Document Coversheet

Study Title:

A Phase 1b/2 Trial of Fludarabine/Melphalan/Total Body Irradiation with Post Transplant Cyclophosphamide as Graft versus Host Disease Prophy

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PROTOCOL TITLE:

A Phase 1b/2 Trial of Fludarabine/Melphalan/Total Body Irradiation with Post Transplant Cyclophosphamide as Graft versus Host Disease Prophylaxis in Matched-Related and Matched-Unrelated Allogeneic Hematopoietic Cell Transplantation

ROSWELL PARK STUDY NUMBER:

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Confidentiality Statement

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ABBREVIATIONS

alloHCT	Allogeneic hematopoietic cell transplantation
AML	Acute Myeloid Leukemia
ANC	Absolute Neutrophil Count
BM	Bone Marrow Hematopoietic Cell Transplant
BMT	Blood or Marrow Transplantation
BU	Busulfan
CLL	Chronic Lymphocytic Leukemia
CMV	Cytomegalovirus
CY	Cyclophosphamide
DLI	Donor Lymphocyte Infusion
DLT	Dose Limiting Toxicity
DVT	Deep Vein Thrombosis
EBV	Epstein Barr Virus
FK506	Tacrolimus
FLU	Fludarabine
G-CSF	Filgrastim-sndz (or other approved biosimilar is acceptable)
GM-CSF	Sargramostim
GVNH	Graft-versus- Host Disease
GVL	Graft-versus- Leukemia
GVT	Graft-versus- Tumor
HCT	Hematopoietic Cell Transplantation
HLA	Human Leukocyte Antigen
MDS	Myelodysplastic Syndrome

MEL	Melphalan
MHC	Major Histocompatability
MM	Multiple Myeloma
MMF	Mycophenolate Mofetil
MPD	Myeloproliferative Disorder
MTD	Maximum Tolerated Dose
MUD	Matched Unrelated Donor
NHL	Non Hodgkin Lymphoma
NRM	Non Relapse Mortality
OS	Overall Survival
PB	Peripheral Blood
PBHCT	Peripheral Blood Hematopoietic Cell Transplant
PMN	Polymorphonuclear Cell
PNH	Paroxysmal Nocturnal Hemoglobinuria
RIC	Reduced Intensity Conditioning
RRT	Regimen Related Toxicity
SOP	Standard Operating Procedure
SOS	Sinusoidal Obstructive Syndrome
TRM	Transplant Related Mortality
VOD	Veno-occlusive Disease

1 OBJECTIVES

1.1 Primary Objective

- To determine the cumulative incidence of extensive chronic graft versus host disease (GVHD) at 1 year after transplantation utilizing the novel conditioning/GVHD prophylactic regimen for patients undergoing allogeneic hematopoietic cell transplantation, in patients who do not progress before Day 100.

1.2 Secondary Objectives

- To evaluate clinical response, engraftment rate, progression-free survival (PFS) at one year and, overall survival (OS).
- To determine the cumulative incidence of relapse.
- To evaluate the Day 100 transplant-related mortality rate.
- To determine the cumulative incidence of grade III-IV acute GVHD.

2 BACKGROUND

Allogeneic hematopoietic cell transplantation (alloHCT) is a potentially curative treatment for many of the hematologic malignancies[1]. Although there have been significant advances towards the success of this procedure including improvements in HLA typing, donor selection, and infectious antibiotics, graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality. Despite the use of current GVHD prophylactic regimens, acute GVHD still occurs in 40-50% of allogeneic transplants and chronic GVHD occurs in approximately 10-80% of transplants[2]. To improve the safety and efficacy of alloHCT, there needs to be a balance between allowing enough alloreactivity to induce graft-versus-tumor (GVT) effect while minimizing the occurrence of graft-versus-host disease. Additionally, the effects of the agents used in GVHD prophylaxis on immune reconstitution must also be considered. As such, there remains a need to develop new GVHD regimens. Recently, several studies have evaluated the efficacy of post-transplant cyclophosphamide as GVHD prophylaxis.

Preclinical studies using post-transplant cyclophosphamide in rodents showed that it could alleviate GVHD after major histocompatibility (MHC) mismatched lymphocyte infusion. Also, similar to human leukocyte antigen (HLA) -matched transplants, it could induce tolerance to grafts despite mismatches at multiple minor histocompatibility antigens[3].

