Once daily intravenous Busulf ex as part of reduced-toxicity conditioning for patients with relapsed/refractory Hodgkin's and non-Hodgkin's lymphomas undergoing allogeneic hematopoietic progenitor cell transplantation - A multicenter phase II study.

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Schema

Once daily intravenous Busulfex as part of reduced-toxicity conditioning for patients with relapsed/refractory Hodgkin's and non-Hodgkin's lymphomas undergoing allogeneic hematopoietic progenitor cell transplantation - A multicenter phase II study.

PRIMARY OBJECTIVES:

1. To assess 1-year PFS of patients with chemotherapy refractory Hodgkin and NHL undergoing reduced-toxicity conditioning (RTC) with once daily intravenous Busulfex and fludarabine.

SECONDARY OBJECTIVES:

1. To record 1 and 2 year overall survival following transplantation.
2. To record 2 year PFS.
3. To assess NRM following RTC transplantation at day +100 and 1-year.
4. To assess relapse rate following transplantation at day +100 and 1-year.
5. To assess disease response rate following transplantation at day +100 and at 1-year.
6. To correlate OS, PFS, RR, NRM following HSCT with systemic busulfan exposure.
7. To assess rates of acute and chronic GVHD.
8. Time to successful neutrophil engraftment.
9. Time to successful platelet engraftment.
10. To assess rates of primary and secondary graft failure.
11. To assess rates of primary and secondary graft rejection.
12. To assess rates of pulmonary toxicity and VOD post transplantation, and assess correlation with Busulfex exposure levels.
13. To assess lineage specific chimerism kinetics of donor cells following once daily IV Busulfex based RTC at days +30, +100, +180 and +365.
14. To correlate chimerism kinetics following transplantation with Busulfex exposure levels.
15. To determine immune reconstitution pattern at days +30, +100, +180, and +365.
16. To evaluate biologic & genetic markers associated with the malignancy, GVHD and/or the treatment.

STUDY DESIGN:

This is a phase II study of allogeneic hematopoietic progenitor cell transplantation (HPCT) following reduced toxicity conditioning with once daily intravenous Busulfex and fludarabine in patients with relapsed/chemotherapy refractory Hodgkin's and non-Hodgkin's lymphomas.

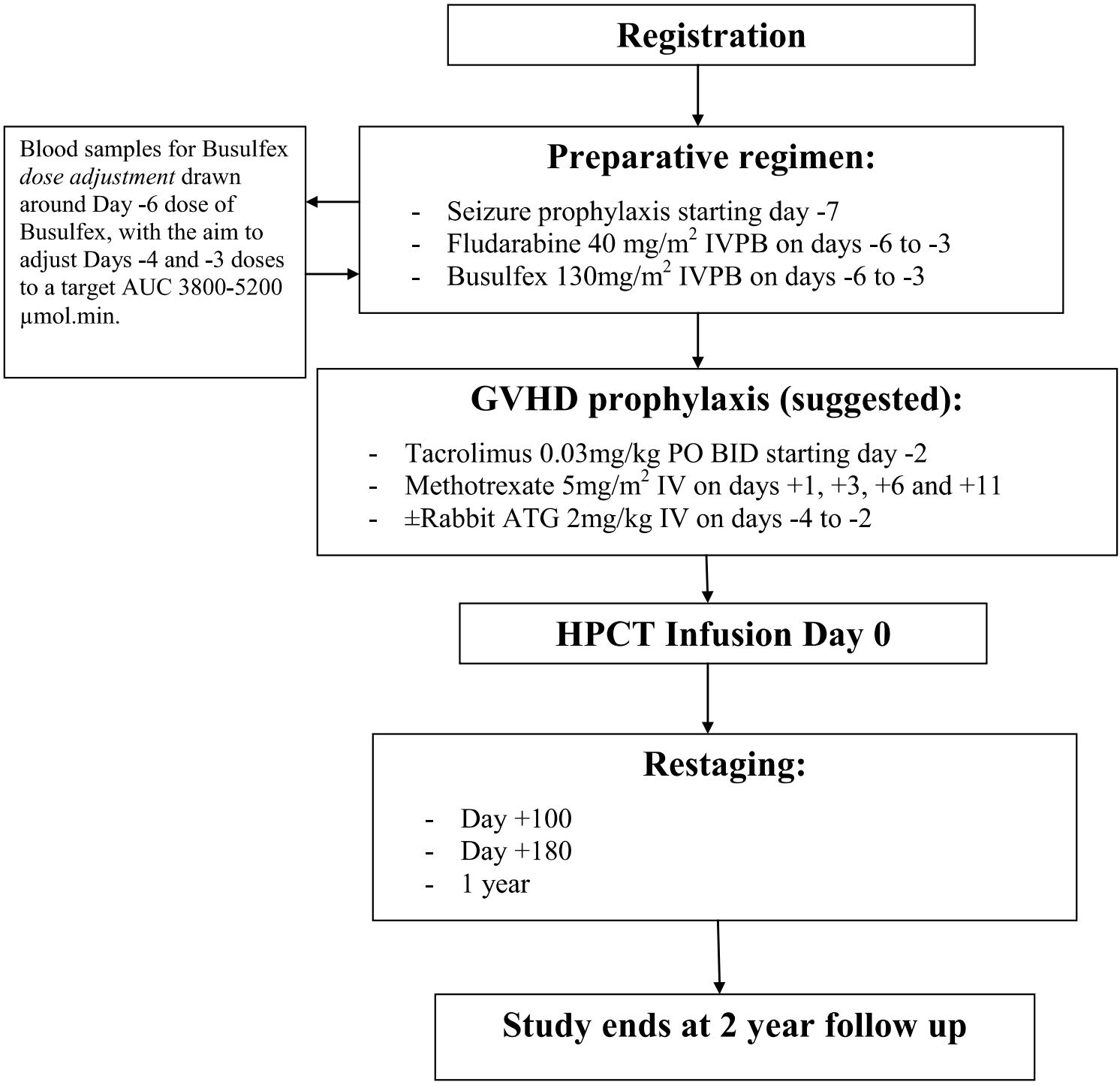
INCLUSION CRITERIA:

1. Patients aged 18-70 years of age are eligible.
2. Eligible histologies include:
   - B-cell, T-cell or NK-cell NHL refractory to frontline or salvage therapy defined as failure to achieve complete or partial remission according to standard criteria [12].
   - Diffuse large B-cell lymphoma relapsing within 12months of finishing a rituximab containing first line chemotherapy regimen (regardless of response to salvage chemotherapy) or with evidence of c-myc. Hodgkin lymphoma which is chemorefractory after at least two prior therapies.
   - Hodgkin and NHL in an untreated relapse.
   - Transformed NHL or chronic lymphocytic leukemia undergoing Richter's transformation (regardless of response to last chemotherapy). Patients with chemosensitive relapsed NHLs or Hodgkin lymphoma, but considered ineligible for curative therapy with autologous transplantation, because of (a) inability to collect stem cells, (b) prior autografting, (c) presence of myelodysplasia or (d) histology not considered curable with autografting in opinion of treating physician will be eligible.
3. All patients must have at least one suitable HLA-matched sibling or volunteer unrelated donor available (according to institutional guidelines). HLA typing should be performed at least at serological level for HLA-A, -B, and –C and at allele level for HLA–DRB1. One antigen or allele level mismatch will be permitted between the donor and the recipient; however each donor/recipient pair must match at HLA-DRB1 at allele level.
4. Patient must be able to provide informed consent.
5. Left ventricular ejection fraction $\geq$ 40%. No uncontrolled arrhythmias or uncontrolled New York Heart Association class III-IV heart failure.
6. Bilirubin, AST, and ALT $\leq$ 3 x normal; and absence of hepatic cirrhosis.
7. Adequate renal function as defined by a serum creatinine clearance of $\geq$ 40% of normal calculated by Cockcroft-Gault equation.
8. DLCO (diffusion capacity; corrected for hemoglobin) or FEV1 $\geq$ 50% of predicted.
9. Karnofsky performance status $\geq$ 70.
10. A negative pregnancy test will be required for all women of child bearing potential. Breast feeding is not permitted.

EXCLUSION CRITERIA:

1. Patients eligible for potentially curative therapy with autologous transplantation.
2. Patients with lymphoblastic lymphoma.
3. Patients with positive HIV serology.
4. Clinical evidence of uncontrolled bacterial, viral or fungal infection at the time of transplant conditioning.
5. Prior allogeneic transplantation.

Treatment Plan



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1. INTRODUCTION:

1.1. Autografting for Relapsed Hodgkin's and non-Hodgkin's Lymphomas:

High dose therapy and autologous hematopoietic progenitor cell transplantation (HPCT) is considered standard therapy for patients with relapsed chemosensitive Hodgkin's Lymphoma and diffuse large B-cell lymphoma (DLBCL), and appears to be curative for 40-45% of the patients [1-3]. Relapsed non-Hodgkin's lymphoma (NHL) patients with other aggressive histologies including Peripheral T-cell lymphoma (PTCL), transformed B-cell lymphoma, mantle cell lymphoma (MCL) and Burkitt's lymphoma (BL) generally do not achieve sustained remissions following autologous transplantation [4-6]. Results of autologous HPCT in relapsed Hodgkin's and indolent or aggressive NHL patients who are *refractory* to salvage chemotherapy have been uniformly disappointing.

1.2. Allogeneic HSCT for Relapsed Chemosensitive Lymphomas:

Allogeneic HPCT is a potentially curative modality for a number of hematological malignancies including indolent and aggressive lymphomas. The advantages of an allogeneic graft include a tumor free graft, and a potential immune mediated graft-versus-lymphoma (GVL) effect. Despite the inherent risk of increased transplant related morbidity and mortality associated with allogeneic HPCT, select relapsed aggressive NHL patients, especially the subgroup with chemosensitive disease, can achieve long term remissions following allografting [7-11]. Hodgkin's or NHL patients who are refractory to salvage chemotherapy (i.e. patients failing to achieve at least a partial remission following salvage therapy as specified by Cheson et al. criteria [12] have poor prognosis if treated with conventional chemotherapy regimens alone.

1.3. Allogeneic HPCT for Chemorefractory Lymphomas:

There are only limited data available for the outcomes with allogeneic HPCT for this high-risk group. Doocey et al. reported 5-year OS of 48% in a cohort of 44 aggressive NHL patients (including 9 with chemorefractory disease) [9]. Similarly French [10] and Japanese [8] registry data have reported OS rates of 41% and 42% respectively with allografting in aggressive NHL. Although these studies included patients with refractory disease, their outcomes were not reported separately. Interestingly in Doocey et al's report [9], the relapse rate in patients with

refractory disease was not significantly different from patients with chemosensitive disease. Various other investigators have retrospectively reported encouraging results with allogeneic HPCT in patients with relapsed and/or refractory Hodgkin's and NHL (13-18), none focusing primarily on refractory lymphomas.

