8.3.3.2. Yes/No for the following categories: PCP prophylaxis or therapy, MAC prophylaxis or therapy, Chemotherapy, and treatment for wasting.

8.3.4. Obtain history for recent (previous three months) symptoms suggestive of CMV retinitis.

8.3.5. Obtain history for recent (previous three months) immunizations.

8.3.6. Measure and record weight and height.

8.3.7. Administer quality of life questionnaire, preferably at the entry visit but no more than 1 week after entry.

8.3.8. Ophthalmologic examination with direct and indirect dilated examination by an experienced ophthalmologist should be obtained within 3 weeks prior to or after entry.

8.4. Follow-up clinic visits.

8.4.1 Laboratory Specimens: At weeks 1 (± 1 day), 2, 3, 4 (± 2 days) and every 3 (± 1 month) months after the first enrollment transfusion episode begins, blood specimens will be obtained, processed and frozen for batch shipment to the central laboratory. In addition, central lab blood specimens will be obtained within 72 hours pre-transfusion and at weeks 1 (± 1 day), 2, 3 and 4 (± 2 days) following the beginning of the second transfusion episode (see section 7.1). If the pre-transfusion blood draw is missed at the second transfusion episode, the weekly blood draws should be skipped and obtained instead at the third transfusion episode. If the second transfusion episode begins during the first 28 days of the initial episode, cancel any remaining weekly blood draws associated with the first episode and start over for the second episode. If there is a quarterly clinical visit during the 28-day period following the second transfusion episode, both the weekly blood draws and the quarterly blood draws should be obtained, if possible. The weekly blood draw and local (CBC with differential and platelets) samples should take precedence over the quarterly central labs if both cannot be obtained. Blood specimens will also be obtained for local testing at entry and at each quarterly visit. These have lower priority than specimens for the Central Lab.

8.4.1.1 Obtain 7 ml EDTA (purple) tube , 7 ml ACD (yellow) tube, and 7 ml red top tube for each scheduled central laboratory blood draw. These specimens will be sent to the clinical site lab which will aliquot them and ship to central lab as per Laboratory Procedures section of the VATS Study Manual.

8.4.1.2 Parameters measured at Central Lab:

1. EDTA blood: CD4 and CD8 counts (and activated lymphocyte subsets), donor WBC survival, IL-6, TNF, and storage.
2. ACD blood: Quantitative CMV testing, HIV plasma RNA PCR, and storage.
3. Serum: TNF-R EIA, β_2-microglobulin EIA, EPO RIA, CMV EIA screen, HIV-1 EIA screen, and storage.

8.4.1.3 Obtain EDTA (purple top) tube for local CBC with differential and platelet count testing every 3 months during the study.

8.4.2. Patients will be contacted every 3 months (±1 month) (i.e., at 3, 6, 9, 12 months, etc.) to collect clinical data. Every attempt should be made to schedule these contacts close to the center of the two-month window. Collection of the "data from patients" is preferably done in person. However if patients refuse blood draws or in person visits, data may be collected via telephone, (see section 7.2) chart review, or contact with patient's provider. Additionally, if certain data is obtained by patient's report, it must be verified by review of medical record (or contact with patient's care provider).

8.4.2.1. Mortality Status. If dead obtain date of death and causes. If possible obtain autopsy report.

8.4.2.2. Assess Karnofsky status.

8.4.2.3. Assess development of new serious HIV-related complications (section 7.5) since last interview. Collect and submit documentation of results of diagnostic tests required for any new serious HIV related complications.

8.4.2.4. Assess transfusion at other sites since last interview.

8.4.2.5. Assess development of renal failure requiring dialysis since last interview.

8.4.2.6. Obtain start and stop dates for medications (see section 6.1) since last interview.

8.4.2.7. Assess use of other medications since last interview.

8.4.2.8. Assess receipt of influenza or pneumococcal vaccine since last interview.

8.4.2.9. Obtain history of symptoms suggestive of CMV retinitis since last visit.

8.4.2.10. Obtain weight. (A home scale is acceptable.)

8.4.2.11. Administer the quality of life questionnaire.

8.4.3. Every 6 months (e.g., 6, 12 months, etc.), patients should have an evaluation by an ophthalmologist. If the patient misses the ophthalmologic exam, obtain it at the next clinical visit. The evaluation would include dilated indirect ophthalmoscopy performed by an experienced ophthalmologist. (This evaluation is for research purposes and is funded by the study.)

