UNIVERSITY OF MINNESOTA BONE MARROW TRANSPLANTATION PROGRAM

Autologous Peripheral Blood Stem Cell Transplant for Patients with Lymphoma

CPRC# 2005LS048
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Confidential

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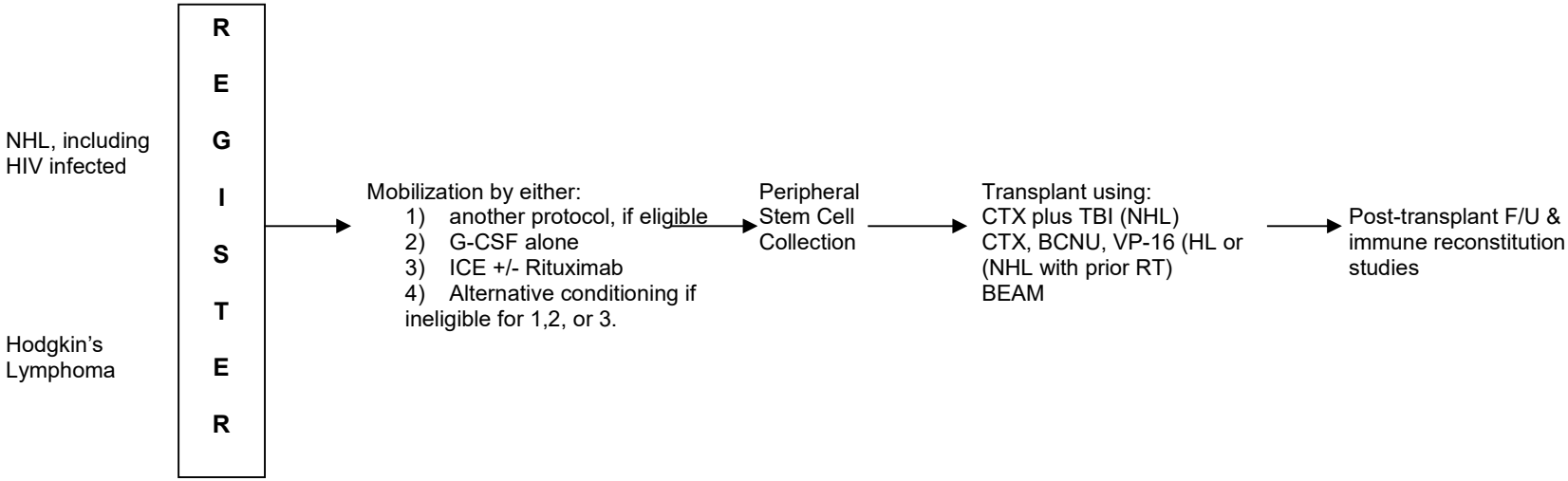
Revision History

date	Revision details	Consent change/ version date
08/23/05	original	
1/30/07	1. Clarification of minimum cell dose required for individuals requiring bone marrow harvest. 2. Addition of Rituximab therapy post-transplant for CD20+ NHL 3. Addition of risk of JC virus with progressive multifocal leukoencephalopathy due to Rituximab.	
2/15/2007	Revised eligibility (p.7) and cell collection sections (p.11) to allow for identical twin donor	
8/10/2007	Revised mobilization to allow for patients who have already failed (R)ICE to use an alternate conditioning regimen.	
7/15/2009	Dr. Bachanova replaces Dr Tomblyn as PI	
4/1/2010	Standardized the cytoxan dosing in obesity (sections 5.3.1 and 12.2)	
9/23/2010	Changed upper age eligibility to 75 Deleted Trec blood draws and analysis	
05/17/2013	Add BEAM as an alternative preparative regimen for patients unable to received additional radiation therapy and/or high dose cyclophosphamide update registration, event reporting and toxicity sections	Yes
8/5/2013	Fixed cut & paste error in the BEAM appendix – toxicity chart for BCNU	Yes
1/16/2014	Fixed additional cut & paste error in the BEAM appendix – toxicity chart for BCNU; correct BCNU (carmustine) infusion time – over 2 hours, not 1 hour	Yes

SCHEMA



Other related Activities: <u>For calculation of PBSC harvest</u>

Record each day of leukapheresis:

- the pbCD34/μL prior to collection,
- the total number of $CD34^+$ cells harvested,
- the volume of apheresis plus the patient's height and weight

<u>Evaluations for immune reconstitution</u>

- Quantitative Immunoglobulins and CD4 and CD 8 –prior to mobilization, day + 100 and then q 3 mon for 1 yr
  For HIV-positive patients, CD4 and CD8 analysis at d+28 and d+60 as well.

1.0 OBJECTIVES

Autologous hematopoietic stem cell transplant (HSCT) is appropriate care for many patients with Hodgkin (HL) and non-Hodgkin Lymphomas (NHL).

1.1 Primary Objective

1.1.1 To determine the disease free survival and overall survival after autologous HSCT for NHL and HL

1.1.2 To verify the safety and efficacy of autologous PBSC HSCT in patients with HIV disease and relapsed lymphoma

1.2 Secondary Objectives

1.2.1 To evaluate immune reconstitution in HIV positive patients undergoing autologous HSCT and compare to immune reconstitution in HIV negative patients

1.2.2 To predict the adequacy of PBSC harvest prior to flow analysis of a PBSC yield (see Section12.2)

1.2.3 To determine the time to engraftment for neutrophils and platelets

2.0 BACKGROUND AND RATIONALE

The role of high-dose chemotherapy and HSCT in the treatment of relapsed lymphoma is well established[1,2]. Since that time, continued improvements in survival, supportive management and transplant techniques have developed. Consequently, it is important to continue to scientifically study issues related to autologous HSCT for patients with lymphomas.

Peripheral blood stem cells (PBSC) are now used almost exclusively as the source of hematopoietic reconstitution for patients treated with autologous HSCT for both NHL and HL. The use of PBSC instead of bone marrow has resulted in more rapid hematopoietic engraftment[3,4]. A limitation to autologous HSCT is sufficient harvest of the 2×10^6 $CD34^+$ cells/kg minimum threshold considered necessary for satisfactory engraftment [5]. Optimal estimation of harvest yield prior to the actual collection is difficult. Surrogate markers have been studied including the white blood cell and platelet counts on the day of leukapheresis and the numbers of

circulating $CD34^+$ cells[6,7]. The most useful marker to date is the peripheral blood $CD34^+$ cells/μL[8].

Even with this surrogate marker, some patients do not mobilize adequate numbers of PBSC to proceed with transplant and alternative means of hematopoietic stem cell harvest must be undertaken or alternative therapy sought. In a previous study of 175 patients with lymphoma undergoing PBSC harvest and autologous HSCT at the University of Minnesota, 41 (23%) had inadequate PBSC collections defined as total collection of less than 2×10^6 $CD34^+$/kg. Twenty-eight of these patients went on to bone marrow harvest[9]. With a reliable means to predict the harvest yield, these patients may have been spared treatment delay and the morbidity and expense of additional leukaphereses. Instead there may have been consideration for initial bone marrow harvest or allogeneic transplant.

The kinetics and yield of mobilization are dependent upon the volume of apheresis and the collection efficiency as well as the peripheral blood $CD34^+$ cells/μL [10,11]. Consequently, even with sufficient circulating peripheral blood $CD34^+$ cells, patients still may not harvest well due to poor collection efficiency. It would be valuable to have a means to estimate stem cell yield accurately to determine if the minimum threshold of PBSC could be obtained with further collection procedures rather than aborting the whole process.

A previously published retrospective analysis conducted by Gidron *et al* found that, even in the setting of a low peripheral blood CD34+ cell count, adequate PBSC could be harvested if the collection efficiency and numbers of harvests was adequate[12]. They retrospectively reviewed 485 PBSC harvests where the peripheral blood $CD34^+$ count was known. In 104 harvests, the circulating peripheral blood $CD34^+$ cells were < 5/μL, a cut-off thought indicative of poor collection. However, over 60% of these harvests contained more than 0.3×10^6 CD34/kg such that a person undergoing leukapheresis for 6-7 days would obtain an adequate graft to move forward to transplant. Using this data, they determined an equation useful to estimate the PBSC harvest based on collection efficiency, volume of leukapheresis, the peripheral blood $CD34^+$ cell count/μL, and the patient's ideal body weight (IBW). This study will attempt to validate that formula in a prospective study. That validation will allow for better estimates of stem cell harvest for patients planned for autologous HSCT for the management of lymphoma.