A single institution study conducted at Ohio State University reported outcomes of allogeneic HPCT in patients with chemotherapy refractory aggressive NHL [19]. The study retrospectively analyzed outcomes of 46 patients with chemorefractory, aggressive NHL patients who had either stable disease (SD, n=32) or progressive disease (PD, n=14), respectively, following last salvage treatment. The median age was 46 years (range 22-63 yrs). 39 patients received matched sibling allografts, while 7 underwent unrelated donor transplantation. Diagnoses included diffuse large B-cell lymphoma (n=18), Burkitt's lymphoma (n=3), transformed B-cell lymphoma (n=5), mantle cell lymphoma (n=11) and peripheral T-cell lymphoma (n=9). The median number of prior therapies was 3 (range 2-8). Median follow-up of surviving patients is 5-years. 5-year overall survival (OS), progression free survival (PFS), and relapse rate for the whole cohort (n=46) were 38%, 34%, and 35% respectively. The rate of grade II-IV acute graft-versus-host disease (GVHD) was 43%. Of the 33 evaluable patients 75% developed chronic GVHD. Overall non-relapse mortality rate was 34%. The 5-year OS and PFS rates for patients with SD and PD were 46% vs. 21% (p-value=0.01; log-rank test), and 46% vs. 7% (p-value=0.0002; log-rank test) respectively. This study hints at potential of allogeneic transplant as curative therapy for a subset of chemorefractory patients with SD. These preliminary, encouraging results warrant confirmation in a well-designed clinical trial.

Thomas et al. [20] retrospectively report outcomes of fludarabine/melphalan/alemtuzumab based RIC in a cohort of 48 consecutive patients with relapsed/refractory DLBCL (30 patients with de novo disease and 18 patients with transformed follicular lymphoma) with a median follow-up of 52 months. Patients had experienced treatment failure with a median of five lines of prior therapy, including autologous transplantation in 69%, and 17% of patients were chemotherapy refractory at transplantation. Median age was 46 years, and 38% of patients had matched/mismatched unrelated donors. GVHD prophylaxis was with cyclosporine. All patients were successfully engrafted. Only 17% of patients developed grade 2 to 4 acute GVHD, with 13% experiencing extensive chronic GVHD. Four-year estimated nonrelapse mortality was 32%, and relapse risk was 33%. Twelve patients received donor lymphocyte infusions +/-

chemoimmunotherapy for relapse, and five patients obtained durable remissions, giving current PFS and OS rates at 4 years of 48% and 47%, respectively. Patients who had chemotherapy-sensitive disease before RIT had current PFS and OS rates at 4 years of 55% and 54%, respectively. Chemotherapy-refractory patients had a poor outcome. The inferior outcome of chemorefractory patients in Thomas et al's [20] study compared to Ohio State University's experience [19] may be due to less intense conditioning regimen employed and use of alemtuzumab (with possible impairment of GVL effects) in the former.

Fred Hutchinson Cancer Research Center reported outcome of nonmyeloablative allogeneic HPCT for chemotherapy-refractory indolent or transformed NHL [21]. Sixty-two patients with indolent or transformed NHL were treated with allogeneic HPCT from related (n = 34) or unrelated (n = 28) donors after conditioning with 2 Gy of total-body irradiation with or without fludarabine. Nine unrelated donors were mismatched for >/= one HLA antigen. Sixteen patients had histologic transformation before HPCT. 63% of patients had chemotherapy sensitive disease at transplantation, while 23% were chemotherapy refractory and 14% had an untreated relapse. Median age was 54 years, and patients had received a median of six lines of treatment before HPCT. Median follow-up time after HPCT was 36.6 months. At 3 years, the estimated OS and PFS rates were 52% and 43%, respectively, for patients with indolent disease, and 18% and 21%, respectively, for patients with transformed disease. Patients with indolent disease and related donors (n = 26) had 3-year estimated OS and PFS rates of 67% and 54%, respectively. The incidences of grade 2 to 4 acute GVHD, grade 3 and 4 acute GVHD, and extensive chronic GVHD were 63%, 18%, and 47%, respectively. Intriguingly while the risk of relapse was increased in patients with transformed (HR = 4.85; 95% CI, 1.5 to 15; *P* = .001) or chemotherapy-refractory disease (HR = 5.37; 95% CI, 1.7 to 17; *P* = .005); however, chemotherapy sensitivity did not have a significant impact on OS or PFS. These data argue that allogeneic HPCT can produce durable disease-free survival in patients with chemotherapy refractory lymphomas.

1.4. Busulfan:

Busulfan, a potent, cytotoxic, bifunctional alkylating agent that causes myeloablation, has been used clinically since the 1950s to treat hematological malignancies and myeloproliferative syndromes. Busulfan produces deoxyribonucleic acid (DNA) cross-linking and chromosomal

damage that can be lethal to rapidly dividing cells. At the low end of the active dose range, busulfan causes a selective depression of granulocytopoiesis. Increasing doses lead to progressive general myelotoxicity culminating in marrow ablation due to cell death. High doses cause significant DNA damage and are myeloablative. The selectivity of busulfan for marrow cells has several explanations. One is based on the susceptibility of relatively undifferentiated stem cells during the G-phase of the cell mitotic cycle to alkylation by busulfan. Busulfan treatment during the G-phase halts further differentiation and progression of the cell through the cell cycle. Microscopic examination of stem cell populations indicates arrested cell division with polyploidy and cell death [22]. Busulfan was used initially at low oral doses for palliative care, then at high oral doses in combination with other alkylating agents having immunosuppressive properties to promote the engraftment of bone marrow following allogeneic or autologous transplantation. In more recent years, busulfan has been used as one component of a chemo- plus radiotherapy or chemotherapeutic-based conditioning regimen prior to transplant. Busulfan's narrow therapeutic window as well as unpredictable intestinal absorption and erratic bioavailability led to the development of a parenteral formulation [23]. Subsequently, intravenous (IV) busulfan (under the name of Busulfex®) was approved in1999 in the US and Canada as a pretransplant conditioning agent.

1.5. Once Daily I.V. Busulfex for Allogeneic HPCT:

Fludarabine has a long plasma half-life, allowing once-daily administration. Fludarabine and once daily busulfan have demonstrated synergistic cytotoxicity [24]. Russell et al [25], have reported safety and efficacy data with a once-daily intravenous busulfan and fludarabine conditioning regimen for allogeneic HPCT in patients with hematologic malignancies. Their conditioning regimen comprised fludarabine 50 mg/m^2 on days -6 to -2 plus i.v. busulfan 3.2 mg/kg daily in a 3-hour infusion on days -5 to -2. The regimen was given to 70 patients aged 15 to 64 years (median, 41 years) with hematologic malignancy. Acute GVHD prevention comprised antithymocyte globulin 4.5 mg/kg over 3 days pretransplantation, cyclosporin A, and short-course methotrexate with folinic acid. Hepatic toxicity was transient and there was no clinically diagnosed veno-occlusive disease. Incidence of acute GVHD grades II to IV was 8% and chronic GVHD at 2 years is 38%. With a median follow-up of 16 months (range, 6-27 months), transplantation-related mortality at 100 days and 2 years was 2% and 5% for matched

related donor HPCT and 8% and 19% for alternate donors HSCT, respectively (P = not significant). Projected disease-free and OS rates at 2 years were 74% and 88% for low-risk disease, 26% and 37% for advanced AML, and 65% and 71% for other high-risk disease, respectively. Pharmacokinetic studies were done using 11 samples with the first and fourth doses of busulfan. Kinetics were linear, and for the first and fourth doses, the half-lives were 2.60 +/- 0.44 and 2.57 +/- 0.36 hours, respectively. Clearances were 106.77 +/- 16.68 and 106.86 +/- 21.57 mL/min per m^2, peak concentrations (Cmax) were 3.92 +/- 0.31 and 3.96 +/- 0.28 mcg/mL, and busulfan areas under the plasma concentration versus time curve (AUC) were 4866.51 +/- 771.42 and 4980 +/- 882.80 µmol.min, respectively. Day 4 pharmacokinetic values were very similar to those on day 1 for every patient. That report demonstrated the combination to be efficacious, with impressively low treatment-related mortality.

de Lima et al. [26] used intravenous busulfan and fludarabine as conditioning therapy for allogeneic HSCT for acute myelogenous leukemia and myelodysplastic syndrome. Fludarabine 40 mg/m^2 and intravenous busulfan 130 mg/m^2 were given once daily for 4 days, with tacrolimus-methotrexate as GVHD prophylaxis. 74 patients with AML and 22 patients with MDS were enrolled. Patients had a median age of 45 years (range, 19-66 years). Only 20% of the patients were in first complete remission (CR) at transplantation. Donors were HLA-compatible related (n = 60) or matched unrelated (n = 36). The CR rate for 54 patients with active disease was 85%. At a median follow-up of 12 months, 1-year regimen-related and treatment-related mortalities were only 1% and 3%, respectively. Two patients had reversible hepatic VOD. Actuarial 1-year OS and event-free survival were 65% and 52% for all patients, and 81% and 75% for patients receiving transplants in CR. The mean and median daily AUCs were 4891 and 4871 µmol.min, respectively (range, 2931-8271 µmol.min). The results strongly suggested that intravenous busulfan-fludarabine is an efficacious, reduced-toxicity, conditioning regimen for patients with AML or MDS undergoing HPCT.

1.6. Monitoring Busulfan levels:

In an interesting retrospective study of patients undergoing allogeneic HPCT with once-daily IV busulfan (3.2 mg/kg days -5 to -2) and fludarabine (50 mg/m^2 days -6 to -2), Geddes et al [27] reported that patients with an AUC >6000 µmol.min had lower OS. NRM at 100 days and PFS at 3 years were better with AUC < or =6000 µmol.min. No such data is available for lymphoid

malignancies. Moreover no studies to date have prospectively explored whether Busulfex dose reduction based on high AUC could reduced NRM and pulmonary and hepatic toxicity associated with this regimen. Despite encouraging safety and efficacy of once daily IV busulfan based conditioning allogeneic HPCT, no prospective trials have been performed in patients with relapsed, refractory Hodgkin's or NHLs, to our knowledge. The current study will be a multicenter, open label phase II study of allogeneic transplantation following reduced toxicity conditioning with once daily IV Busulfex, and fludarabine for patients with chemotherapy refractory lymphoid malignancies. Median daily AUC values following once-daily Busulfex doses of 130mg/m^2 (roughly equivalent to 3.2mg/kg i.v dose) range from approximately 4800 µmol.min to 5200 µmol.min [25, 26]. While it is suggested that AUC of >6000 µmol.min are associated with negative outcomes, lower Busulfex exposure levels can potentially lead to poor donor-cell engraftment and more importantly suboptimal disease control in this high-risk group before generation of a robust graft-versus-lymphoma effect by donor stem cells. Hence we hypothesize that a strategy of therapeutic monitoring of Busulfex drug levels and dose adjust to a target AUC of 3800-5200 µmol.min, will ensure uniform engraftment of donor cells, provide adequate disease control while minimizing toxicity associated with higher Busulfex exposure values.