Section 9 -- Study Parameters

Schedule of Clinical and Laboratory Evaluations

		Transfusion Episode Begins	Days after 1st & 2nd Transfusion Episodes Begin				Every 3 Months After 1st Transfusion
	Entry	**Day 0**	**7** (± 1)	**14** (± 2)	**21** (± 2)	**28** (± 2)	**Months 3-24** (± 1 month)
Consent	X						
Randomization	X						
Medical History	X						X
Medication History	X						X
Eye Symptom History	X						X[6]
Weight	X						X
Height	X						
Karnofsky	X						X
QOL	X[9]						X
Platelets	X[4]						X
CBC with differential	X[4]						X
CMV Antibody	X[5]						
Dilate direct/indirect ophthalmoscope	X[1]						X[8]
Central Labs (7ml EDTA 7ml ACD 7ml red top)		X[2,3]	X[3]	X[3]	X[3]	X[3]	X
Transfusion		**X[7]**					

1 Initial eye exam within ± 3 weeks of enrollment.
2 Must be drawn pre transfusion for 1st and 2nd transfusion episodes only.
3 Pre transfusion and weekly blood draws performed following the 1st and 2nd transfusion episodes only: If pre-transfusion blood draw is missed at 2nd transfusion episode, skip weekly bloods and obtain instead at 3rd transfusion episode.
4 Within 72 hours prior to enrollment transfusion.
5 If not drawn pre-randomization or results not available in chart for abstraction.
6 Refer to Ophthalmologist if CMV symptoms exist
7 Ordinarily day 0 of the first transfusion episode will be the same as the day of entry into the trial.
8 Completed every 6 months, instead of every 3 months, plus when clinically indicated.
9 Preferably prior to or during initial transfusion, but can be up to 1 week post initial transfusion.

Section 10 -- Forms Submission & Specimen Shipping Schedule

10.1 VATS data collection forms will be supplied to sites by the coordinating center (NERI). All completed forms should be mailed to NERI once a week as follows:

Attn: VATS Data Manager
New England Research Institutes
9 Galen St.
Watertown, MA 02172

Sites should xerox and retain a copy of all forms in their patient study files and mail the originals to NERI.

10.2 Patient blood samples, red blood cell and platelet unit segments will be shipped to the Central Laboratory (Irwin Memorial Blood Center) on a regular schedule. Complete shipment content logs are to be enclosed in the shipment, with NCR forms and copies of logs forwarded to the Data Manager at NERI (see address above). The central lab will provide all materials needed for storage and shipment of samples, except for dry ice. Shipments are to be made via Federal Express to:

Irwin Memorial Blood Center
Attn: VATS Central Lab, Megan Laycock
270 Masonic Ave.
San Francisco, CA 94118
Phone: (415) 749-6645

Refer to Laboratory Procedures section of the VATS Study Manual for complete specimen collection, storage and shipping information.

Section 11 -- Statistical Considerations

11.1 Design

This trial will be a two-group, double-blind, randomized trial comparing standard non-leukoreduced red blood cell (RBC) transfusions with leukoreduced RBC transfusions in 640 patients. Randomization will be stratified by CD4 history (or total lymphocyte count; see below) and by CMV end organ disease. All patients must be CMV seropositive.