Lymphoma is a frequent complication of HIV disease. With the advent of highly active anti-retroviral therapy (HAART), transplant is being investigated for patients with HIV and lymphoma[13-15]. A small case series (n=9) from the City of Hope demonstrated adequate engraftment similar to non-HIV infected patients[16]. All patients had either NHL or HL and were on HAART therapy. Patients were conditioned with cyclophosphamide, BCNU, and etoposide (CBV). The median time to neutrophil and platelet engraftment was 11 and 10 days, respectively. Furthermore, despite a transient decrease in $CD4^+$ counts to a nadir of 138/μL at

two months post-transplant, the majority recovered pre-transplant $CD4^+$ levels at a median of 14 months post-transplant. Infections during the neutropenic period included bacteremias, *Clostridium difficile* colitis, and an episode of culture negative sepsis. Three patients subsequently developed opportunistic infections including zoster, cytomegalovirus viremia, and *Pneumocystis carinii* pneumonia in a patient who stopped his prophylactic antimicrobials. Re and colleagues published another small series (n=16) with similar results[17]. In both series, there were no deaths due to treatment or opportunistic infection. Furthermore, in the series from Krishan *et al*, seven of the nine patients were in remission at a median of 19 months after transplant. While the literature supports the safety and benefit of transplantation, the impact on immune reconstitution in HIV patients is unclear.

3.0 ELIGIBILITY AND EXCLUSION CRITERIA

3.1 Age: All patients must be less than 75 years of age at the time of enrollment in the study.

3.2 Karnofsky performance status: >80% (>60% if poor performance status is related to lymphoma) [Appendix I]

3.3 Life expectancy: Greater than 8 weeks.

3.4 No evidence of serious organ dysfunction that is not attributable to tumor including:

3.4.1 Renal: Creatinine ≤ 2.0 mg/dl or creatinine clearance > 50 ml/min.

3.4.2 Hepatic: No history of severe prior or ongoing chronic liver disease. Total bilirubin ≤ 2.0 mg/dl, AST and alkaline phosphatase <5x upper limit of normal.

3.4.3 Cardiac: Patients must be free of symptoms of uncontrolled cardiac disease including unstable angina, decompensated congestive heart failure, or arrhythmia. The ejection fraction by gated cardiac blood flow scan (MUGA) must be >45%.

3.4.4 Pulmonary: Patients must have no significant obstructive airways disease (FEV_1 must be ≥ 50%) and must have acceptable diffusion capacity (corrected DLCO > 50% of predicted).

3.4.5 Hematologic: Patients must have (1) hemoglobin > 8 gm/dL without transfusion and off erythropoietin for 14 days or Aranesp for 21 days; (2) WBC > 2.5 x 10^9/L with an ANC > 1.5 x 10^9/L off G-CSF or GM-CSF for 10 days or Neulasta for 21 days; (3) Platelets > 100 x 10^9/L without transfusion; (4) Bone marrow cellularity of > 20% with <10% involvement with tumor

3.4.6 Central nervous system: Patients with a history of CNS involvement by lymphoma or with relapsed primary CNS lymphoma will be

eligible. Patients with active CNS disease are eligible if they have completed a standard treatment for CNS lymphoma and have no evidence of progressive CNS disease at the time of enrollment.

3.4.7 Infection: Patients with serious uncontrolled infections at the time of transplant will be excluded

3.5 Patients must be at least 4 weeks from previous chemotherapy; 6 weeks from nitrosoureas.

3.6 Hepatitis B: Patients who are carriers of Hepatitis B will be included in this study. These patients are not eligible to receive rituximab as a component of their chemotherapy mobilization[18].

3.7 HIV disease

Patients with HIV disease are eligible for this study provided that:

3.7.1 Patients will be seen in the infectious disease(ID)/HIV clinic prior to enrollment on study for the purpose of determining eligibility and for local coordination of HIV care during the peri-transplant period.

3.7.2 Must be on a maximally active anti-HIV regimen to control disease as determined appropriate by the ID/HIV physicians. For the majority of patients, this will be a highly active anti-retroviral therapy (HAART)-type therapy including a protease inhibitor.

3.7.3 $CD4^+ \geq 50/\mu L$

3.7.4 HIV RNA viral load ≤ 100,000 copies per mL on each of samples 4 weeks apart. The most recent level must be within one month of enrollment.

3.8 Non-Hodgkin's lymphoma (NHL)

Patients with chemo-sensitive histologically confirmed NHL will be eligible for this treatment protocol contingent on histologic subclassification.

NHL patients with resistant or refractory lymphoma (no PR following up to three cycles of combination chemotherapy) will be ineligible for transplant in this trial.

3.8.1 Precursor B-cell or Precursor T-cell NHL

3.8.1.1 Lymphoblastic lymphoma

- All patients will be eligible in second or greater complete remission (CR) or first or subsequent partial remission (PR).
- Patients with any high-risk features will be eligible in first complete remission
- High risk features include:
  - Stage IV;

- LDH >2x normal;
- ≥ 2 extranodal sites.

3.8.2 Mature B-cell Lymphomas

3.8.2.1 Small lymphocytic lymphoma (SLL) or Chronic Lymphocytic Leukemia (CLL)

- Patients will be eligible in ≥ CR1 with molecularly negative disease
- Patients in CR with molecularly positive disease or in PR will be excluded from autologous transplant.

3.8.2.2 Follicular Lymphoma

- Patients will be eligible in ≥ first CR/PR (if treatment is delayed until clinically required).

Patients who are treated at diagnosis (without clinical symptoms necessitating treatment, such as B symptoms, bulky disease, marrow or other organ compromise) will be eligible in ≥ second CR/PR.

3.8.2.3 Diffuse Large B-cell Lymphoma

- All patients will be eligible in ≥ CR2 or ≥ PR1.
- Patients with a high intermediate or high IPI (≥ 2 for age-adjusted IPI or ≥3 for IPI) at diagnosis will be eligible in first CR.

3.8.2.4 Mantle Cell Lymphoma

- All patients will be eligible in first or greater CR or PR.

3.8.2.5 Burkitt's/Burkitt's like

- All patients except localized lymphoma will be eligible anytime after initial therapy (after achievement of first complete remission), or in partial remission if they fail to achieve CR.
- Patients with localized (stage I or Ziegler stage A) will be eligible only if they fail to achieve CR1 or after relapse

3.8.3 Mature T-cell lymphoma

- T-cell lymphomas including Primary T-cell not otherwise specified, angioimmunoblastic, and ALK-

positive anaplastic large cell, will be eligible after initial therapy, whether or not CR is achieved.

- Mycosis fungoides/Sezary syndrome will be eligible in ≥CR2/PR2

3.9 Hodgkin's lymphoma (HL)

Patients with histologically proven HL will be eligible for transplantation after failing prior therapy.

Patients with resistant disease (initial or at relapse): those who fail to achieve an objective partial response to three cycles of combination non-cross resistant chemotherapy will be ineligible for transplant in this trial.

3.9.1 For stage I/II patients treated with primary radiation, they must have failed (no CR or progression after CR) at least one salvage combination chemotherapy treatment regimen.

3.9.2 For advanced (stage III/IV) Hodgkin's disease, patients must have failed an adriamycin containing regimen (ABVD) or an alternative non-cross resistant regimen (eg MOPP).

3.9.3 Patients with any high-risk features will also be eligible, including those who:

- Fail to achieve complete remission with initial combination chemotherapy
- Patients with bulky disease after initial therapy (chemotherapy or radiation) defined as residual mediastinal mass ≥ 5 cm or other residual mass ≥ 10 cm accompanied by other features of persisting disease (e.g., Gallium or PET scan positive; high LDH; enlarging on serial x-rays or biopsy positive) will be eligible. If possible, persistent disease should be proven by biopsy.