1.7. Inclusion of Women and Minorities:

It is the policy of the Mary Babb Randolph Cancer Center to strive for gender and minority patient participation that represents the population of West Virginia in all clinical investigations. Between January 1st 2009 and December 31st 2009, 125 patients were enrolled onto Phase I/II/III trials at the Mary Babb Randolph Cancer Center. Of these patients 73% percent were female (n=92) and 2.4 percent were members of minority ethnic groups. It is anticipated that a similar or greater proportion of patients on this study will be female and/or members of ethnic minorities.

2. OBJECTIVES:

2.1. Primary Objectives:

1. To assess 1-year progression free survival (PFS) of patients with chemotherapy refractory Hodgkin's and non-Hodgkin's lymphoma (NHL) undergoing reduced-toxicity conditioning (RTC) with once daily intravenous (IV) Busulfex and fludarabine.

2.2. Secondary Objectives:

2. To record 1 and 2 year overall survival (OS) following transplantation.
3. To record 2 year PFS.
4. To assess non-relapse mortality (NRM) following RTC transplantation at day +100 and 1-year.
5. To assess relapse rate following transplantation at day +100 and 1-year.
6. To assess disease response rate following transplantation at day +100 and at 1-year.
7. To correlate OS, PFS, RR, NRM following HPCT with systemic busulfan exposure.
8. To assess rates of acute and chronic graft-versus-host disease (GVHD).
9. Time to successful neutrophil engraftment.
10. Time to successful platelet engraftment.
11. To assess rates of primary and secondary graft failure.
12. To assess rates of primary and secondary graft rejection.
13. To assess rates of pulmonary toxicity and veno-occlusive disease (VOD) post transplantation, and assess correlation with Busulfex exposure levels.
14. To assess lineage specific chimerism kinetics of donor cells following once daily IV Busulfex based RTC transplantation at days +30, +100, +180 and +365.
15. To correlate chimerism kinetics of donor cells following transplantation with Busulfex exposure levels.
16. To determine immune reconstitution pattern following transplantation at days +30, +100, +180, and +365.
17. To evaluate biologic & genetic markers associated with the malignancy, GVHD and/or the treatment.

3. ELIGIBILITY CRITERIA:

3.1. Inclusion Criteria:

1. Patients aged 18-70 years of age are eligible.
2. Eligible histologies include:
    - B-cell, T-cell or NK-cell NHL refractory to frontline or salvage therapy defined as failure to achieve complete or partial remission according to standard criteria [12].
    - Diffuse large B-cell lymphoma relapsing within 12months of finishing a rituximab containing first line chemotherapy regimen (regardless of response to salvage chemotherapy) or with evidence of c-myc. Primary refractory NHL (regardless of response to salvage chemotherapy).
    - Hodgkin lymphoma which is chemorefractory after at least two prior therapies.
    - NHL in an untreated relapse.
    - Transformed NHL or chronic lymphocytic leukemia undergoing Richter's transformation (regardless of response to chemotherapy). Patients with chemosensitive relapsed NHLs or Hodgkin lymphoma, but considered ineligible for curative therapy with autologous transplantation, because of (a) inability to collect stem cells, (b) prior autografting, (c) presence of myelodysplasia or (d) histology not considered curable with autografting in opinion of treating physician will be eligible.
3. All patients must have at least one suitable HLA-matched sibling or volunteer unrelated donor available (according to institutional guidelines). HLA typing should be performed at least at serological level for HLA-A, -B, and –C and at allele level for HLA–DRB1. One antigen or allele level mismatch will be permitted between the donor

and the recipient; however each donor/recipient pair must match at HLA-DRB1 at allele level.

4. Patient must be able to provide informed consent.
5. Left ventricular ejection fraction ≥ 40%. No uncontrolled arrhythmias or uncontrolled New York Heart Association class III-IV heart failure.
6. Bilirubin, AST, and ALT ≤ 3 x normal; and absence of hepatic cirrhosis.
7. Adequate renal function as defined by a serum creatinine clearance of ≥ 40% of normal calculated by Cockcroft-Gault equation.
8. DLCO (diffusion capacity; corrected for hemoglobin) or FEV1 ≥ 50% of predicted.
9. Karnofsky performance status ≥ 70.
10. A negative pregnancy test will be required for all women of child bearing potential. Breast feeding is not permitted.

3.2. Exclusion Criteria:

1. Patients eligible for potentially curative therapy with autologous transplantation.
2. Patients with lymphoblastic lymphoma.
3. Patients with positive HIV serology.
4. Clinical evidence of uncontrolled bacterial, viral or fungal infection at the time of transplant conditioning.
5. Prior allogeneic transplantation.

4. REGISTRATION PROCEDURE:

4.1. **Registration – WVU Patients**

- All source documents that support eligibility including a signed informed consent/HIPAA and signed eligibility checklist. These must be available for review and verification.

- At the point of registration, the research nurse or data manager will register the patient in the electronic database, including demographic, consent and on-study information. The patient will be assigned a unique sequence number for the study.

4.2. Registration Procedures – Collaborating Institutions

- The collaborating institution must first have written documentation of site activation to enroll patients into the trial. The notification will be sent to the PI of the collaborating site as soon as all required regulatory information has been accepted by the MBRCC CTRU.
- The collaborating institution must contact the Network Coordinator at least 24 hours prior to registering/randomizing a patient. After speaking with the Network Coordinator, the following documents should be faxed to (304) 293-8698 to begin the registration process:
    - The dated and signed informed consent
    - A physician signed eligibility checklist
    - All source documents that validate eligibility
    - The demographic form
- Written confirmation will be sent to the site once the patient has been enrolled into the study to issue the unique patient identifier and the dose/level/cohort as applicable.

5. TREATMENT PLAN:

All therapy (including methotrexate and tacrolimus) and growth factor doses will be based on patients' actual weight up to 120% of ideal body weight, above which it will be based on **adjusted ideal body weight** (ideal weight plus 50% of the difference between ideal and actual weight). For patients whose actual weight is less than ideal, then use actual weight.

5.1. Seizure Prophylaxis:

Seizure prophylaxis for busulfan will be according to the individual transplant center's guidelines. Phenytoin is discouraged for use as prophylaxis for busulfan-induced seizures as it is known as a potent inducer of hepatic drug-metabolizing enzymes causing

increased clearance of busulfan [28,29]. Recommended seizure prophylaxis options are as following:

1. Lorazepam can be given in a per os (PO) dose of 0.5 mg every 6 hours beginning the evening on Day −7 before the conditioning regimen of busulfan until the morning of Day −2, one day after the last day of busulfan administration [28]. OR;
2. Levetiracetam at 500 mg PO BID beginning the evening on Day −7 before the conditioning regimen of busulfan until the morning of Day −2, one day after the last day of busulfan administration [29].

5.2. Preparative Regimen:

1. Fludarabine 40 mg/m^2/day IVPB given over 60 minutes daily X 4 days on Days -6 through -3.
2. Each fludarabine dose will be immediately followed by Busulfex 130 mg/m^2/day IVPB over 3 hours daily X 4 days on Days -6 through -3. The drug will be infused via a controlled-rate infusion pump through a central venous catheter (CVC).

5.3. Busulfex therapeutic drug monitoring and dose adjustment:

A total of 6 blood samples (4-mL Sodium Heparin green top blood tubes) for Busulfex's *therapeutic drug monitoring and dose adjustment* will be collected surrounding the Day -6 Busulfex dose at the following times:

(1) at 3 hours after initiation of busulfan infusion (i.e., at the end of the infusion),

(2) at 195 minutes after the initiation of busulfan infusion (i.e., 15 minutes after completion of the busulfan infusion

(3) at 4 hours after initiation of busulfan infusion (i.e., one hour after completion of the busulfan infusion, ±10mins),

(4) at 5 hours after initiation of busulfan infusion (i.e., 2 hours after completion of the busulfan infusion, ±10mins),

(5) at 6 hours after initiation of busulfan infusion (i.e., 3 hours after completion of the busulfan infusion, ±10mins),
(6) at 8 hours after initiation of busulfan infusion (i.e., 5 hours after completion of the busulfan infusion, ±10mins),

Blood for Busulfex levels can be drawn from the patient's central line using a lumen not used for the busulfan infusion. **Make sure that the all the drug has been delivered and the line has been thoroughly flushed with saline before drawing the end of infusion sample**.. Samples will be transported to the laboratory in ice, and cryopreserved as previously described [31] (for details see Appendix C). The reference laboratory will determine busulfan systemic exposure and provide recommendations to maintain the individual dose to a targeted AUC of 3800-5200 μmol.min. It is expected that this information will be available to the inpatient transplant physician to adjust 3rd and 4th doses of Busulfex on days -4 and -3.

All blood specimens for Busulfex level monitoring for patient undergoing transplantation at WVU will be dispatched to and processed at Seattle Cancer Care Alliance (SCCA) Clinical Laboratories (for details see Appendix C). While it is recommended that all participating sites use SCCA clinical labs for Busulfex PK studies; however use of a different clinical laboratory of this purpose, will ultimately be at the discretion of individual transplant center.

5.4. GVHD Prophylaxis:

GVHD prophylaxis will be according to the individual transplant center's guidelines. However a combination of tacrolimus, mini-dose methotrexate with or without thymoglobulin (ATG) is strongly recommended as following:

1. Tacrolimus starting on Day -2 at 0.03mg/kg PO BID. Tacrolimus dosing is to be adjusted to maintain target serum levels of 5-12 ng/mL. Serum levels are not to exceed 15 ng/mL. Begin tapering between Day +90 to +120 in the absence of GVHD as tolerated with a goal of stopping by Day +150 to +180. Please note that concurrent use of agents such as itraconazole, voriconazole or fluconazole

(at doses >200 mg) may inhibit the metabolism of tacrolimus, and thus increase tacrolimus levels. Hence, it is recommended to check tacrolimus levels weekly when these agents are initiated concurrently. In addition, the initial dose of tacrolimus may be decreased according to institutional policies.