The CD4 stratification is based on a cut point of 50 cells/μL. Because there will be insufficient time to measure CD4 lymphocyte counts at the time of randomization, assignment to CD4 strata will be based on either patient report or Total Lymphocyte Count (TLC) as a readily available proxy of absolute CD4 count. Specifically, if by available documentation or patient report the absolute CD4 count was known to be < 50 at any time in the past, the patient will be assigned to the < 50 CD4 stratum. If by documentation or patient report, the absolute CD4 count was never below 50 and there was either documentation or patient report of an absolute CD4 ≥ 50 within the past month, the patient will be assigned to the ≥ 50 CD4 stratification. If there is insufficient information for assigning a stratum as outlined above, the patient will be assigned to a CD4 stratum based on TLC measured within 1 week prior to randomization. The TLC cut point chosen for this study is < 1000/μL· Previous research[58] had modeled TLC as a predictor of absolute CD4 at a CD4 cut point of 200 cells/μL. To estimate the operating characteristics of TLC for a CD4 cut point of 50 cells/μL, a dataset from one of the VATS sites was analyzed. 164 patients with CD4 counts less than 100 and date matched TLC measurements were analyzed in classification tables varying by TLC cut points (1000, 1250, and 1500). A dataset restricted to patients with < 100 CD4 cells corresponds roughly to the CD4 distribution anticipated among VATS eligible patients. A survey of VATS sites during the design of the trial indicated that very few patients undergoing first RBC transfusions had CD4 ≥ 100 and that the median count was < 50. The "optimal" TLC cut point for predicting CD4 < 50 depends on the prevalence or prior probability of CD4 < 50 , the sensitivity and specificity corresponding to specified TLC cutpoints and relative costs of misclassification. In the analysis data set the prior probability of CD4 < 50 was, 62%. Overall, TLC performed well in ROC analyses (AUC=0.82). At the chosen cut point of TLC=1000, the sensitivity and specificity in predicting CD4 < 50 were 69% and 81% respectively.

Randomization will also be stratified on CMV end organ disease. New CMV disease among patients without prior CMV disease, and CMV disease progression among patients with prior CMV disease constitute an important endpoint in this trial. Stratifying on past CMV disease will ensure that patients with and without prior CMV disease will be allocated equally to the two treatment groups.

11.2 Sample Size Considerations

The total accrual goal for this trial is 640 patients (320/arm) accrued over 22 months, with 12 months of additional follow-up. This sample size ensures 83% power for the primary endpoint of overall survival. Details of this calculation follow.

Preliminary data from three of the VATS sites and from ACTG trial 993 suggest a baseline median survival from first transfusion of less than one year. One site provided raw data on 45 cases at first transfusion. The estimated median survival was 7 months with 6, 12 and 18-month survival probabilities of 53%, 34% and 26%, respectively. Another site reported that after one year follow-up of 15 consecutive first-transfusion cases, 9 had died, for a one-year survival of 40%. A third site reported a median survival of 11 months and a 34-month survival of 20%. The 6, 12 and 18-month survival probabilities from first transfusion, among patients in ACTG 993 (a trial for patients with CD4 counts <50/μL) were 73%, 53% and 39%, respectively[59]. Although the data from the VATS sites suggest shorter durations of survival (medians of 7 to 11 months) than the ACTG trial (median>12 months), it is projected that the ACTG results may reflect more accurately what to expect in the VATS, since patients who enter clinical trials often have a better prognosis than patients in consecutive series. A median survival of 12 months in the control group will be assumed. An improvement from 12 months in the non-leukoreduced to 16 months in the leukoreduced RBC group would be considered an important treatment difference.

Assuming an annual accrual rate of 350 per year, 640 patients can be accrued in 22 months. With a fixed sample size design, 640 patients provide 84% power for a two sided $\alpha = .05$ test, using sample size formulae of Schoenfeld and Richter [61,65].

However a group sequential procedure based on the methodology of Lan and DeMets[62] using an O'Brien-Fleming boundary[63] will be used to monitor the trial for possible early stopping, with formal interim analyses at 22, 28 and 34 months from study activation. Repeated looks at the data result in a slight loss of power. This design has 83% power while controlling Type I error at 5% (two-sided), as detailed in the table.

Months	Nominal P-Value	Pr(Reject $H_0 \mid H_1$)	Number Deaths	Info Time
22	.008	.36	258	.61
28	.026	.33	357	.84
34	.041	.14	427	1.00
		.83 (= power)		

So, for example, if a log rank test has p-value <.008 at 22 months the stopping rule would dictate termination of the trial with an overall false-positive rate of 5%. Of course in practice, crossing a sequential boundary should trigger further review of other endpoints, and should not be considered as a hard and fast rule for early stopping. Assuming medians of 12 and 16 months in the two treatment groups, a total of 427 events would be expected after 34 months. The "information time" is the fraction of these 427 events expected at each interim analysis. This results in 83% power for detecting a four month difference in median survival of 12 versus 16 months. The calculations are based on a constant failure rate assumption, although the actual analysis will be with a log rank test. The calculations are relevant for the power of a log rank test, if the hazard ratio is the same as the ratio of medians assumed above, i.e., $1.33 = 16 \div 12$. In the actual trial, the exact numbers of events in the "number deaths" column will not be observed at the specified time points. The Lan and DeMets procedure can then be used to determine the appropriate nominal p-values at the times of the interim analyses, depending on the actual numbers of events observed.