3.9.4 Patients should receive chemotherapy to attempt to achieve CR or minimal disease state for all patients pre-transplantation. The use of up to three cycles of non-cross resistant combination chemotherapy is advised.

3.9.5 Residual areas of limited disease should receive radiotherapy following and not prior to transplantation (See Section 5.4).

3.10 All responses are to be determined using the Response Criteria for Non-Hodgkin's Lymphoma[19](Appendix II) and will include PET/CT prior to HCT.

3.11 All participating patients must exercise informed voluntary consent and sign a consent form approved by the University of Minnesota Committee on the Use of Human Subjects in Research.

3.12 Patients may be transplanted under this protocol using a syngeneic (identical) twin donor.

3.13 Exclusions

3.13.1 Patients eligible for any higher priority transplant protocols
3.13.2 Women who are pregnant or breast feeding
3.13.3 Patients with chemotherapy resistant disease

4.0 SUBJECT REGISTRATION

Patients will be registered to this study in OnCore at the time of consent signing.

5.0 TREATMENT PLAN

5.1 Peripheral Blood Stem Cell (PBSC) Mobilization

All patients will have PBSC collected by leukapheresis. Patients may be eligible and enrolled on other higher priority PBSC mobilization studies.

Patients not requiring further disease reduction (i.e. in CR or very good PR after salvage chemotherapy) can be mobilized with G-CSF alone at a dose of 10µg/kg/day sub-cutaneously (see Appendix III) [9].

- CR or CRu: definition per Appendix II
- Very good PR: defined as minimal residual disease with all nodal sites ≤2 cm and no morphologic bone marrow involvement

Patients with stage IV disease at the time of diagnosis or more than 1 year from time of diagnosis to planned harvest should be considered for chemotherapy priming if not eligible for alternative PBSC mobilization studies [9].

If patients have progressed through the (R)ICE regimen, then an alternative conditioning regimen will be used at the discretion of the treating physician. One suggestion is to use Cyclophosphamide 4000mg/m^2 as per protocol MT2003-13. R-DHAP and Gemcitabine based regimen (Gemcitabine, Vinorelbine, Dexamethasone) are also permitted. Rituximab 375mg/m^2 may be co-administered if the patient has a CD20+ lymphoma.

5.1.1 Mobilization with G-CSF alone

G-CSF 10µg/kg/day subcutaneously q day each morning (prior to harvest on days of apheresis) until completion of harvest

Leukapheresis will begin on day 5 of G-CSF administration

If patients do not adequately mobilize with G-CSF alone, they will then receive chemo-mobilization (see 5.1.2)

5.1.2 Chemo-mobilization using ICE ± Rituximab (R-ICE)

Patients requiring further disease reduction (i.e. PR after salvage chemotherapy) or others eligible will receive chemo-mobilization using Ifosfamide, Carboplatin, and Etoposide (ICE) ± Rituximab (R-ICE). This may be administered either as an inpatient or outpatient.

Patients with $CD20^+$NHL or lymphocyte predominant HL will have Rituximab included in their mobilization chemotherapy. Patients who are carriers of Hepatitis B are not eligible to receive Rituximab regardless of their disease type.

Chemotherapy for mobilization will be administered as follows:

Day	Drug	Administration
1	Rituximab ($CD20^+$ NHL or lymphocyte predominant HL pts. only)	375mg/m^2 on day 1 infused over 6-8 hours. Infusion should start at 50mg/hr and increase by 50mg/hr every 30 minutes as tolerated to a maximal infusion rate of 400mg/hr Premedication with acetaminophen 650mg and benadryl 50mg orally. Hydrocortisone 50mg IV may be administered if necessary for infusion reactions.
Day 2	Ifosfamide	1665 mg/m^2/day IV over 2 hours
	MESNA	1665 mg/m^2/day IV over 2 hours given concomitantly with Ifosfamide
	Etoposide (VP-16)	100mg/m^2/day IV over 30 minutes after Ifosfamide
	Carboplatin	AUC 5 (max dose 350mg/m^2; see Appendix III) IV over 1 hour after Etoposide
Day 3, 4	Ifosfamide	1665 mg/m^2/day IV over 2 hours
	MESNA	1665 mg/m^2/day IV over 2 hours given concomitantly with Ifosfamide
	Etoposide (VP-16)	100mg/m^2/day IV over 30 minutes after Ifosfamide

Start Day 5	G-CSF	10µg/kg/day SQ (dosed per appendix III) until completion of leukapheresis

Ifosfamide will be dosed based on actual body weight (ABW) unless the patient is 20% or more of ideal body weight (IBW) [See section 12.2 for IBW formula]. If more than 20% of ideal body weight, an adjusted ideal body weight (AIBW) will be used for dosing. This is calculated as:

AIBW = IBW + 1/3(ABW - IBW)

Leukapheresis will begin the first day after the ANC ≥ 1.0 x 10^9/L. This will likely be between day +12 and day +15 after the initiation of chemotherapy.

5.1.3 Supportive Care during Chemotherapy mobilization

5.1.3.1 IV Hydration: All patients will be given a minimum of 1 Liter of Normal Saline daily (days 1 – 4) during chemotherapy mobilization. Additional IV hydration can be given at the physician's discretion.

5.1.3.2 Prophylactic antiemetics will be administered as follows: day 2 (prior to Ifosfamide, VP-16, and Carboplatin): Ondansetron 32mg IV and dexamethasone 20mg IV plus lorazepam 1mg po/IV 30 minutes before chemotherapy. days 3 and 4: Ondansetron 8mg oral dissolving tablet (ODT) every 8 hours and dexamethasone 10 mg IV 30 minutes before chemotherapy. Lorazepam and prochlorperazine can be given IV/PO every 6 hours as needed.

5.1.3.3 For patients with uric acid > 5.0 mg/dl on the first day of chemotherapy, add allopurinol 300 mg/d or 150 mg/M^2/d po for 7 days or longer if hyperuricemia (> 10 mg/dl) persists.

5.1.3.4 Beginning day 5 of mobilization, patients will be monitored closely (at least q 48h but daily if medically necessary) with careful observation for fever, infection, or anemia or thrombocytopenia needing transfusion support.

5.1.3.5 All blood products administered must be irradiated before infusion and if the patient is CMV seronegative, must be CMV-safe (CMV-seronegative or leukocyte depleted by filtration).

5.1.3.6 Antibiotic prophylaxis starting on the first day of chemotherapy will include Levofloxacin 500mg po daily and Fluconazole 200mg po daily. Acyclovir 400mg po TID should be given to if patient's are seropositive for

HSV. Antibiotic prophylaxis will continue until the ANC ≥ 1.0 x 10^9/L

5.1.3.7 Neutropenic fever or signs of infection should be investigated promptly and treated empirically with broad spectrum IV antibiotics.

5.1.3.8 CBC differential and platelets shall be monitored at least q 48 hours

5.2 Peripheral Blood Stem Cell Collection

5.2.1 Patients must have satisfactory venous access using appropriate **large bore vascular access catheters** suitable for repeated leukapheresis. (Hickman/Leonard types are not acceptable; Quinton or Davol double lumen subclavian 13.5 French dialysis catheters are required.)

5.2.2 Collection starts on the first day after the ANC ≥ 1.0 x 10^9/L after R-ICE or on day 5 of G-CSF if not chemo-mobilized

A peripheral blood $CD34^+$ (pbCD34) cell count/μL will be drawn in clinic daily prior to apheresis starting the first day of leukapheresis and continuing through the entire period of leukapheresis

5.2.3 Peripheral blood stem cell collection proceeds for a minimum of 3 days regardless of the measured pre-collection pbCD34/μL unless the goal (5 x 10^6 $CD34^+$/kg) collection is met (see Section 5.2.4). However, if patients do not collect a minimum of 0.1 x 10^6 $CD34^+$ cells/kg on the first day of apheresis or a total of 0.3 x 10^6 $CD34^+$ cells/kg by the completion of day 2 of apheresis, the leukapheresis procedure will be aborted and alternative means of hematopoietic stem cell collection will be determined as defined below

Decisions regarding continued PBSC collection beyond 3 days should be based on the following:

5.2.3.1 If the pbCD34/μL is continuing to increase and the goal of 5 x 10^6 $CD34^+$cells/kg has not been reached, then further collections should proceed. This increase should be by at least 1/μL/day to continue as long as the minimum pbCD34/μL is ≥5.