2. Methotrexate (5mg/m^2 on days +1, +3, +6 and +11). Hydrate intravenously and induce diuresis. Methotrexate will be held if the serum creatinine >3.0mg/dL. If the creatinine level is >1.5 mg/dL, then administer leucovorin 10mg IV or PO q6 hours for four doses beginning 24 hours after methotrexate.
3. In patients receiving allografts from unrelated donors or from one antigen mismatched sibling donors it is recommended to give rabbit antithymocyte globulin (Thymoglobulin) at 2.0 mg/kg/day IV over 6 hours x 3 doses on Days -4 through -2 [30]. Patients must be premedicated with acetaminophen 650mg PO, diphenhydramine 25-50 mg PO/IV, and methylprednisone 1mg/kg (dexamethasone 20mg IV) at initiation and midway through thymoglobulin administration each day. After the first dose, subsequent administration of antithymocyte globulin may be infused over 4 hours.

5.5. <u>Allogeneic hematopoietic progenitor cell infusion:</u>

1. On Days 0 (and +1; if necessary) a (recommended) minimum total cell dose of CD34+ cells of 2 x 10/kg^6 (actual weight - recipient) will be infused. There is no maximum CD 34+ cell dose.
2. G-CSF (5 mcg/kg/day subcutaneously) to promote recovery of neutrophils is at the discretion of treating physician.

5.6. <u>Antibiotic Prophylaxis:</u>

Antibiotic prophylaxis will be according to the individual transplant center's guidelines. Following represent recommended options as prophylactic antibiotics:

1. Herpes prophylaxis: Patients with a history of herpes simplex infection or seropositivity should receive prophylaxis according to institutional guidelines through Day +100. One regimen that may be used is acyclovir 200-400 mg PO TID Days -3 through Day +100. Valacyclovir 500mg PO QD may be used

instead of acyclovir. Prophylaxis may be extended beyond Day +100 at the discretion of the treating physician.

2. Candida prophylaxis: Candida prophylaxis will occur according to institutional guidelines through Day +100. A suggested regimen is fluconazole or itraconazole 200- 400 mg PO daily or voriconazole 200-300 mg PO twice daily (or 3- 6 mg/kg IV q12 hours) on Days -2 through +100. Low dose amphotericin B (10-20 mg/day) IV or echinocandins may also be used.
3. Pneumocystis Pneumonia (PCP) Prophylaxis: PCP prophylaxis will occur according to institutional guidelines through Day +100. One regimen that may be used to prevent PCP is cotrimoxazole (Bactrim®) administered as one double strength tablet daily on 3 days weekly, beginning on Day +21 through Day +100. If, at this time, CD4 lymphocytes are < 200/μL, then prophylaxis should be continued until CD4 lymphocytes are 200/μL. Patients allergic to cotrimoxazole should receive dapsone, atovaquone or inhaled pentamidine instead.
4. CMV Infections: No routine prophylaxis for CMV will be initiated. Surveillance for CMV using CMV PCR is required weekly beginning on Day +7 through Day +100. Patients with positive CMV PCR (>5000copies/ml) should receive treatment according to institutional practice. One regimen is ganciclovir 5mg/kg IV BID x 14 days (or appropriate doses of valganciclovir or foscarnet).
5. EBV Monitoring: For patient receiving antithymocyte globulin, it is strongly recommended that Epstein Barr Virus (EBV) surveillance of peripheral blood be performed in the form of EBV DNA PCR monitoring. EBV DNA PCR monitoring should be performed at least every two weeks from neutrophil engraftment through Day +100. If the EBV DNA copy number exceeds 5000/mL on two consecutive samples, it is recommended that rituximab 375 mg/ m2 IV be given to prevent post transplant lymphoproliferative disease (PTLD). Further rituximab dosing should be based on response to first dose as measured by a change in the EBV DNA copy number. Patient s with a positive EBV DNA PCR and signs or symptoms possibly attributable to PTLD (e.g., fever,

lymphadenopathy) should undergo a CT scan of the chest, abdomen, and pelvis to rule out PTLD.

5.7. Dose modification and management of toxicity:

5.7.1. Tacrolimus

Tacrolimus may cause hypertension, renal insufficiency (usually reversible), seizures, liver function abnormalities, hemolytic uremic syndrome (rare), hyperglycemia, and hypomagnesemia. Tacrolimus dose adjustments will be made to achieve target trough levels of 5-12 ng/mL. Serum levels are not to exceed 15 ng/mL.

5.7.2. Methotrexate

Methotrexate may cause mucositis and cytopenias. In the presence of worsening renal insufficiency or the development of effusions or ascites, the addition of leucovorin is permitted at the discretion of the physician (see Section 5.3). If the creatinine is > 1.5 mg/dL, leucovorin 10 mg IV or PO may be given every 6 hours for four doses beginning 24 hours after methotrexate. Methotrexate will not be given if the creatinine is > 3.0 mg/dL. Individual doses of methotrexate will not be altered.

5.8. Data Safety Monitoring Plan

This protocol will adhere to the policies of the Mary Babb Randolph Cancer Center Data and Safety Monitoring Plan, guidelines in accordance with NCI regulations. The Data and Safety Toxicity Committee will review all serious adverse events and toxicity reports as well as continuing reviews. Collaborating sites will be monitored routinely including

conference calls, DSTC assessment of adverse events and unexpected events, eligibility verification, and site visits if necessary.

6. MEASUREMENT OF EFFECT:

6.1. Follow up schedule:

The schedule for follow up on the study is shown in the table below.

Study Visit	Target Day Post-Transplant
1 week	7 ± 2 days
2 week	14 ± 2 days
3 week	21 ± 7 days
4 week	28 ± 7 days
5 week	35 ± 7 days
6 week	42 ± 7 days
7 week	49 ± 7 days
8 week	56 ± 7 days
9 week	63 ± 7 days
10 week	70 ± 7 days
11 week	77 ± 7 days
12 week	84 ± 7 days
100 day	100± 10 days
6 month	180 ± 28 days
12 month	365 ± 28 days
24 month	730 ± 28 days

6.2. GVHD Assessment:

It is recommended that following neutrophil engraftment, patients are monitored for development of acute and chronic GVHD at least once a week until day +100. After Day 100, patients should be assessed at each study visit for the presence of GVHD. Acute GVHD will be assessed by consensus criteria (Appendix A) (32) and graded on BMT CTN MOP suggested grading sheets (Appendix A). Chronic GVHD diagnosis and grading will be according to NIH Criteria. Please see Appendix B (33-34).

Clinical Grading of Chronic GVHD (According to Appendix B)

None

Mild chronic GVHD involves only 1 or 2 organs or sites (except the lung: see below ‡), with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites)

Moderate chronic GVHD involves: (1) at least 1 organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site) or (2) 3 or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). ‡A lung score of 1 will also be considered moderate chronic GVHD.

Severe chronic GVHD indicates major disability caused by chronic GVHD (score of 3 in any organ or site). ‡A lung score of 2 or greater will also be considered severe chronic GVHD.

6.3. Engraftment Assessment:

Neutrophil engraftment will be defined as first of three consecutive days with ANC ≥ 500 x 10^9/L post-conditioning regimen induced nadir. Similarly platelet engraftment is defined as first day of platelet count ≥ 20,000 x 10^9/L, without transfusion for 7 consecutive days. Lineage specific chimerism analysis (CD3 and CD33 subsets), quantitative immunoglobulins and immune reconstitution will be performed on days +30, +100, +180 and +365.

6.4. Veno-occlusive disease:

Veno-occlusive disease (VOD) will be **diagnosed** according to Baltimore criteria (35) as following:

Development of hyperbilirubinemia with serum bilirubin > 2 mg/dL within 21 days after transplantation and at least 2 of the following clinical signs and symptoms:

- Hepatomegaly, usually painful
- > 5% weight gain
- Ascites

For VOD, **severity** will be graded according to the McDonald criteria (36) as following:

• **Mild disease**: If patients showed no apparent adverse effect from liver disease; required no medications for diuresis of excessive fluid or for hepatic pain; and had completely reversible signs, symptoms, and laboratory abnormalities.
• **Moderate disease:** If patients had an adverse effect from liver disease; required sodium restriction and diuretics to minimize signs of fluid excess (edema, ascites, cardiopulmonary congestion) or medication to alleviate pain from hepatomegaly; and eventually showed a complete resolution of all signs of liver damage (a return of weight to baseline, a decrease in liver size, and a decrease in total serum bilirubin to < 34.2 μmol/L [2 mg/dL]).
• **Severe disease:** If patients showed an effect from liver disease, and signs, symptoms, and laboratory values did not resolve before day 100 or the patient died, whichever occurred first. Death is not a requirement for assignment to the severe VOD category.

6.5. Response Assessment and Criteria:

Disease response will be assessed by serial CAT or CT/PET imaging with or without bone marrow aspiration & biopsy (if clinically indicated) at days +100 (±28 days), +180 (±28 days) and +365 (±28 days). Additional imaging and clinical investigations to assess disease relapse and/or progression are permitted if clinically indicated (at the discretion of treating oncologist). The standard revised response criteria (International Harmonization Project) (12) for lymphoma presented in Appendix D, will be used to assess disease response throughout the study per the Study Calendar (please see section 7).

6.6. Removal of Patients from Protocol therapy:

6.6.1. Disease progression or relapse or death

Disease progression, relapse or death constitutes attainment of the primary endpoint. At that time, protocol therapy will be discontinued and patients may be treated at the discretion of the treating physician. Relapsing patients will be removed from protocol therapy and followed for survival and secondary malignancy.

6.6.2. Extraordinary medical circumstances

If, at any time, the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, protocol therapy shall be discontinued. In this event:

- Notify the Study Chair.
- Document the reason(s) for discontinuation of therapy in patient records.
- Follow the patient for survival, progression, relapse, and secondary malignancies, if possible.

7. Study Parameters (Study Calendar):

7.1. Patient Study Calendar:

The table below summarizes the patient clinical assessments over the course of the study.

Study Assessment	Baseline[1]	Conditioning Phase Days					Day 0	Days Post-Transplantation														
		-7	-6	-5	-4	-3		7	14	21	30	35	42	49	56	63	70	77	84	100	180	365/ 730
Informed Consent	X																					
History & physical exam[2]	X										X					X				X	X	X
Vital signs[3]	X	X	X	X	X	X	X				X					X				X	X	X
Karnofsky performance status	X										X					X				X	X	X
CBC, differential, & platelets	X	X	X	X	X	X	X	X[4]	X[4]	X[4]	X[4]					X				X	X	X
Serum chemistries panel[5]	X	X					X	X	X	X	X					X				X	X	X
Infectious disease titers[6]	X[6]																					
LVEF[7]	X																					
DLCO[7]	X																					
Ann Arbor stage (at transplant)	X																					
Disease risk score[15] (at transplant)	X																					
CMV quantitative PCR										X	X	X	X	X	X	X	X	X	X	X		
Bone marrow aspirate/biopsy[8,13]	X																			X	X	X
β-HCG serum pregnancy test [9]	X																					
Quantitative immunoglobulins[10]											X									X	X	X
Peripheral blood for chimerism	X[11]										X									X	X	X
Immune reconstitution panel[12]											X									X	X	X
CD34/CD3 cell dose infused							X															

Study Assessment	Baseline[1]	Conditioning Phase Days					Day 0	Days Post-Transplantation													
		-7	-6	-5	-4	-3		7	14	21	28	35	42	49	56	63	70	77	84	100	180	365 & 730
Seizure prophylaxis		X																				
PK Sample Submission[14]			X																			
PK-directed IV Bulsulfex dose					X	X																
Fludarabine/Busulfex ±ATG conditioning			X	X	X	X																
HPCT infusion							X															
ANC and platelet recovery[16]																						
CT or PET/CT chest/abdomen/pelvis[17]	X																			X	X	X
Acute/chronic GVHD assessment								X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity assessment							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Research specimens (Voluntary)[13]	X						X				X					X				X	X	X

Notes

[1]Baseline refers to the period prior to conditioning. Assessments should be made within 8 weeks prior to 1st day of conditioning.