The basic analysis of viral activation will involve calculating, within each patient, the ratio of HIV viral load (as measured by RNA PCR copy number) measured one week after initial transfusion, divided by the pre-transfusion copy number. It will be assumed in the following that the logarithm of this ratio is normally distributed, and a two-sample T-test will be used for comparing groups with respect to log ratio. Thus, the endpoint for each patient is the change in viral load on a logarithmic (base 10) scale. Even if the normal distribution is not a good approximation in this case, the T-test is known to be quite robust to violations of this assumption with the sample sizes being considered.

In pilot data from 10 cases given non-leukoreduced transfusions, the mean change in viral load from pre-transfusion to 7 days post-transfusion was .15 logs (base 10) with a standard deviation of .484 logs, i.e., about a half a power of 10. Six hundred forty cases will provide 97% power for detecting a .15 log difference between the treatments with respect to mean log change in viral load. Even if 50% of cases are missing sufficient data to evaluate this endpoint, (320 cases with data) the power would still be 79%. A treatment difference of .15 logs is a 1.41-fold difference. However, it is felt that a much larger difference, on the order of at least 5-fold difference (.7 logs), would likely be required to have a clinically meaningful effect. Even with a standard deviation of 1.0 logs there is >93% power for detecting a .7 log treatment difference with a much smaller sample size of 100 patients (50 per group).

Note that the primary clinical endpoint is driving the sample size requirements for the trial rather than the primary laboratory endpoint. This reflects the absence of knowledge about whether a biological effect on viral load would

have any clinical implication for the patient. Thus, the early stopping rule is specifically designed to be based on the clinical endpoint. Even if an early, convincing treatment difference in viral activation is observed, this should not be sufficient for early termination of the trial.

The trial will also have high statistical power for the secondary endpoint of time to first serious HIV complication or death. A median duration of time to first serious HIV complication or death of 7 months will be assumed, based on the following argument. Data from the Community Programs for Clinical Research on AIDS (CPCRA) was used to estimate how much shorter the duration of time to serious HIV complication will be relative to the time to death[60]. The CPCRA paper gives cumulative mortality estimates and cumulative incidence estimates for time to disease progression, defined as time to the first AIDS defining event. Estimates are given separately by gender and CD4 strata. Assuming the hazard functions for the two time-to-event distributions are proportional, one can estimate the hazard ratio from the estimates. Hazard ratio estimates for CD4 strata less than 100 primarily ranged from about 2 to 3. Combining these hazard ratios with the estimated 12 month median survival in the VATS, implies a median time to AIDS defining event of 12/3=4 to 12/2=6 months (assuming exponential distributions). However, the CPCRA events included all AIDS defining conditions. Thus we will use an estimate of 7 months median for the primary endpoint in this trial, which includes only "serious" HIV-related conditions. An improvement from 7 months in the non-leukoreduced to 10 months in the leukoreduced RBC group would be considered an important treatment difference. The sample size of 640 will provide > 95% power for this treatment difference.

11.3 Analysis Plans

The primary analysis for the time-to-event endpoint will be the logrank test. The primary analysis for the change in viral load will be the t-test. In other analyses proportional hazards models will be used to adjust treatment comparisons for covariates and to explore the effects of covariates themselves. Short-term changes in viral load will be based on log change from baseline, using two-sample t-tests or a Wilcoxon test if Normal assumptions are violated even after transformations. Covariates will be incorporated using Analysis of Variance. Random effects models will be used to analyze slopes of cytokines, lymphocyte markers and viral load over time. Incidence of individual events will be summarized by calculating number of events per Person-year and compared with Poisson methods. For example, the number of new diagnoses of CMV end organ disease, or diagnoses of CMV progression, will be counted and divided by the number of person-years of followup in order to estimate an incidence rate for CMV events, and compared with Poisson methods. (Note that a single person can have more than one CMV event.) In addition, person-specific incidence rates for each of several event types will be compared with two-sample Wilcoxon tests. The test statistics can be combined into an overall test using methods of Wei and Johnson[66.]