- For example, if on day 2, the pbCD34/μL is 5 and on day 3, the pbCD34/μL is 6, then, if necessary, the collection could proceed to day 4. If the pbCD34/μL on day 4 has declined or reached a plateau, a fifth day of harvest would not be done. However, if the pbCD34/μL is 7, then apheresis could continue if necessary.
- See Section 12.1 for formula to calculate pbCD34/μL

5.2.3.2 If the patient was mobilized with G-CSF alone, then growth factor should be stopped and the patient should proceed to chemo-mobilization (see 5.2.4)

5.2.3.3 If the patient was chemo-mobilized, then a recovery period of 1 – 2 weeks should be given and decision made for alternative means of harvest (see 5.2.4)

5.2.4 Targets

Goal collection: 5 x 10^6 CD34/kg actual body weight
Minimal peripheral blood collection to proceed to transplant: 2 x 10^6 CD34/kg actual body weight.
Minimum collection to proceed to transplant if patient requires a bone marrow harvest (see below): 1 x 10^6 CD34/kg actual body weight.

If < 2 x 10^6 CD34/kg actual body weight are collected, alternative harvesting procedure after ANC>1.5 x 10^9/L and Plt > 100 x 10^9/L off G-CSF will be performed

- If patient had previously undergone G-CSF mobilization, then chemotherapy mobilization using ICE ± Rituximab per 5.1.2
- If patient had previously undergone chemotherapy mobilization then, if bone marrow biopsy is ≥20% cellular and without morphologic evidence of lymphoma, patients will be given GM-CSF at 10µg/kg/day for 5 days. On day 5, patients will undergo a 1.5L bone marrow harvest. For patients requiring a bone marrow harvest, the minimum $CD34^+$ cell/kg dose is 1 x 10^6 CD34/kg actual body weight.
- If patients do not achieve the minimal collection goal with combination of growth factor and chemotherapy mobilization and do not meet the bone marrow biopsy criteria to move forward with bone marrow harvest, they will be removed from the transplant portion of the study. Data collected for harvest will be used in the evaluation of appropriate secondary endpoints.

5.2.5 Syngeneic (identical) twin donors will be mobilized according to standard clinical practice (as outlined in allogeneic protocol MT2001-02)

5.3 Transplant Admission

5.3.1 Non-Hodgkin's lymphoma including irradiation

For patients who have had greater than the following pre-transplant radiotherapy limits, treatment will be per section 5.3.2:

i) > 1000 cGy to whole lung, kidney, or abdominal bath.
ii) > 3000 cGy to spinal cord, myocardium, mediastinum, lumbar periaortic lymph nodes.
iii) > 3600 cGy to whole brain.

Day	Procedure
Day -8	Admission
Day -7, -6	Cyclophosphamide 60 mg/kg i.v. over two hours daily x 2 days (total 120 mg/kg).
Day -5	Rest
Day -4, -3, -2, -1	Total body irradiation (TBI) 165 cGy b.i.d. x 4 days (total 1320 cGy).
Day 0	Reinfuse all previously collected PBSC.
Day +5	Begin G-CSF 5µg/kg/day SQ rounded to the nearest vial size. Continue G-CSF until ANC > 1500/µl x 3 consecutive days. If ANC falls <1000/µL, restart G-CSF.

Cyclophosphamide dosing is calculated based on ABW (actual weight) unless ABW is > 150% of IBW (Ideal Body Weight). In this case the dose should be computed using adjusted body weight.

Ideal body weight is calculated using:

50kg + [2.3kg x (height in inches - 60)] for men

45.5kg + [2.3kg x (height in inches -60)] for women

Adjusted body weight = IBW + 0.5(ABW - IBW)

TBI will be given by 6, 18, or 24 MV x-ray beams through right and left lateral fields prescribed at the umbilicus at midplane. Aluminum compensators will be used as necessary to deliver a uniform dose within ±10% of the prescribed dose. A beam spoiler will be used to prevent skin sparing. The dose rate will be 10-19 cGy/min. Total dose will be 1320 cGy administered in 8

fractions over 4 days. The interval between fractions should be at least 6 hours.

5.3.2 Non-Hodgkin's Lymphoma. Radiation-free and Cyclophosphamide-free regimen

For patients with NHL

- ineligible to receive total body irradiation because of prior radiation (as above) to central nervous system, lungs, or heart in excessive doses, or
- patients who are not candidates for high dose cyclophosphamide due to excessive risk of toxicity, the following conditioning regimen will be used.

Treatment will be administered per current institutional guidelines.

Day	Procedure
Day -7	Admission
Day -6	BCNU 300 mg/m^2 IV over 2 hours
Day -5	Etoposide 100 mg/m^2 IV over 2 hours BID AraC 100 mg/m^2 IV over 1 hour BID
Day -4	Etoposide 100 mg/m^2 IV over 2 hours BID AraC 100 mg/m^2 IV over 1 hour BID
Day -3	Etoposide 100 mg/m^2 IV over 2 hours BID AraC 100 mg/m^2 IV over 1 hour BID
Day -2	Etoposide 100 mg/m^2 IV over 2 hours BID AraC 100 mg/m^2 IV over 1 hour BID
Day -1	Melphalan 140 mg/m^2 IV over 1 hour
Day 0	Re-infuse all previously collected PBSC
Day +5	Begin G-CSF 5µg/kg/day SQ rounded to the nearest vial size. Continue G-CSF until ANC > 1500/µl x 3 consecutive days. If ANC falls <1000/µL, restart G-CSF.

BEAM conditioning regimen can be used for HL patients only if they are ineligible for CBV (detailed below).

5.1.3 Hodgkin's Lymphoma Without Irradiation

For patients with HL the following conditioning regimen will be used.

Day	Procedure
Day -7	Admission
Day -6, -5, -4, -3	Cyclophosphamide 1.5 gm/M^2 over 2 hours at 10 a.m. daily x 4 days. (Total dose of cyclophosphamide 6 gm/M^2)
Day -6	BCNU (carmustine) 300 mg/M^2 over 2 hours, 9 am.
Day -6, -5, -4	Etoposide (VP-16) 150 mg/M^2 intravenously over 4 hours every 12 hours starting at 6AM and 6PM, for 6 total doses. (Total dose: 900 mg/M^2)
Day -2, -1	Rest
Day 0	Reinfuse all previously collected PBSC
Day +5	Begin G-CSF 5µg/kg/day SQ rounded to the nearest vial size. Continue G-CSF until ANC > 1500/µl x 3 consecutive days. If ANC falls <1000/µL, restart G-CSF.

Cyclophosphamide will be dosed based on actual body weight (ABW) unless the patient is 20% or more of ideal body weight (IBW) [See section 12.2 for IBW formula]. If more than 20% of ideal body weight, an adjusted ideal body weight (AIBW) will be used for dosing. This is calculated as:

AIBW = IBW + 1/3(ABW - IBW)

5.1.4 Supportive Care

5.1.4.1 All patients with uric acid > 5.0 mg/dl will receive allopurinol (300 mg/d or 150 mg/M^2/d po) beginning on admission and continuing to Day -1 unless hyperuricemia (>10 mg/dl) persists. Allopurinol may be stopped when uric acid ≤ 5mg/dL.

5.1.4.2 Vigorous intravenous hydration (2000-3000 ml/m2/day) should be given from 4 hours before the first cyclophosphamide dose until 24 hours past the second dose. Adequate diuretics should be given and patients urged to urinate every 1-2 hours to ensure urinary output of at least 200 ml every two hours and to maintain appropriate fluid balance. Patients should be weighed b.i.d. during cyclophosphamide administration to aid in managing fluid balance. Mesna should be given totaling the same number of milligrams as the cyclophosphamide dose each day. See MT(S)9006 for Mesna dosing.