[2]History is only required at baseline.

[3]Vital signs: blood pressure, pulse rate, respiratory rate, temperature.

[4]Daily during the post-infusion period while hospitalized (recommended) and once weekly after discharge until Day 28.

[5]Serum chemistries panel: electrolytes, BUN, ALT, AST, creatinine, bilirubin, alkaline phosphatase, LDH, albumin. Electrolytes to include sodium, potassium, chloride, carbon dioxide, and calcium.

[6]Infectious disease titers: Cytomegalovirus (CMV) antibody test, hepatitis panel (Hepatitis B including HBsAg, HBcAb; Hepatitis C Ab), HIV including HIV testing, syphilis, Epstein-Barr Virus (EBV) IgG and IgM, herpes simplex virus (HSV) IgG and IgM. If not available at baseline this can be performed at any time prior to the transplant.

[7]To be determined by MUGA or echocardiogram. If LVEF and/or DLCO are outside of baseline window of 8 weeks they do not need repeated unless the patient has received chemotherapy.

[8]Required at baseline. On Days +100, +180 and +365, bone marrow biopsy will be performed if clinically indicated. May be waived by the study Principal Investigator if outside of the baseline window of 8 weeks.

[9]Females of reproductive potential only.

[10]Quantitative immunoglobulins = IgG, IgM and IgA levels.

[11]Chimerism testing to be performed prior to study from BOTH donor and recipient at any time point. Chimerism testing should be sorted (lineage specific) to assess myeloid and lymphoid cell chimerism. If the chimerism is outside of the baseline window of 8 weeks it does not need repeated.

[12]Peripheral blood flow cytometry to assess number of CD3+, CD4+, CD8+, CD20+, CD15/56+ cells.

[13]For patients transplanted at MBRCC peripheral blood and bone marrow aspirate specimens in two blue top (10 mL; ACD containing) tubes each will be drawn at indicated time points and stored in **Dr. Laura Gibson's** laboratory. Please note that bone marrow aspirate specimens will ONLY be obtained during a bone marrow biopsy performed for routine clinical care. For patients transplanted at the Georgia Health Sciences University, specimens will be stored in Dr. Farrukh Awan's laboratory.

[14]Obtain blood for pharmacokinetics into a green top tube: at 3hrs, 3hrs & 15mins, 4hrs, 5hrs, 6hrs, and 8hrs after initiation of busulfan infusion. For details please see section 5.3 and Appendix C. Please note that the individual transplant center can send PK specimens to a reference laboratory of their choice.

[15]For patients with follicular lymphoma FLIPI score, for ones with Hodgkin lymphoma IPS (Hasenclever index), for all other NHLs IPI score (please see Appendix E).

[16]Record time to neutrophil engraftment defined as first of three consecutive days with ANC $\geq 500 \times 10^9$/L, and platelet engraftment defined as first day of platelet count $\geq 20{,}000 \times 10^9$/L, without transfusion for 7 consecutive days.

[17] CT/PET scans may be waived by the study Principal Investigator if outside the baseline window of 8 weeks.

8. Drug Formulation and Procurement

8.1. Busulfan (Busulfex®)

AVAILABILITY

Busulfan is commercially available as 60 mg/10mL ampoules. Please refer to the agent's package insert for additional information.

PREPARATION

Dilute busulfan injection in 0.9% sodium chloride injection or dextrose 5% in water. The dilution volume should be ten times the volume of busulfan injection, ensuring that the final concentration of busulfan is 0.5 mg/mL.

STORAGE & STABILITY

Store unopened ampoules under refrigeration at 2° C to 8°C. The diluted solution is stable for up to 8 hours at room temperature (25° C) but the infusion must also be completed within that 8-hour time frame. Dilution of busulfan injection in 0.9% sodium chloride is stable for up to 12 hours at refrigeration (2°C-8°C) but the infusion must also be completed within that 12-hour time frame.

ADMINISTRATION

Intravenous busulfan should be administered via a central venous catheter as a 3 hour infusion every 24 hours for 4 consecutive days for a total of 4 doses.

TOXICITY

Severe myelosuppression with marrow ablation, alopecia, and mild nausea/vomiting are expected. Alopecia may not be completely reversible. Liver toxicity including severe or fatal veno-occlusive disease (<5%) may occur. Pulmonary toxicity is rare in this schedule. It is expected that patients may require mouth care including narcotic analgesia, and may require parenteral nutrition. Darkening of the skin may occur and may last several months. Seizures may occur (<5%). Busulfan causes immunosuppression and risk of opportunistic infection even after resolution of neutropenia. Busulfan is expected to cause nearly universal infertility in the doses used, although men may occasionally father children.

8.2. Fludarabine Monophosphate (Fludara®)

AVAILABILITY

Fludarabine monophosphate is commercially available as FLUDARA IV as a white, lyophilized powder. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol and sodium hydroxide to adjust pH. Store at 15-30°C (59-86°F). Please refer to the agent's package insert for additional information.

STORAGE & STABILITY

Reconstituted FLUDARA IV is chemically and physically stable for 24 hours at room temperature or 48 hours if refrigerated. In addition, reconstituted FLUDARA IV contains no antimicrobial preservative and thus care must be taken to assure the sterility of the prepared solutions and should be discarded eight hours after initial entry.

PREPARATION

FLUDARA IV should be prepared for parenteral use only by aseptically adding Sterile Water for Injection, USP. When reconstituted with 2 ml of Sterile Water for Injection, USP, each ml of the resulting solution will contain 25 mg of Fludarabine Phosphate, 25mg of mannitol, and sodium hydroxide to adjust the pH to 7-8.5. The product may be further diluted for intravenous administration to a concentration of 1 mg/ml in 5% Dextrose for Injection USP or in 0.9% Sodium Chloride, USP.

ADMINISTRATION

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Fludarabine will be delivered as a piggy-back via an ongoing IV line, over a period of 60 minutes.

TOXICITY

Myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache,

agitation, hearing loss, transient episodes of somnolence and fatigue, autoimmune hemolytic anemia, autoimmune thrombocytopenia, paresthesias, peripheral neuropathy, renal and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine monophosphate. Such toxicity has only been rarely demonstrated at the 25-30mg dosage of fludarabine monophosphate. Very rarely described complications include transfusion-associated graft versus host disease, thrombotic thrombocytopenic purpura, and liver failure. Tumor lysis syndrome, complicating fludarabine monophosphate therapy has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed in both pre-treated patients receiving fludarabine and in individuals receiving fludarabine combined with other agents (corticosteroids, mitoxantrone, and cyclophosphamide).

8.3. Tacrolimus (Prograf®)

AVAILABILITY

Tacrolimus is a commercially available macrolide compound with potent immunosuppressant properties. Tacrolimus is available for oral administration as capsules containing the equivalent of 0.5 mg, 1 mg, or 5 mg of anhydrous tacrolimus. For IV use, tacrolimus is available as a sterile solution in 1mL ampoules containing the equivalent of 5 mg of anhydrous tacrolimus per mL. The oral absorption of tacrolimus is erratic and incomplete; absolute bioavailability is approximately 25%; peak serum levels are seen 1 to 3 hours after an oral dose, and therapeutic trough blood concentrations have ranged from 5 to 20 ng/mL; tacrolimus is extensively metabolized in the liver, with only small amounts of unchanged drug (2% or less) being recovered in the urine; the elimination half-life of tacrolimus is approximately 10 hours. Tacrolimus suppresses both humoral (antibody) and cell-mediated immune responses. The compound is chemically distinct from cyclosporine but both agents elicit similar immunosuppressant effects. The immunosuppressive activity of tacrolimus is,

however, more marked than that of cyclosporine. Please refer to the agent's package insert for additional information.

PREPARATION FOR IV USE

Tacrolimus concentrate for injection must be diluted prior to IV infusion. For IV infusion, the concentrate is diluted with 0.9% sodium chloride or 5% dextrose injection to a concentration of 4-20 µg/mL. Preparation of the solution in polyethylene or glass containers allows storage for 24 hours beyond which unused solution should be discarded. A plasticized polyvinyl chloride (PVC) container should not be used because stability of the solution is decreased and polyoxyl 60 hydrogenated castor oil contained in the formulation may leach phthalates from PVC containers. Tacrolimus concentrate for injection and diluted solutions of the drug should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

ADMINISTRATION

Tacrolimus is to be initiated on D ay -2. Begin tapering between Day +90 to +120 in the absence of GVHD as tolerated with a goal of stopping by Day +150 to +180. See protocol text for tapering instructions, and for instructions for patients who are unable to take oral tracolimus.

STORAGE & STABILITY

Store tacrolimus capsules at controlled room temperature, 15-30°C (59-86°F) (Prod Info Prograf®, 1997). An extemporaneous suspension of tacrolimus with a final concentration of 0.5 milligrams/milli liter was stable for 56 days when it was stored at 24-26°C in glass or plastic amber prescription bottles.