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APPENDIX I
MODEL CONSENT FORM
Viral Activation Transfusion Study (VATS)

1. Purpose and Background

Drs. _____ and _____ from the AIDS Clinic at _____ are conducting a study of blood transfusion in patients infected with human immunodeficiency virus (HIV), the virus that causes AIDS. This multicenter study is sponsored by the National Heart, Lung, and Blood Institute.

Although blood transfusion is common in people with AIDS or HIV infection, there has been little study of its consequences in these patients. A laboratory study has shown that blood transfusion may temporarily increase the level of HIV virus in the blood stream, perhaps because of the small number of white blood cells contained in the blood transfusion.

Filtered blood is used commonly in patients who are prone to transfusion reactions. This study will see if filtering the blood to remove white blood cells will make a difference in the severity of my HIV disease. The study will also measure whether the level of HIV or other viruses such as cytomegalovirus (CMV) in my blood changes after blood transfusions.

I am being asked to participate in the study because I am HIV infected and my doctor has decided that I need a blood transfusion.

2. Procedures

If I agree to participate in the study, the following will happen:

a) The research nurse will review my medical record and will ask me questions about my medical condition. I must be HIV and CMV positive to be in the study. Approximately 3 tablespoons of blood will be drawn for laboratory tests. The blood specimen will be tested for HIV, CMV, a complete blood count and a T-cell count. I will receive counseling about HIV before and when I receive the HIV results.

b) I will be randomized to receive either a regular red blood cell transfusion or a transfusion of blood that has been filtered to remove white blood cells. Randomization is the process of assignment by chance, like flipping a coin. I will have an equal chance of getting either type of blood transfusion. Neither I nor my doctor will know which type of blood I get.

If I need another blood transfusion later in the course of the study, I will receive the same kind of transfusion. However, if medically indicated, the study doctor or my own doctor may recommend that I receive filtered blood. If this happens I will remain in the study.

c) The study will continue for up to three years. I will be seen every three months by the study nurse and I will be asked questions about my medical condition, feelings, and activity level, and my medical chart will be reviewed. The visit with the research nurse every three months will take 1/2 hour per visit.

I will also have my eyes examined by an eye doctor at the beginning of the study and every six months while I am in the study. This will require a separate appointment of less than one hour. My pupils will be dilated for this exam, and I won't be able to drive for a couple of hours.

d) Blood drawing:

Blood will be drawn before I get my transfusion, one, two, three, and four weeks after the transfusion, and then every three months after the transfusion. Each blood test will require a brief visit at the clinic [LOCAL OPTION (a home visit may be arranged instead)]. The amount of blood to be drawn for the study is 20 ml (4 teaspoons) for each of the 5 samples before and after transfusion (total of 100 ml, about 7 tablespoons) plus an additional 30 ml (2 tablespoons) every three months.

If I receive a second blood transfusion during the three years of the study (but more than a week after first one), I will again have additional blood drawn before and at one, two, three, and four weeks after the blood transfusion (a total of 7 more tablespoons).

My blood will be tested to measure the level of HIV, CMV, a complete blood count, T-cell count, and other tests of immune function. Some of the blood will be frozen for future research tests.

e) If I need a transfusion of platelets (blood clotting cells), they will be filtered to remove white blood cells.

f) While I am on this study, I will make every effort to have all of my transfusions at the study clinic or hospital. However, if I must receive a transfusion at another hospital, I or my care giver will call the study nurse at this phone number _____.

g) I may take other AIDS or HIV medicines prescribed by my doctor while I am in the study. However, it is requested that **I not take a new AIDS medicine** such as AZT, DDI, DDC, D4T, or 3TC, or a new immune modulator drug (GM-CSF, interferon, etc.), **for two weeks following any blood transfusion**. I may take erythropoietin (procrit or epoetin - a hormone for increasing my red blood cells) while in the study.