5.1.4.3 Prior to infusion of PBSC on day 0, patients will receive 6 hours of hydration with D_5 1/2NS + 20mEq KCL/L at a rate of 150ml/hr. Patients will be administered acetaminophen 650mg po and diphenhydramine 50mg po 30 minutes prior to PBSC infusion

5.1.4.4 Patients will be eligible for additional supportive care study protocols including, but not limited to, those related to prophylaxis and treatment of infection, mucositis, transfusions, nutritional support, graft failure, and/or post-transplant therapy or immune reconstitution.

5.1.4.5 All blood product support will be irradiated to prevent inadvertent blood donor lymphoid engraftment. Standard BMT protocol blood product support techniques will be used, including packed red cell transfusions to maintain hemoglobin > 8.0 g/dl, platelet transfusion support to maintain platelet counts >10,000/µl. Leukocyte transfusions can be used if clinically indicated.

5.1.4.6 HIV positive patients will remain on optimized antiretroviral medication regimen, which will usually be HAART therapy. As there are no IV alternatives, HAART therapy will be held if patient is unable to tolerate oral medications and re-instituted as soon as the patient can tolerate oral therapies.

5.1.4.7 All patients will receive standard antimicrobial prophylaxis per institutional protocols (See Infection Prophylaxis and Therapy Guidelines for BMT Recipients at UMMC)

5.1.4.8 All patients will receive antiemetic support per institutional protocols (see Antiemetic Guideline for BMT Recipients at UMMC).

5.2 Post Transplant Irradiation

Patient's will be eligible and should receive post-transplant irradiation after disease re-staging at Day +28 per Appendix III and when ANC > 1500/µL and Plts are > 100,000/µL. Persisting nodal masses ≥ 2 cm will be treated with additional localized external beam irradiation with a target treatment of 2000 – 3600 cGy given as 150 – 200 cGy/day 5 days/week. Sites suspicious for neoplastic involvement but <2 cm and potentially amenable to radiation should have biopsy confirmation of neoplastic involvement before treatment.

5.3 Post Transplant Rituximab Therapy

Patients with $CD20^+$ NHL will be eligible for post-transplant Rituximab therapy if they have recovered from transplant. Particularly patients with mantle cell lymphoma with high risk features prior to transplant (high MIPI and positive preHCT PET or marrow MRD positive) may benefit from Rituximab maintenance. Treatment will be started between day + 45 and day +90 after transplant and will be repeated at day +180 ± 14 days.

5.3.3 Indications for Rituximab therapy

- 5.3.3.1 Hematologic: ANC > 1500/µL and Plts are > 100,000/uL
- 5.3.3.2 Recovery from all other transplant toxicity
- 5.3.3.3 Absence of active uncontrolled infections

5.3.4 Dosing

- 5.3.4.1 Rituximab 375mg/m2 once every 2 months..

Rituximab is administered on **day 1** infused over 6-8 hours. Infusion should start at 50mg/hr and increase by 50mg/hr every 30 minutes as tolerated to a maximal infusion rate of 400mg/hr.

Premedication with acetaminophen 650mg and benadryl 50mg orally for each infusion. Hydrocortisone 50mg IV may be administered if necessary for infusion reactions.

6 REQUIRED OBSERVATIONS (APPENDIX V)

In addition to complete history and physical examination with detailed recording of initial sites and characteristics of disease at diagnosis, immunophenotyping (molecular biologic or cytogenetic studies as available) as well as previous chemotherapy and radiation and the clinical responses to this treatment, patients will undergo multi-organ screening to assess their eligibility for initiation into the study treatment protocol. An attempt should be made to obtain the scheduled activity as close as possible to the scheduled date. However, scheduling difficulties (for procedures and around weekends) may make this difficult. Therefore the scheduled activity will be obtained within 14 days of that schedule.

6.1 Staging studies prior to enrollment to define the extent of disease will include unilateral bone marrow aspiration and bone marrow biopsy, PET/CT scans of chest, abdomen, pelvis (and neck if indicated), serum protein electrophoresis, LDH, β_2-microglobulin.

6.1.3 If patient has previous PET negative disease, then PET scan is not required for eligibility or follow-up

6.1.4 If patient has had no prior bone marrow involvement, then bone marrow biopsies will be performed to determine eligibility, at day+100, and then at 1 year unless otherwise clinically indicated.

6.2 PBSC collection data: For each day of leukapheresis, the pbCD34/μL prior to collection, the total number of $CD34^+$ cells harvested, the patient's actual weight, the amount of CD34+ cells/kg of actual body weight and the volume of apheresis will be recorded. The patient's ideal body weight will be calculated and included in the database.

6.3 Disease staging will be performed prior to enrollment in study; at Day +28, at Day +100, at Day +180, at 1 year and then at 6 month intervals in the second post-transplant year.

6.4 All diagnostic bone marrow aspiration and biopsy studies (at study entry and thereafter) shall include a unilateral aspirate and bilateral bone marrow biopsies for light microscopic morphologic studies; aspirate for immunophenotyping (lymphoma panel, Cell Marker Lab); aspirate to Molecular Diagnostics lab for gene rearrangement studies (if appropriate); and cytogenetic studies to evaluate for development of myelodysplasia.

6.5 HIV-positive patients should have HIV disease assessed (HIV viral load and CD4 count) prior to stem cell mobilization, at day +28, day +60, day +100 and then q 3 months during the first post-transplant year and then at 6 month intervals in the second and third post-transplant years, and then yearly thereafter. If there is evidence of an increasing HIV viral load, the patient's HIV physician will be consulted

6.6 Evaluations for immune reconstitution

6.6.1 Quantitative Immunoglobulins (IgG, IgM, IgA) should be measured prior to mobilization and post-transplant at day +100, day +180, and at 1 year.

6.6.2 Evaluation of CD4 and CD8 cells should be measured prior to mobilization and post-transplant at day +100, day +180, and 1 year. For HIV-positive patients, CD4 and CD8 analysis will be performed at day +28 and day +60 as well.

7 ENDPOINTS

7.1 Primary Endpoints

7.1.1 The percent of patients achieving complete response, the duration of response and, ultimately, progression free and overall survival will be evaluated.

7.1.2 Safety and outcomes of autologous transplant in the management of lymphoma in HIV-positive patients with adequate control of HIV disease.

7.2 Secondary Endpoints

7.2.1 Prospective validation of the previously published formula used to estimate targeted collection of PBSC (Section 12.0).

7.2.2 Evaluation of immune reconstitution post-transplant in patients with HIV disease compared to HIV negative patients.

7.2.3 The time to hemopoietic recovery after transplantation (first of three consecutive days of ANC ≥ 0.5 x 10^9/L; time to red cell transfusion-independence [no transfusions for 30 days]; time to platelet independence of 20 x 10^9/L and 50 x 10^9/L [no transfusions for 15 days]).

8 TRANSPLANT RELATED TOXICITIES AND COMPLICATIONS

8.1 G-CSF

G-CSF		
Common	**Less Common**	**Rare**
none	• bone or muscle pain • injection site reaction (redness, pain, or swelling)	• allergic reaction • spleen enlargement or rupture • serious lung problems (ARDS) • coughing up blood

8.2 Side Effects Of The Chemotherapy For Mobilization

Ifosphamide		
Common	**Less Common**	**Rare**
• nausea • vomiting • hair loss • bladder irritation and	• pain or inflammation where the drug was injected • low platelet count with increased risk of bleeding	• tiredness (fatigue) • confusion • sleepiness • seizures

Ifosphamide		
Common	**Less Common**	**Rare**
bleeding, blood in the urine • frequent or painful urination • abnormal kidney function • poor appetite • diarrhea • low white blood cell count with increased risk of infection	• agitation • dizziness • constipation	• hallucinations (seeing or hearing things that aren't there) • severe kidney damage or kidney failure • allergic reaction • abnormal heart rhythm • death due to effects on the brain or other causes