TOXICITY

In patients receiving tacrolimus, 5% to 47% experienced anemia, 8% to 32% experienced leukocytosis, and 14% to 24% experienced thrombocytopenia. Rare cases of microangiopathic hemolytic anemia have been reported. Mild to moderate

hypertension was reported in 38% to 50% of patients receiving tacrolimus. Mild to moderate hypertension is a common adverse effect associated with tacrolimus therapy. Chest pain was reported in 19%. Antihypertensive therapy may be required. The most common adverse effects of tacrolimus have involved the central nervous system, and include headache (37% to 64%), tremors (48% to 56%), insomnia (32% to 64%), paresthesia (17% to 40%); and dizziness (19%). Tremor and headache may respond to a dosage reduction. Agitation, anxiety, confusion, seizures, depression, hallucinations, myoclonus, neuropathy, psychosis, incoordination, and abnormal dreams have been reported in 3% to 15% of tacrolimus-treated patients. Hyperkalemia (13% to 45%), hypokalemia (13% to 29%), hypophosphatemia (49%), and hypomagnesemia (16% to 48%) have been associated with tacrolimus therapy. In addition, hirsutism occurs only rarely with tacrolimus. Hyperuricemia has been reported in greater than 3% of tacrolimus-treated patients. Gastrointestinal adverse effects of tacrolimus have included nausea (32% to 46%), vomiting (14% to 29%), anorexia (7% to 34%), constipation (23% to 35%) and diarrhea (37% to 72%). Gingival hyperplasia observed in patients treated with cyclosporine has not been reported with tacrolimus therapy. Nephrotoxicity was reported in 36% to 40% and 52% of liver and kidney transplant patients receiving tacrolimus. Overt nephrotoxicity is usually seen early after transplantation and is characterized by an increased serum creatinine and a decrease in urine output. Hematuria has been reported in greater than 3% of tacrolimus-treated patients (Prod Info Prograf®, 1997). Abnormal liver function tests have been reported in 6% to 36% of patients receiving tacrolimus; ascites was reported in 7% to 27% of these patients. Other miscellaneous effects that have occurred in clinical trials include pain (24% to 63%), fever (19% to 48%), asthenia (11% to 52%), back pain (17% to 30%), and peripheral edema (12% to 36%). The incidence of hyperglycemia is 17% and may require therapy with insulin. Other less frequently occurring effects (greater than 3%) include abscess, chills, peritonitis, and photosensitivity reactions. Anaphylaxis has been reported in a few patients receiving intravenous tacrolimus. Tacrolimus contains castor oil which has been associated with anaphylaxis in other drugs containing castor oil derivatives. The incidence of bloodstream infection is 22%. Most infections are due to bacteria (81%), followed by

candidemia (14%), and cryptococcemia (5%). The source of bloodstream infection was primarily intravascular catheter, accounting for 39% of cases.

8.4. Methotrexate (Amethopterin®; MTX)

AVAILABILITY

Commercially available in 2mL, 4mL, 8mL, 10mL vials, or 1 g vials or preserved with benzyl alcohol. Please refer to the agent's package insert for additional information.

PREPARATION

The 1 gm vial may be diluted in 10 mL of saline or D5W.

COMPATIBILITY

Additive incompatibility: bleomycin, prednisone.

STORAGE & STABILITY

Stability and compatibility of methotrexate sodium solutions depend on several factors including the formulation of methotrexate sodium used, presence of preservatives, concentration of drug, specific diluents used, resulting pH, and temperature; the manufacturer's labeling and specialized references should be consulted for specific information. Methotrexate sodium solutions should be inspected visually for particulate matter and discoloration whenever solution or container permits.

ADMINISTRATION

Administer via slow IV push. Hydrate intravenously and induce diuresis.

TOXICITY

Hematologic including leukopenia (1.5%), thrombocytopenia (5%; nadir 5-12 days; recovery 15-27 days), anemia (nadir 6-13 days), pancytopenia (1.5%); gingivitis, glossitis, pharyngitis, stomatitis, enteritis; nausea/vomiting, anorexia, diarrhea;

hematemesis, melena; acute and chronic hepatotoxicity; transaminases increase 1-3 days after administration, hepatic fibrosis and cirrhosis with long-term therapy; pulmonary toxicity including pneumonitis, pulmonary fibrosis that is not dose dependent and may not be fully reversible; pruritis, urticaria, photosensitivity; CNS: drowsiness, blurred vision, tinnitus, malaise, seizures; nephropathy: cystitis, dysuria, azotemia, hematuria, renal failure; diabetes; when administered it may cause headache, back pain, rigidity.

DRUG INTERACTIONS

Aminoglycosides may cause decreased absorption of methotrexate, and increased renal toxicity. Folic acid may decrease response to methotrexate. The use of NSAIDs may increase methotrexate levels. Probenecid, salicylates, sulfonamides may increase therapeutic and toxic effect of methotrexate. Procarbazine can cause increased nephrotoxicity. Theophylline may increase plasma levels. Alcohol may result in increased hepatotoxicity. Thiazides may cause granulocytopenia. Food will delay absorption, and decreases methotrexate peak.

8.5. Antithymocyte Globulin (Rabbit) (Thymoglobulin®; rabbit ATG)

AVAILABILITY

Antithymocyte globulin is commercially available as a lyophilized powder for reconstitution containing 25 mg per vial. Each vial of powder is supplied with 5mL diluent.

STORAGE & STABILITY

Intact vials should be stored under refrigeration and protected from light. Do not freeze. Reconstituted solutions should be used within 4 hours. Further diluted solutions for infusion should be used immediately after dilution.

PREPARATION

Remove the ATG rabbit plus diluent from the refrigerator and allow them to reach room temperature prior to reconstitution. Reconstitute each 25 mg vial with 5mL of the diluent provided (sterile water for injection, USP). Rotate the vial gently to dissolve the powder. The resultant solution contains 5mg/mL of ATG rabbit. Withdraw the calculate dose and inject into D5W or NS for IV infusion. The final concentration should be 0.5 mg/mL. The solution should be administered through a 0.22 micron filter.

ADMINISTRATION

ATG rabbit will be administered IV at a dose of 2.0 mg/kg/day for 3 days (on Days -4, -3, and -2). The first dose should be infused over at least six hours, and subsequent doses over at least 4 hours. Infuse through a 0.22 micron in-line filter. Acetaminophen 650 mg PO, diphenhydramine 25-50 mg PO/IV, and methylprednisolone 1 mg/kg IV should be administered at the initiation and midway through each antithymocyte globulin (rabbit) infusion to minimize infusion reactions.

TOXICITY

Infusion reactions such as fever and chills are common, occurring in more than 10% of patient s. Steroids, antihistamines and acetaminophen will be given, as described above, to minimize infusion reactions. Hypersensitivity reactions, including anaphylaxis, occur less frequently and may also be minimized with steroids and antihistamines. Immunosuppression from antithymocyte globulin (rabbit) is associated with an increase in opportunistic infections, including fungal, viral, and pneumocystis infections.

8.6. Filgrastim (G-CSF: Granulocyte Colony-Stimulating Factor, Neupogen®)

AVAILABILITY

G -CSF is commercially available in 1.0 and 1.6mL vials containing 300 µg and 480µg G-CSF, respectively. Please refer to the agent's package insert for additional information.

STORAGE & STABILITY

G-CSF is available as a sterile buffered protein solution and must be stored at 2-8°C. DO NOT ALLOW THE DRUG TO FREEZE.

ADMINISTRATION

Each vial should be entered only once, and the remainder of the vial discarded and not re-entered a second time. The daily dose should be injected subcutaneously in one or two sites. Standard dosing is 5µg/ kg daily as a subcutaneous injection. **TOXICITY**

Chills, nausea, anorexia, myalgias, bone pain, local injection site pain or inflammation, abnormal liver function tests, thinning of hair, and enlargement of the spleen. Rarely fluid retention and pericardial effusion. All of these are generally reversible when the drug is discontinued.

9. Statistical Considerations:

This is a multicenter phase II study of once daily intravenous Busulfex as part of reduced toxicity conditioning for patients with chemorefractory Hodgkin and non-Hodgkin lymphomas. The primary objective of this study is assess 1 year PFS of patients with refractory lymphomas undergoing allogeneic HSCT following conditioning with once daily Busulfex, fludarabine with or without thymoglobulin. Literature review suggests that for chemotherapy refractory relapsed Hodgkin and NHL patients 1 year PFS is below 15% (P0=0.15). We hypothesize that RIC transplantation should improve the 1 year PFS to this high risk group to 35% (P1=0.35). A sample size of 32 achieves 90% power to detect a

difference (P1-P0) of 0.2500 using a two-sided binomial test. The target significance level is 0.0500. The actual significance level achieved by this test is 0.0477. These results assume that the population proportion under the null hypothesis is 0.1000.

OS and progression free survival PFS will be estimated using the Kaplan-Meier method. OS is defined as the time from transplant to death, and surviving patients will be censored at last follow-up. PFS is defined as the time from transplantation to disease progression/relapse and/or death. OS and PFS data between subgroups will be analyzed by the log-rank test. NRM is defined as death from any cause other than disease progression or relapse. Cumulative incidences of NRM and relapse will be calculated with relapse or death as a competing event, respectively [37]. Comparisons between estimates of cumulative incidence will be made by using the Gray's test. The cumulative incidence of acute or chronic GVHD will be calculated with relapse or death without GVHD as competing events [37,38]. We anticipate that a maximum of 32 patients will be accrued to the study. Descriptive statistics (i.e. means, standard deviations, 95% confidence intervals for continuous variables, and frequencies for discrete data) will be computed for all correlative laboratory parameters. Given the small sample size in this phase II trial, a total of 32 patients limits our ability to perform inferential tests.

Accrual Estimate: 32 patients.

Accrual Period: Until 12/31/2013.

Safety/Stopping Rules:

Rates of non-relapse mortality and grade III-IV acute GVHD will be monitored on 6 monthly basis. The study will be halted for review by the data safety and toxicity committee (DSTC) if any of the following criteria are met.

1. Non-relapse mortality of >40% at day 100 after transplantation.
2. Cumulative incidence of grade III-IV acute GVHD is >40% at day 100 after transplantation.

Follow-Up Period : One year to evaluate progression free survival.

10. Records to be Kept:

Particular attention should be given to the specification of which data elements should be included in the Mary Babb Randolph Cancer Center Clinical Trials Research Unit OnCore database. The investigator will maintain adequate records in the Investigator Site File to enable the conduct of the study to be fully documented. All documents related to the conduct of the study will be kept on file in a readily accessible order. This file must be safely archived after the termination of the study.

Patient data collected on Case Report Forms (CRFs) should be sent to the Network Coordinator at the CTRU by fax at (304) 293-8698 according to CRF submission guidelines provided by WVU. Data will be entered into the OnCore database.

Data Acquisition and Submission:

- Informed consent, including HIPAA authorization, must be obtained on all subjects prior to their participation.
- Always keep the original signed and dated consent form, sending a copy to the MBRCC CTRU with the source documents and eligibility checklist. A physician must sign the checklist confirming eligibility and intent to register the patient.
- In the event that the consent is signed, but later is either withdrawn or inactivated – even if the patient did not begin treatment – send a copy of the signed and dated consent to the MBRCC CTRU.
- Case report forms will be provided by MBRCC CTRU specific for this study.
- Submit original forms, done in black ink written legibly.
- Amended data should be identified as such, and the change(s) written (preferably) in red ink, initialed and dated.
- The source documentation for each subject should be clearly written/typed, dated and signed; all printouts, test reports, and procedures should be signed and dated.