3. Risk and Discomforts

a) **HIV testing**: Since I already have HIV disease, the only risk is of a false test result. I will be counselled before and after the test, and any unexpected results will be repeated.

b) [OPTIONAL PER LOCAL IRB] **Randomization**: I will be assigned to either filtered or unfiltered blood by chance. The type of blood transfusion I get may be less effective or have more side effects than the other type.

c) **Blood Filtration**: There is the very small possibility of a reaction to the filter materials. The filtration will be done in the blood bank under sterile conditions and will not change the time or procedure of the actual blood transfusion.

d) **Blood Transfusion**: The risk of blood transfusion include the small possibility of a transfusion reaction caused by my body reacting the blood from another person which results in fevers, chills, or shortness of breath, and very rarely blood in the urine or kidney failure. Blood transfusion can also occasionally cause mild heart failure due to fluid overload. However, my doctor has determined that I require a blood transfusion so these risks would occur whether or not I entered the study.

e) **HIV Therapy**: I am requested not to start new anti-HIV, or immune modulator drugs for a two week period after each transfusion. There is a small possibility that my HIV disease, or overall condition could worsen during this time. However, this risk is probably small, and if my doctor feels the medicine is important I may take it and stay in the study.

f) **Blood Drawing**: The risk of drawing blood includes temporary discomfort from the needle stick, bruising, and rarely infection.

g) **Confidentiality**: Participation in research may mean a loss of confidentiality (or privacy). My research records will be kept as confidential as possible by using code numbers, and no names will be used in any publications from the study. The study personnel, New England Research Institute (the coordinating center) and the National Heart, Lung, and Blood Institute may need to review my medical records now and in the future to check the study data.

4. Treatment and Compensation for Injury

If I am injured as a result of being in the study, treatment will be available. The cost of such treatment maybe covered by _____ depending on a number of factors. The _____ does not normally provide any other form of compensation for injury. For further information, I may call or write _____.

5. Benefits

[It is not known if I will receive any direct benefits from participating in this study]. I will receive a T-cell count and periodic eye exams from the study. The knowledge gained from the study may help to better understand what type of blood transfusion should be used in AIDS patients.

6. Alternatives

If I choose not to participate in the study, I will receive a regular blood transfusion according to the usual practices at my hospital, and I will continue in my regular HIV medical care. I will be informed of any significant new findings that may affect my willingness to participate in this study.

7. Costs

Any additional costs of being in the study will be covered by the study. I, or whoever usually pays for my medical care, will still be responsible for the costs of my medical treatment, including the cost of the blood units (but not the filtration) and administration of transfusions.

8. Reimbursement

In return for my time, effort, and travel expenses, I will be reimbursed $100 for completing all four follow up blood draws following each of two transfusion episodes. If I do not complete all four visits, I will receive $15 per blood draw. I will also receive $25 for completing each of the three-month study visits. The money will be paid by check within six weeks.

9. Questions

This study has been explained to me by Dr. _____ or Dr. _____ and my questions were answered. If I have any other questions about the study, I may call Dr. _____ at phone number _____. If for any reason I do not wish to speak with Dr. _____, I may call the Institutional Review Board at _____.

10. Consent

I have been given copies of this consent form and the experimental subjects' bill of rights to keep.

11. Voluntary Participation

Participation in research is entirely voluntary. I have the right to decline to participate or to withdraw at any point in this study without jeopardy to my medical care. If I wish to participate, I should sign below.

_____ _____

Signature Date

_____

Print Name

_____ _____

Person Obtaining Consent Date

Appendix II

Karnofsky Performance Scale

Able to carry on normal activity; no special care is needed	100	Normal; no complaints; no evidence of disease.
	90	Able to carry on normal activity.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work, able to live at home and care for most personal needs; a varying amount of assistance is needed.	70	Cares for self, unable to carry on normal activity or to do active work.
	60	Requires occasional assistance but is able to care for most needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospitalization is indicated, although death is not imminent.
	20	Very sick; hospitalization necessary; active supportive treatment is necessary.
	10	Moribund; fatal processes progressing rapidly
	0	Dead