Carboplatin		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • low red blood cell count (anemia), which can make you tired, dizzy, or easily out of breath • brittle hair • kidney function can be altered at high doses • fetal abnormalities if taken while pregnant	• nausea • vomiting • loss of appetite • diarrhea • constipation • taste changes • allergic reaction • sensation of pins and needles in hands and/or feet related to nerve irritation • temporary or permanent infertility (inability to have children)	• confusion • changes in vision or vision loss (which improves after drug is stopped) • ringing in ears or hearing loss, which may be permanent • rash • dehydration • sores in the mouth or throat • severe allergic reaction* • kidney damage (may go away when drug is stopped) • liver problems • hair loss or thinning, including face and body hair • dizziness • death due to allergic reaction, infection, or other causes

Etoposide		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • low blood platelet count with increased risk of bleeding • nausea and/or vomiting • loss of appetite • hair loss, including face and body hair	• constipation • diarrhea • fever and chills • lowered red blood cell count (anemia)	• low blood pressure while drug is being given • sores in mouth and throat • changes in how foods taste • rash, which can become serious • itching • numbness and tingling in hands and/or feet • allergic reactions (may include chills, fever, rapid heart rate, trouble breathing, dizziness) • increased risk of a second cancer

Rituximab		
Common	**Less Common**	**Rare**
• mild allergic reaction with first infusion (may include fever, headache, chills, itching, hives, nausea, shortness of breath)	• allergic reaction with second and later infusions • low white blood cell count with increased risk of infection • cough • rash, itching • nausea • vomiting • diarrhea • muscle aches • runny nose • sinus infection	• serious allergic reaction, with hives, trouble breathing, tightness in the chest or throat, heart attack, or shock • serious skin reaction • kidney damage • low platelet count with increased risk of bleeding • blockage or hole in the bowel, with abdominal (belly) pain • low red blood cell count (anemia) with tiredness and weakness • death due to allergic reaction, infection, lung damage, tumor lysis syndrome, serious skin rash, bowel obstruction, liver failure from reactivated hepatitis B, and other causes

8.3 Peripheral Blood Stem Cell Collections

Risks to the apheresis process including:
1) bruising and/or bleeding where the needles are inserted for the apheresis,
2) reaction to citrate
3) loss of platelets with the of stem cells collection.
4) loss of blood (up to ½ pint) if the tubing breaks – this is very rare

8.4 Side Effects Of The Preparative Agents For Transplantation

8.4.1 Cyclophosphamide, BCNU and Etoposide

Cyclophosphamide		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • hair loss or thinning, including face and body hair (usually grows back after treatment) • nausea • vomiting • loss of appetite • sores in mouth or on lips • bleeding from bladder, with blood in urine	• allergic reaction with second and later infusions • low white blood cell count with increased risk of infection • cough • rash, itching • nausea • vomiting • diarrhea • muscle aches • low platelet count (mild) with	• heart problems with high doses, with chest pain, shortness of breath, or swollen feet • severe allergic reactions • skin rash • scarring of bladder • kidney damage (renal tubular necrosis) which can lead to kidney failure • heart damage, with trouble getting your breath, swelling

Cyclophosphamide		
Common	**Less Common**	**Rare**
• diarrhea • long-term or short-term infertility (inability to have children) in women and men	increased risk of bleeding • darkening of nail beds • acne • tiredness • infection • fetal changes if you become pregnant while taking cyclophosphamide	of feet, rapid weight gain • scarring of lung tissue, with cough and shortness of breath • second cancer, which can happen years after taking this drug • death from infection, bleeding, heart failure, allergic reaction, or other causes

BCNU (Carmustine)		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • nausea • vomiting • loss of appetite • diarrhea • headache • pain along vein while the drug is being given • irritation of vein used for giving the drug • fetal abnormalities if pregnancy occurs while taking this drug	• scarring of lung tissue, with cough and shortness of breath, which can happen years after treatment • flushing of skin • redness of the eyes (just after infusion) • tiredness (fatigue) • loss or thinning of hair (including hair on the face and body) • low red blood cell counts (anemia) causing tiredness and other symptoms	• temporary kidney damage • hardening of vein used for injection • liver abnormalities, which usually get better when the drug is stopped • death due to lung damage or other problems

Etoposide		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • low blood platelet count with increased risk of bleeding • nausea and/or vomiting • loss of appetite • hair loss, including face and body hair	• constipation • diarrhea • fever and chills • lowered red blood cell count (anemia)	• low blood pressure while drug is being given • sores in mouth and throat • changes in how foods taste • rash, which can become serious • itching • numbness and tingling in hands and/or feet • allergic reactions (may include chills, fever, rapid heart rate, trouble breathing, dizziness) • increased risk of a second cancer

Total Body Irradiation (TBI)		
Common	**Less Common**	**Rare**
• nausea and vomiting • diarrhea • cataracts • sterility • endocrinopathies • growth failure • intestinal cramps • mucositis	• parotitis • interstitial pneumonitis • generalized mild erythema • veno-occlusive disease	• dysphagia • vertebral deformities • nephropathy • risk of 2nd malignancy years later (when given along with chemotherapy)

8.4.2 BEAM

BCNU (Carmustine)		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • nausea • vomiting • loss of appetite • diarrhea • headache • pain along vein while the drug is being given • irritation of vein used for giving the drug • fetal abnormalities if pregnancy occurs while taking this drug	• scarring of lung tissue, with cough and shortness of breath, which can happen years after treatment • flushing of skin • redness of the eyes (just after infusion) • tiredness (fatigue) • loss or thinning of hair (including hair on the face and body) • low red blood cell counts (anemia) causing tiredness and other symptoms	• temporary kidney damage • hardening of vein used for injection • liver abnormalities, which usually get better when the drug is stopped • death due to lung damage or other problems

Etoposide		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • low blood platelet count with increased risk of bleeding • nausea and/or vomiting • loss of appetite • hair loss, including face and body hair	• constipation • diarrhea • fever and chills • lowered red blood cell count (anemia)	• low blood pressure while drug is being given • sores in mouth and throat • changes in how foods taste • rash, which can become serious • itching • numbness and tingling in hands and/or feet • allergic reactions (may include chills, fever, rapid heart rate, trouble breathing, dizziness) • increased risk of a second cancer

Ara-C		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • low red blood cell count (anemia) with symptoms like weakness, tiredness, shortness of breath • nausea • vomiting • stomach pain • tiredness (fatigue) • sores in mouth or on lips	• diarrhea • loss of appetite • rash • hair loss or thinning (may include face and body hair) • fever • muscle and bone aches • liver damage • blood clots and inflammation of the vein where the drug was given	• red or swollen eyes • sleepiness • muscle weakness • trouble walking • trouble writing • slurred speech • kidney damage • fetal changes that may lead to birth defects, prematurity, or serious illness in the newborn if you become pregnant while taking this drug • allergic reaction with itching, dizziness, trouble breathing, or swelling of the face, mouth, or throat • death due to infection, bleeding, or other causes

Melphalan		
Common	**Less Common**	**Rare**
• nausea (at higher doses) • vomiting (at higher doses) • low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • anemia (low red blood cell count) with symptoms like tiredness, paleness, or	• short-term or long-term infertility (inability to have children) • weakness	• severe allergic reaction • loss of appetite • scarring (fibrosis) or inflammation of lungs • hair loss, including face and body hair • rash • itching • second type of cancer (may

Melphalan		
Common	**Less Common**	**Rare**
trouble catching breath		happen years after treatment) • death from lung damage or other causes

8.5 Possible Risks of Autologous Transplant:

During the cell infusion: nausea, chills

During the post-transplant period:

- low blood counts increasing the risk of infection and bleeding
- suppression of the immune system
- risks of cancer recurrence

9 ADVERSE EVENT REPORTING

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 3.0 (CTCAE) and reported on the schedule below. A copy of the CTCAE can be downloaded from the CTEP home page (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf).

9.1 Definitions

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Life-Threatening Adverse Event Or Life-Threatening Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death.

Event Attribution Categories:
CTCAE does not define an AE as necessarily 'caused by a therapeutic intervention.' The clinical investigator must assign attribution for an adverse event after naming and grading of the event.