- Baseline and on-study forms are due within 6 weeks of registration. Please use the date the consent was signed as the On-Study date.
- Source documents include, but are not limited to: medical records; chemotherapy treatment records/notes; radiation treatment records/notes; laboratory/pathology reports; radiology reports; EKGs, MUGA, etc. reports; correspondence related to patient care; home care documents.

10.1. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Definitions

The following are definitions of adverse events as defined by 21CFR312.32.

Types of Adverse Events

Adverse Event means any untoward medical occurrence associated with the use of a drug in humans, whether or not consider drug related.

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. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse event or serious suspected adverse reaction: An adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator or sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect.

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent of the outcomes listed above.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, it is not consistent with the risk information currently described.

Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse Event Grading

Grade	Description
0	**No AE** (or within normal limits).
1	**Mild**; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	**Moderate**; minimal, local or noninvasive intervention (e.g., packing cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL).
3	**Severe** or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling;

limiting self-care ADL.

4 **Life-threatening** consequences; urgent intervention indicated.

5 **Death** related to AE

Adverse Event Attribution

Relationship	Attribution	Description
Unrelated to investigational agent/intervention	Unrelated	The AE *is clearly **NOT** related* to the intervention
	Unlikely	The AE *is **doubtfully related*** to the intervention
Related to investigationl agent/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

Reporting of Adverse Events

Because all of the study transplant recipients will be receiving potentially toxic preparative therapy, significant regimen related toxicity is expected. These risks are listed in the consent form. A study specific toxicity CRF will be designed to capture information regarding these expected events. Transplant is also related to a degree of mortality and this will also be captured by a study designed CRF. Unexpected adverse events will be reported throughout the study.

<u>Unexpected Adverse Events</u>

Grades 3-5 (severe, life threatening, disabling, or fatal) require expedited reporting and will be submitted to the DSTC within 24 hours of discovery for review. If the Grade 3-5 event is determined to be an unanticipated problem, the event will be forwarded to the WVU IRB for review as required by their policy. Unexpected adverse events, regardless of severity, will be reported to the DSTC and reviewed on a quarterly basis.

Expected Adverse Events

All fatal (grade 5) expected adverse events require expedited reporting and will be reported to the DSTC within 24 hours for review. Expected adverse events that are being captured on the study toxicity form will be reported at the time of the form's scheduled due date.

All DSTC reports and recommendations will be submitted to the IRB for their review.

Adverse Events Occurring after the End of the Study

Follow-up of AEs

Any unexpected AEs ongoing at the time of study discontinuation will be followed until resolution or stable for at least 2 months.

FDA Reporting Procedures

Commercial Agents: Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. In some cases an agent obtained commercially may be used for indications not included in the package label. The following procedures should be followed to determine if an adverse is reportable to the FDA:

Refer to the pharmaceutical section of the protocol to determine if an agent is investigational or commercial.

- **WHAT TO REPORT:** An unexpected, life-threatening (Grade 4) or unexpected, fatal (Grade 5) adverse event with an attribution of possible, probable or definite.

- **WHEN TO REPORT:** These events should be reported within (7) working days.

- **WHERE TO REPORT:** These adverse events with commercial agents must be reported to the FDA using the MedWatch form. A copy of the MedWatch form can be obtained from theFDA's MedWatch web site at www.fda.gov/medwatch. You can mail the reports to the address below or fax it 1-800-332-0178.

MedWatch
5600 Fishers Lane
Rockville, MD 20852-9787

11. Patient Consent and Peer Review Statement

11.1. Subject Information and Informed Consent

Written informed consent must be obtained from the subject prior to study participation.

The informed consent document must be signed and dated by the subject and properly witnessed (if applicable) before initiation of any study procedures including any change in medication or initiation of study drug dosing.

Subjects must be consented in accordance with all local regulatory and legal requirements. This process must include a verbal explanation of the nature, scope, and possible consequences of the study provided in plain language. The information should be presented by the investigator unless a designee is permitted by local regulations. The potential study subject should be encouraged to ask questions about the study.

The informed consent document must be prepared in accordance with GCP guidelines and with local regulatory and legal requirements. A copy of the signed consent form will be given to the subject and the original document must be safely archived by the investigator so that the forms can be retrieved at any time for monitoring, auditing, and inspection purposes.

The informed consent will be updated as appropriate (e.g., due to protocol amendment or if significant new safety information that may be relevant to consent of the subjects becomes available). If the informed consent is revised, it is the investigator's responsibility to ensure that an amended consent form is reviewed and signed by all subjects subsequently entered into the study and those currently in the study, if applicable per local IRB and/or federal requirements.

11.2 **Peer Review Statement**

This study was reviewed and approved by the Mary Babb Randolph Cancer Center Protocol Review and Monitoring Committee (PRMC) on 6/15/2010

12. References:

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13. Appendix A - Assessment of Acute GVHD

Clinical Acute GVHD Assessment

Date _____ Patient ID _____ Karnofsky/Lansky _____

	Code							Differential Diagnosis						
	0	1	2	3	4	5		GVHD	Drug Rxn	Cond Reg	TPN	Infect	VOD	Other
Skin	☐	☐	☐	☐	☐		% body rash:____	☐	☐	☐		☐		_____
Lower GI	☐	☐	☐	☐	☐	☐	Vol: _____	☐	☐	☐	☐	☐		_____
Upper GI	☐	☐						☐	☐	☐	☐	☐		_____
Liver	☐	☐	☐	☐	☐		Max bili: _____	☐	☐	☐	☐	☐	☐	_____

Treatment: ☐ CSA ☐ Tacrolimus ☐ Pred ☐ Methylpred ☐ Ontak
☐ Pentostatin ☐ MMF ☐ Etanercept ☐ Other _____

Code Definitions:

Skin:
0 No rash
1 Maculopapular rash, <25% of body surface
2 Maculopapular rash, 25-50% of body surface
3 Generalized erythroderma
4 Generalized erythroderma with bullous formation and desquamation

Lower GI (Diarrhea):
0 None
1 ≤500 mL/day or <280 mL/m^2
2 501-1000 mL/day or 280- 555 mL/m^2
3 1001-1500 mL/day or 556- 833 mL/m^2
4 >1500 mL/day or >833 mL/m^2
5 Severe abdominal pain with or without ileus, or stool with frank blood or melena

Upper GI:
0 No protracted nausea and vomiting
1 Persistent nausea, vomiting or anorexia

Liver (Bilirubin):
0 <2.0 mg/dl
1 2.1-3.0 mg/dl
2 3.1-6.0 mg/dl
3 6.1-15.0 mg/dl
4 >15.1 mg/dl

Signature _____

TABLE 1.3.1 – GVHD STAGING

Stage	Skin	GI	Liver
1	< 25% rash	Diarrhea > 500ml/d or persistent nausea	Bilirubin 2-3mg/dl
2	25-50%	> 1000 ml/d	Bilirubin 3-6 mg/dl
3	> 50%	> 1500 ml/d	Bilirubin 6-15 mg/dl
4	Generalized erythroderma with bullae	Large volume diarrhea and severe abdominal pain ± ileus	Bilirubin > 15 mg/dl

TABLE 1.3.2 – CONSENSUS GVHD GRADING (PRZEPIORKA, ET. AL., 1995)

Grade	Skin	GI	Liver
I	Stage 1-2	0	0
II	Stage 3 or	Stage 1 or	Stage 1
III	---	Stage 2-4	Stage 2-3
IV	Stage 4	---	Stage 4

14. Appendix B - Grading of Chronic GVHD (NIH Criteria)

Check all that apply	Score 0 - None	Score 1 - Mild	Score 2 - Moderate	Score 3 - Severe
Skin: *Clinical features:* ❑Maculopapular rash ❑Lichen planus-like features ❑Papulosquamous lesions or ichthyosis ❑Hyperpigmentation ❑Hypopigmentation ❑Keratosis pilaris ❑Erythema ❑Erythroderma ❑Sclerotic features ❑Poikiloderma ❑Pruritus ❑Hair Involvement ❑Nail Involvement % BSA involved __%	❑No symptoms	❑< 18% BSA with disease signs but NO sclerotic features	❑19-50% BSA, ❑Involvement with superficial sclerotic features "not hidebound" (able to pinch)	❑> 50% BSA ❑Deep sclerotic features "hidebound" (unable to pinch) ❑Impaired mobility, ulceration or severe pruritis
Mouth:	❑No symptoms	❑Mild symptoms with disease signs but not limiting oral intake significantly	❑Moderate symptoms with disease signs WITH partial limitation of oral intake	❑Severe symptoms with disease signs WITH major limitation of oral intake
Eyes: Mean tear test (mm): ❑> 10 ❑6-10 ❑≤ 5 ❑Not done	❑	❑Mild dry eyes symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) ❑Asymptomatic signs of keratoconjunctivitis sicca	❑Moderate dry eyes symptoms partially affecting ADL (requiring eyedrops > 3 x per day or punctual plugs) WITHOUT vision impairment	❑Severe dry eyes symptoms significantly affecting ADL (special eyewear to relieve pain) ❑Unable to work because of ocular symptoms ❑Loss of vision caused by keratoconjuctivitis sicca
Pulmonary **FEV1** ❑Not done **Pulmonary Fibrosis** **Bronchiolitis Obliterans** **Supplemental O2 required?** ❑Yes ❑No	❑No symptoms ❑FEV1> 80% ❑None ❑Not assessed ❑None ❑Yes, clinical ❑Yes, histologic ❑Not assessed	❑Mild symptoms (shortness of breath after climbing one flight of steps) ❑FEV1 60-78% ❑ Minimal radiographic findings	❑Moderate symptoms (shortness of breath after walking on flat ground) ❑FEV1 40-51% ❑Patchy or bi-basilar radiographic findings	❑Severe symptoms (shortness of breath at rest; requiring O_2) ❑FEV1 ≤ 39% ❑Extensive radiographic findings
GI Tract:	❑No symptoms	❑Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (< 5%)	❑Symptoms associated with mild to moderate weight loss (5 – 15%)	❑Symptoms associated with significant weight loss > 15% ❑ Requires nutritional supplement for most caloric needs ❑Esophageal dilation
Liver:	❑Normal LFT	❑Elevated Bilirubin, AP, AST or ALT < 2 x ULN	❑Bilirubin 3 – 10 mg/dl; liver enzymes 2-5 x ULN	❑Bilirubin > 10 mg/dL; liver enzymes > 5 x ULN
Genital Tract:	❑No symptoms	❑Symptomatic with mild signs on exam AND no effect on coitus and minimal discomfort with gynecological exam	❑Symptomatic with moderate signs on exam AND with mild dyspareunia or discomfort with gynecological exam	❑Symptomatic WITH advanced signs (stricture, labial agglutination or severe ulceration) AND severe pain with coitus or inability to insert vaginal speculum

Check all that apply	Score 0 - None	Score 1 - Mild	Score 2 - Moderate	Score 3 - Severe
Joints and Fascia:	❑ No symptoms	❑ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) AND not affecting ADL	❑ Tightness of arms or legs ❑ Joint contractures, erythema thought due to fascitis, moderate decreased ROM AND mild to moderate limitation of ADL	❑ Contractures WITH significant decrease of ROM AND significant limitation of ADLs (unable to tie shoes, button shirts, dress self etc.)