Attribution	Description
Unrelated	The AE is clearly NOT related to the intervention
Unlikely	The AE is doubtfully related to the intervention
Possible	The AE may be related to the intervention
Probable	The AE is likely related to the intervention
Definite	The AE is clearly related to the intervention

Unanticipated (unexpected) adverse event or unexpected suspected adverse reaction as defined by the University Of Minnesota IRB are those that are *not* already described as potential risks in the consent form, *not* listed in the Investigator's Brochure or *not* part of an underlying disease.

Expedited (Rapid) Reporting: Certain events may require rapid notification to entities providing patient safety oversight (e.g. IRB) as detailed in section 9.2.

9.2 Adverse Event Reporting Requirements

The reporting period for this study is from initiation of study treatment through engraftment or day 42, whichever occurs earlier; however after this time point, the investigator must report upon knowledge any study treatment related event meeting the expedited reporting criteria below.

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ fax numbers	Copy AE to:
U of MN IRB	**UPIRTSO**: any event which is unanticipated, involved new or increased risk to subjects, and was at least possibly related to study procedures through engraftment or day 42, whichever occurs earlier	10 Working Days	UMCC SAE	MMC 820	MCC SAE Coordinator mcc-saes@umn.edu
	Other Problems or Events meeting the definition of UPIRTSO		UPIRTSO Form		
MCC SAE Coordinator	Any event that counts toward a study stopping rule (see section 11.2.2)	Upon reporting	Study stopping rule form		

The SAE Coordinator will provide the Cancer Center's Data and Safety Monitoring Council (DSMC) with the SAE in an appropriate format depending on the individual SAE (as reported or in a summary format).

10 DATA AND SAFETY MONITORING

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://www.cancer.umn.edu/exfiles/research/dandsmplan.pdf.

For the purposes of data and safety monitoring, this study is classified as moderate risk. Therefore the following requirements will be fulfilled:

- The PI will complete and submit a quarterly Trial Progress Report to the Masonic Cancer Center Data and Safety Monitoring Council (DSMC) with the understanding the Cancer Protocol Review Committee (CPRC) may require more frequent reporting.
- The PI will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable events per the definition of reportable in section 9.2 to the Masonic Cancer Center's SAE Coordinator and the University of Minnesota IRB.

At the time of the IRB continuing review, the Principal Investigator will submit to the CPRC a copy of all documentation submitted to the IRB for continuing review.

11 EXPERIMENTAL DESIGN AND STATISTICAL CONSIDERATIONS

11.1 Estimated Accrual

It is estimated that approximately 30 patients per year will be accrued to this study. Of these 30 patients, approximately 5 per year are expected to be HIV positive.

11.2 Primary Endpoint analyses

11.2.1 Time-to-event analyses of progression-free and overall survival
Progression-free (PFS) and overall survival (OS) will all be assessed by time-to-event analyses. Based on the event rate from prior data collected from this institution on protocol MT1995-27, the anticipated precision of the PFS estimate is ± 2.5% at 1 year and ± 3.3% at 5 years. For OS, the anticipated precision is ±1.5% at 1 year and ± 3.6% at 5 years.

Kaplan-Meier and cumulative incidence plots will describe the distribution of event times. For events experienced by greater than 50% of patients, median event times and confidence intervals will

be reported. Log-rank tests will assess differences between HIV positive and negative patients

11.2.2 Safety of autologous PBSC HSCT in patients with HIV disease and relapsed lymphoma

It is estimated that approximately 5 HIV positive patients per year will be enrolled. Stopping rules will be implemented based on numbers of unexpected adverse events in the cohort of HIV positive patients. Based on current available literature for autologous HSCT in HIV positive patients with lymphoma, morbidity and mortality are similar to non-HIV infected patients (i.e. ≤ 5%). Based on this information, the study would be reviewed if the following occur:

2 events in the first 2 patients enrolled
3 events occurring up to and including15 patients enrolled
4 events occurring up to and including 22 patients enrolled
5 events occurring up to and including 30 patients enrolled

These stopping rules control the chance of stopping to 4.9% if the event rate is at an acceptable 5% while giving an 80.7% chance of stopping if the event rate is 20%. Other probabilities of stopping the study are included in the table below.

Event Rate	Probability of Stopping Accrual	Expected number of subjects enrolled before stopping
8%	16.5%	28
10%	27.5%	26
15%	57.7%	21
18%	72.9%	18
20%	80.7%	16
25%	93%	13
30%	97.9%	10

11.3 Secondary Endpoint Analysis

11.3.1 Comparison of the actual CD34+ cells collected versus the collection estimated by the formula (Section 12.2):

The accuracy of the formula's estimation of CD34+ cell collection will be assessed by a statistical description of the difference between actual and estimated daily collection of CD34+ cells. This difference is the formula's discrepancy.

A repeated measures (variance components) model will be used to account for the correlation of daily differences from the same patient.

Estimates of the mean daily difference and the standard deviation of the daily differences both within and between patients will characterize the formula's performance.

A criterion of acceptable variation in the formula's discrepancy is specified a priori. The lower 95% confidence interval for the predicted discrepancy shall not fall below zero by more than 1/3 the predicted CD34+ cell collection for CD34+ cell collection levels in the range (0.3, 1.5) x 10^6 cells/kg. For example, for a formula-predicted CD34+ cell collection of 0.5 x 10^6 cells/kg, the lower end of the 95% confidence interval around the discrepancy should be ± 0.167, indicating high confidence that actual collection will fall between 0.33 and 0.67 x 10^6 cell/kg.

Tests will also be performed to determine if the mean and standard deviation of the formula's discrepancy vary by pbCD34, collection day, disease stage, type of lymphoma, and HIV status.

11.3.2 Assessment of CD34+ cell collection

CD34+ cell collection data will be described by its distribution, mean, median, range and standard deviation both within patient and across all patient cohorts.
Regression analysis of CD34+ cell collection will be performed to estimate potential effects of collection day, pbCD34 measurement, HIV status and patient and lymphoma characteristics on cell counts.

11.3.3 Immune reconstitution will be compared between HIV negative and positive patients.

Immune reconstitution will be assessed by the time from transplant until measured levels return to the lower of a patient's pre-transplant level and a clinically normal level. This assessment will be performed for CD4, CD8, and quantitative immunoglobulins (IgG, IgM, IgA).
Statistical comparison will be performed by a log-rank test to compare Kaplan-Meier curves describing time-to-reconstitution for each measured quantity.

11.3.4 Descriptive statistics for patient and lymphoma characterization

Patient demographics, clinical history, and attributes of their lymphomas will be described statistically using means, medians, ranges, and standard deviations for quantitative variables and frequencies for categorical variables. Differences between HIV positive and negative patients will be assessed using t-tests and chi-squared tests

11.3.5 Time-to-event analyses of engraftment of neutrophils and platelets

Engraftment will be assessed by time-to-event analyses. Kaplan-Meier and cumulative incidence plots will describe the distribution of event times. For events experienced by greater than 50% of patients, median event times and confidence intervals will be reported. Log-rank tests will assess differences between HIV positive and negative patients.

12 ESTIMATION OF CELLS

12.1 Hypothesis: Using a set volume of apheresis and the estimated collection efficiency (CE) as well as the measured pbCD34/µL and donor ideal body weight (IBW) in kilograms, the number of pbCD34 collected can be accurately estimated prior to harvest

12.2 Formulas:
Prediction of CD34 + cells collected:

(Volume of Apheresis) x (CE) x [(pbCD34/µL) / (IBW)]

- Volume of apheresis will be recorded by the Donor Center for every collection

- Based on recent analyses of stem cell collections, the estimated collection efficiency (CE) at the University of Minnesota donor center is 60 ± 0.5%

- Peripheral Blood (pb)CD34/µL = WBC count x %CD34
  - WBC count is reported in the computer $x10^9$/L
  - %CD34 is reported by the Flow cytometry lab as the percentage of mononuclear cells that are dual positive for CD45 and CD34
  - The factor 10 corrects for conversion of WBC to cells/µL and the %CD34 to an absolute number
  - EXAMPLE: Patient's WBC is reported as 5.7 x 10^9/L and the %CD34 is reported as 0.25% then
    pbCD34/µL = 5.7 x 0.25 = 1.425/µL

- Ideal body weight is calculated using:
  - 50kg + [2.3kg x (height in inches - 60)] for men
  - 45.5kg + [2.3kg x (height in inches -60)] for women
  - Adjusted body weight = IBW + 0.5(ABW - IBW)

13 ADMINISTRATIVE PROCEDURES

13.1 Informed Consent

After the patient is fully evaluated and available clinical data is reviewed by the attending physician, eligible patients are presented with specific details of the treatment plan. The recommended therapeutic steps are discussed thoroughly with the patient and family. The risks of the procedure are outline including the priming of PBSC chemotherapy plus G-CSF, the peripheral stem cell collections, the pre-transplant chemotherapy and/or radiation; the transplantation hospital course; the potential need for local radiation treatment post-transplant and the need for ongoing follow-up evaluation to monitor the patient's post-transplant recovery.

The rationale of the study as well as the hazards of these components of treatment is explained to the patient in full and all questions are answered as completely as possible. Alternative forms of therapy are presented. After this presentation, informed consent is obtained using forms approved by the University of Minnesota IRB: Human Subjects Committee.

13.2 Records Retention

The investigator, through the CTO and BMT research database, will retain study records, including source data and all study correspondence in a secured facility for 6 years from the date of the study file closure with the IRB. In addition, the Clinical Trials Office (CTO) will keep a master log of all patients participating in the study, with sufficient information to allow retrieval of the medical records for that patient.

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Appendix I

Karnofsky Performance Status

KARNOFSKY PERFORMANCE STATUS SCALE DEFINITIONS RATING (%) CRITERIA

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	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; varying amount of assistance 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 of his personal 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; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

Appendix II

Response Criteria for Non-Hodgkin's Lymphoma

Response Category	Physical Examination	Lymph Nodes	Lymph Node Masses	Bone Marrow
CR	Normal	Normal	Normal	Normal
CRu	Normal	Normal	Normal	Indeterminate
	Normal	Normal	> 75% decrease	Normal or indeterminate
PR	Normal	Normal	Normal	Positive
	Normal	≥50% decrease	≥50% decrease	
	Decrease in liver/spleen	≥50% decrease	≥50% decrease	
Relapse/progression	Enlarging liver/spleen; new sites	New or increased	New or increased	Reappearance

1. CR: Complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms if present before therapy, and normalization of biochemical abnormalities (i.e. LDH) attributable to disease. All lymph nodes and nodal masses must have regressed to normal size (< 1.5 cm in their greatest transverse diameter for nodes ≥1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their greatest transverse diameter before treatment must have decreased to < 1 cm in their greatest transverse diameter after treatment. The spleen, if considered to be enlarged before therapy on the basis of a CT scan, must have regressed in size and must not be palpable on physical examination. If the bone marrow was involved by lymphoma before treatment, the infiltrate must be cleared on repeat bone marrow aspirate and biopsy.

2. CRu: Includes patients who fulfill the criteria for CR but with the following features: A residual lymph node mass greater than 1.5 cm in greatest transverse diameter that has regressed by more than 75%. Individual nodes that were previously confluent must have regressed by more than 75% compared with

the size of the original mass. Indeterminate bone marrow (increased number or size of aggregates without cytologic or architectural atypia).

3. PR: At least 50% regression in diameter of major nodal masses. No increase in the size of the other nodes, liver, or spleen. Splenic and hepatic nodules must regress by at least 50%. With the exception of splenic and hepatic nodules, involvement of other organs is considered assessable and not measurable disease. Bone marrow assessment is irrelevant for determination of a PR because it is assessable and not measurable disease; however, if positive, the cell type should be specified. No new sites of disease.

4. Stable Disease:
   This is defined as less than a PR but is not progressive disease.

5. Progressive Disease:
   At least a 50% increase from nadir of any previously identified abnormal node. Appearance of any new lesion during or at the end of therapy.

Appendix III

Calculation of AUC for Carboplatin dosing using Calvert formula

Carboplatin Dose (mg) = target AUC (mg/mL x min) x [GFR (mL/min) +25]

GFR (mL/min) = (wt in kg) x (140 – age)/ [72 x Creatinine]

** for women, multiply result by 0.85

Appendix IV

Cell Collection, Storage and Use/Vascular Access for Apheresis

Peripheral blood stem cells will be collected using available apheresis machines. Currently at the University Hospital, the Fenwal CS3000 is available and will be used. A standard technique, using a modification of program one, run at 1400 rpm, will be used. Procedures will be performed using either antecubital veins or 13.5 Fr **Quinton/Davol** catheters if antecubital veins are not appropriate for apheresis collection. Peripheral blood mononuclear cell collections will be performed on weekends only if clinically appropriate.

Peripheral blood cells will be combined with an equal volume of 20% dimethylsulfoxide (DMSO) in protein containing media for a final concentration of ≤ $300x10^6$ nucleated cells per kg and 10% DMSO. The cell concentrate will then be frozen at a controlled rate in a programmable controlled rate freezer (1°minute through -60°C and 3°minute through -90°C). The frozen cell concentrates will be stored in the vapor phase of liquid nitrogen.

Quality control including careful measurement of CD34%, absolute MNC and NC counts will be performed. The total number of CD34 cells collected (not weight based) as well as the CD34/kg actual body weight will be reported.

See Section 5.2 for guideline for continuation of poor collections.

Appendix V

Dosing of G-CSF and Volume of Apheresis based on patient actual body weight

BODY WEIGHT	DOSE OF G-CSF	VOLUME OF APHERESIS
<40 Kg	300 mcg	6 L
40 – 45 kg	300 mcg	6 L
45 – 50 kg	480 mcg	8 L
51 – 55 kg	480 mcg	8 L
56 – 60 kg	600 mcg	10 L
61 – 65 kg	600 mcg	10 L
66 – 70 kg	600 mcg	10 L
71 – 75 kg	780 mcg	12 L
76 – 80 kg	780 mcg	12 L
81 – 85 kg	900 mcg	12 L
86 – 90 kg	900 mcg	12 L
91 – 95 kg	900 mcg	12 L
>95 kg	1260 mcg	15 L

Appendix VI
Required Observations

	within 30 days of enrollment	Start of mobilization	Daily during apheresis	Prior to Admit for transplant	day +28	day +60	day +100	day +180	1 yr	18 mo	2yr
Medical History	x	x		x							
Physicial Exam	x	x	x	x	x	x	x	x	x	x	x
Weight/Height	x	x	x	x							
Performance status	x	x		x	x	x	x	x	x	x	x
Adverse Event/toxicity notation	x	x	x	x	x	x	x	x	x	x	x
Comprehensive chemistry panel	x			x	x	x	x	x	x	x	x
Hepatic Panel	x			x	x	x	x	x	x	x	x
CBC with diff	x	x	x	x	x	x	x	x	x	x	x
*SPEP	x						x			x	
LDH	x				x		x	x	x	x	x
*$ß_2$-microglobulin	x						x			x	
HIV viral load[2]	x			x	x	x	x	x	x	x	x
Viral Hepatitis serologies	x										
quantitative Immunoglobulin	x						x	x	x		
pb $CD4^+$	x				x[2]	x[2]	x	x	x[2]	x	x[2]
pb $CD8^+$	x				x[2]	x[2]	x	x	x[2]	x	x[2]
%CD34 cells prior to collection	x		x								
Bilateral bm biopsy unilateral aspirate	x				x[1]		x		x		x
CT scan – chest, abdomen, pelvis (neck if indicated)	x				x		x	x	x	x	x
PET scan	x				x		x				
Flow cytometry	x				x[1]		x		x		x
BM Cytogenetics	x						x		x		x
CD34+ cells harvested (total number)			x								
Volume of apheresis			x								

[1] Day 28 bone marrow biopsy and flow cytometry to be performed ONLY if prior history of marrow involvement

[2] Only for HIV-positive patients

[3] If patient is eligible for post-transplant radiation therapy and has prior PET-positive disease.

* for CLL + low grade lymphomas only

An attempt should be made to obtain the scheduled activity as close as possible to the scheduled date. However, scheduling difficulties (for procedures and around weekends) may make this difficult. Therefore, the scheduled activity will be obtained within 14 days of that schedule (30 days for dates after day +100).