Other indicators, clinical manifestations or complications related to Chronic GvHD (check all that apply).
Assign a score to it's severity based on functional impact, where applicable (0= none, 1=mild, 2 = moderate, 3= severe)

❑ Ascites (serositis) ___	❑ Esophageal stricture or web___	❑ Nephrotic syndrome ___	❑ Pleural effusions ___
❑ Cardiac conduction defects ___	❑ Eosinophilia > 500 µl ___	❑ Pericardial effusion ___	❑ Polymyositis ___
❑ Cardiomyopathy ___	❑ Myasthenia Gravis ___	❑ Peripheral neuropathy ___	❑ Progressive onset___
❑ Coronary artery involvement ___		❑ Platelets < 100,000/µl ___	
❑ Other(s): Specify & score___			

Based on observations checked in the above table, select the severity of chronic GvHD for this assessment (Check only one)

☐ **None**

☐ **Mild chronic GVHD** involves only 1 or 2 organs or sites (except the lung: see below ‡), with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites)

☐ **Moderate chronic GVHD** involves: (1) at least 1 organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site) or (2) 3 or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). ‡*A lung score of 1 will also be considered moderate chronic GVHD.*

☐ **Severe chronic GVHD** indicates major disability caused by chronic GVHD (score of 3 in any organ or site). ‡*A lung score of 2 or greater will also be considered severe chronic GVHD.*

15. Appendix C - Busulfex Therapeutic Drug Monitoring

SEATTLE CANCER CARE ALLIANCE

Instructions for Busulfan Pharmacokinetic Sample Processing

New Accounts (For sites using SCCA Laboratories for the first time)

When setting up a new account with us for busulfan analysis, please have the following information ready and call (206) 288-7389.

1. Institution information: Name of the institution, address, and general contact numbers.
2. Billing contact information: Name, title, address with mail stop, phone number and fax number.
3. Caller information: Name, phone number, and fax number.

Please allow 3 to 4 days for a new account to be properly set up. Patient samples can be sent in before the set up process is completed. Please ask for sample acceptance.
Additional Information at http://www.seattlecca.org/busulfan-lab-samples.cfm#setup

Nursing Instructions

1. Complete the enclosed **IV BUSULFEX PHARMACOKINETICS REQUISITION** (next page). Please make sure that all the information is completely filled out. The Actual Time is the time the sample is physically collected from the patient.

Sample Collection and Processing

- Draw minimum 4 mL of blood into 4-mL Sodium Heparin green top blood tubes.
- Busulfan degrades quickly at room temperature. Each sample should be kept in wet ice slurry or refrigerated at all time. Centrifuge them as soon as possible at 4 °C. Separate the plasma from each sample and transfer it to individual 4mL plastic tubes. Immediately freeze these plasma samples at -20 °C.
- All samples have to be labeled with patient name, Medical Record #, date and actual clock time of the blood draw. Two unique identifiers and clock times are REQUIRED or samples can be rejected.
- Samples must be shipped on a minimum of 3 kg of dry ice using an overnight carrier. Samples that arrived thawed will not be analyzed.
- A REQUISITION SHEET (for IV Q24hr) is required to be sent with the samples. Patient identifiers must be matched with those on the collected samples mentioned above. Also, our requisition serves as physician's order and must be signed by the attending physician or designee/caregiver according to the College American Pathologists (CAP) requirement.

Advance notice is required for all sample shipments:

- Tuesday to Saturday arrival: At least 48 hours in advance.
- Sunday, Monday, or holiday arrival: At least 72 hours in advance (Sample acceptance is only on pre-arranged basis).

SHIP TO: Pharmacokinetics Laboratory
Seattle Cancer Care Alliance
825 Eastlake Ave. E. Room G7-405
Seattle, WA 98109-1023

IV BUSULFEX PHARMACOKINETICS REQUISITION

Please provide the following information below.

Patient Name _____ Institution _____

Medical Record # _____ **Required by C.A.P. Must also be on sample labels.**

Actual weight _____ Date of Birth _____

AIBW (wt used for determining dose) _____ Disease _____

Height _____ ICD9 Code _____

Dose number _____ of _____ (Total BU Therapy Doses)

Dose (mg) _____

Date _____

Target range _____

Units (circle one) AUC (uMol*min) Css (ng/mL)

Signature of M.D. or Designee _____

Attending Physician (printed name) _____

Beeper Number _____

Fax Number(s) _____

_____

Q 6 IV Busulfex

Infusion start time: _____

Infusion stop time: _____

	Dose #1 ACTUAL draw time	Follow Up Doses draw time
Pre Infusion	n/a	
*END OF INFUSION		
135 minutes (or EOI + 15 min)		
150 minutes (or EOI + 30 min)		
3 hours from start		n/a
4 hours from start		
5 hours from start		n/a
6 hours from start		

Q 6 Hr Not Applicable

NOTE: All draw times are post START OF INFUSION based on a 2 hour (120 minute) infusion.

Q 24 IV Busulfex

Infusion start time: _____

Infusion stop time: _____

	Dose #1 ACTUAL (clock) draw time	Follow Up Doses draw time
Pre Infusion	n/a	
END OF INFUSION		
195 minutes (or EOI + 15 min)		
4 hr from start		
5 hr from start		
6 hr from start		
8 hr from start		

NOTE: All draw times are post START OF INFUSION.

*Modification to Q6 draw schedule: Always draw the first sample at the end of infusion regardless of the duration of the infusion. Draw the next 2 samples 15 minutes apart AFTER the end of infusion sample. Continue the normal draw schedule, i.e. 3, 4, 5, and 6 hours from the START of infusion. Always note the actual draw times.

*Be sure all of the drug has been delivered and the lines have been thoroughly flushed by Saline before drawing the end of infusion (EOI) sample.

Draw 1-3 mL blood in green top (sodium heparin) tubes. Keep on ice at all times. Centrifuge at 4°C. Remove and freeze plasma in a plastic tube labeled with: patient name, Medical Record #, date and time of draw. Please tape labels on. Send plasma with 5 kg of dry ice priority overnight to the address below.

SHIP TO: Pharmacokinetics Laboratory
Seattle Cancer Care Alliance
825 Eastlake Ave. E. Room G7-405
Seattle, WA 98109-1023

PLEASE CALL AT LEAST 48 HOURS PRIOR TO SAMPLE SHIPPING.
Tracking Numbers REQUIRED.

Questions? Call Matthew Pawlikowski, Lisa Brundardt or Loule Yu at (206) 288-7389, (206) 994-5942 (beeper), or via email: PKLab@seattlecca.org

16. Appendix D - Response Criteria for Lymphoma

Response	Definition	Nodal Masses	Spleen, Liver	Bone Marrow
Complete remission (CR)	Disappearance of all evidence of disease	a) [^{18}F]fluorodeoxyglucose (FDG)-avid or positron emission tomography (PET) positive prior to therapy; mass of any size permitted if PET negative b) Variably FDG-avid or PET negative; regression to normal size on computertomography (CT)	Not palpable, nodules disappeared	Infiltrate cleared on repeat biopsy; if indeterminate by morphology, immunohistochemistry should be negative
Partial remission (PR)	Regression of measurable disease and no new sites	≥50% decrease in sum of the product of the diameters (SPD) of up to 6 largest dominant masses; no increase in size of other nodes a) FDG-avid or PET positive prior to therapy; one or more PET positive at previously involved site b) Variably FDG-avid or PET negative; regression on CT	≥50% decrease in SPD of nodules (for single nodule in greatest transverse diameter); no increase in size of liver or spleen	Irrelevant if positive prior to therapy; cell type should be specified
Stable disease (SD)	Failure to attain CR/PR or PD	a) FDG-avid or PET positive prior to therapy; PET positive at prior sites of disease and no new sites on CT or PET b) Variably FDG-avid or PET negative; no change in size of previous lesions on CT	–	–
Relapsed disease or progressive disease (PD)	Any new lesion or increase by ≥50% of previously involved sites from nadir	Appearance of a new lesion(s) >1.5 cm in any axis, ≥50% increase in SPD of more than one node, or ≥50% increase in longest diameter of a previously identified node >1 cm in short axis. Lesions PET positive if FDG-avid lymphoma or PET positive prior to therapy.	≥50% increase from nadir in the SPD of any previous lesions	New or recurrent involvement.

Source: Cheson B, Pfistner B, Juweid M, Gascoyne R, Specht L, Horning SJ, et al. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007;25(5):579-86.

17. Appendix E – Scoring Systems

International Prognostic Score for Hodgkin's Lymphoma International Prognostic Score (IPS) 1 point per factor (advanced disease)
▪ Albumin < 4 g/dL ▪ Hemoglobin < 10.5 g/dL ▪ Male ▪ Age ≥ 45 years ▪ Stage IV disease ▪ Leukocytosis (white blood cell (WBC) count ≥ 15,000/mm^3 ▪ Lymphocytopenia (lymphocyte count less than 8% of WBC count, and/or lymphocyte count less than 600/ mm^3)

Source: Hasenclever D, Diehl V. A prognostic score for advanced Hodgkin's disease: International Prognostic Factors Project on Advanced Hodgkin's Disease. N Engl J Med 1998:339:1506-1514.

International Prognostic Index for Non-Hodgkin's Lymphoma		
All Subjects	**International Index**	**All Subjects**
Age > 60 years	Low	0 or 1
Serum LDH > 1 x normal	Low intermediate	2
Performance status 2-4	High intermediate	3
Stage III or IV	High	4 or 5
Extra nodal > 1		
Age-Adjusted International Prognostic Index		
Subjects ≤60 years	**International Index**	**Subjects ≤60 years**
Stage III or IV	Low	0
Serum LDH > 1 x normal	Low/intermediate	1
Performance status 2-4	High/intermediate	2
	High	3

Source: A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factor Project. N Engl J Med 1993;329:987-994.

FLIPI Criteria	
Age	≥ 60 years
Ann Arbor stage	III-IV
Hemoglobin level	< 2 g/dL
Serum LDH level	> ULN (upper limit of normal)
Number of nodal sites	≥ 5
Risk Group According to FLIPI Chart	
	Number of Factors
Low	0-1
Intermediate	2
High	≥3

Source: Solal-Celigny P, Pascal R, Colombat P et al. Follicular lymphoma international prognostic index. Blood 2004;104:1258-1265.