Additionally, there are certain features of cyclophosphamide that make it an appealing agent to use for GVHD prophylaxis[2]. Cyclophosphamide is an alkylating agent that exerts it effects predominantly on rapidly proliferating cells whereas hematopoietic cells are resistant to its DNA damaging effects given their ability to convert cyclophosphamide into its inactive metabolite. Thus, engraftment following alloHCT is not effected by cyclophosphamide. Cyclophosphamide is also capable of triggering apoptosis of T cells. This is in contrast to the standard agents used for GVHD prophylaxis including calcineurin inhibitors, sirolimus, and mycophenolate mofetil (MMF), all of which lead to inhibition of the T cells, not cell death. Another mechanism of action of cyclophosphamide is that it leads to clonal deletion of intrathymic host-reactive T cells resulting in maintenance of long-term tolerance. These effects were demonstrated in a mouse model in which cyclophosphamide was shown to selectively cause clonal destruction of both alloreactive donor cells as well as clonal deletion of host-reactive cells. The

implications of these properties of cyclophosphamide are that it can be effective in preventing graft-versus-host disease without compromising immune reconstitution and engraftment.

2.1 Clinical Studies Supporting Post-transplant Cyclophosphamide

The initial studies utilizing post-transplant cyclophosphamide were in haploidentical bone marrow transplants. In a trial by Luznik, et al[4], patients with advanced hematologic malignancies or paroxysmal nocturnal hemoglobinuria underwent haploidentical transplants with T cell-replete bone marrow. A nonmyeloablative conditioning regimen was used consisting of fludarabine, cyclophosphamide, and total body irradiation (TBI). The GVHD prophylaxis regiment was cyclophosphamide 50 mg/kg on days 3 as a single dose or on days 3 and 4 as split doses. Tacrolimus and MMF were initiated the day after completion of cyclophosphamide. Graft failure occurred in 13% of patients evaluated. Non-relapse mortality and relapse at 1 year were 15% and 5% respectively. The incidences of grades II-IV acute GVHD was 36% and grades III-IV was 6%. With regards to chronic GVHD, the incidence was 5% in those patients who received two doses of cyclophosphamide versus 25% in those who received one dose. This study demonstrated the efficacy of post-transplant cyclophosphamide in improving rates of extensive chronic GVHD (although associated with relatively high relapse rates).

Since this study, there have been additional trials with variation of the transplant regimen aiming to improve on both the GVHD and relapse rates. Solomon, et al[5] performed haploidentical transplants for patients with hematologic malignancies. T cell-replete grafts were used from peripheral blood stem cells (PBSCs). They used a myeloablative conditioning regimen consisting of fludarabine, busulfan, and cyclophosphamide with GVHD prophylaxis consisting of cyclophosphamide on days 3 and 4 with tacrolimus and MMF starting at day 5. The incidences of grades II-IV acute GVHD was 30% and grades III-IV was 10%. Chronic GVHD occurred in 35% of patients. The one-year estimate of relapse was 40%. Another study by Castagna, et al[6] was a retrospective comparison of bone marrow versus peripheral blood as the hematopoietic cell source in haploidentical transplants. A nonmyeloablative conditioning regimen was used and GVHD prophylaxis included post-transplant cyclophosphamide. The incidence of acute GVHD was 33% for PBSC and 25% for BM and chronic GVHD was 13% for both. The 1 year NRM was 12% for PBSC and 22% for BM with similar rates of relapse between the two modalities.

Based on these results, subsequent studies further expanded the use of post-transplant cyclophosphamide as GVHD prophylaxis. Luznik, et al[7] evaluated the use of post-transplant cyclophosphamide as single agent GVHD prophylaxis in patients receiving myeloablative alloHCT from HLA matched related and matched unrelated donors. The patients received myeloablative conditioning with busulfan and cyclophosphamide followed by stem cells from T cell-replete bone marrow. GVHD prophylaxis consisted of cyclophosphamide at 50 mg/kg/d on days 3 and 4. The results of this study showed that the incidence of grade II-IV acute GVHD was 43% and grades III-IV was 10%. The incidence of chronic GVHD was 10%. The rate of relapse at 2 years was 44% for all patients and 26% for those patients who were in complete remission (CR) prior to transplant.

More recently, Mielcarek, et al[8] performed a study where 43 patients with high risk hematologic malignancies underwent allogeneic hematopoietic cell transplantation with allografts from related and unrelated donors. They used a myeloablative conditioning regimen consisting of fludarabine and busulfan. GVHD prophylaxis consisted of post-transplant cyclophosphamide at 50mg/kg/day on days +3 and +4 along with cyclosporine starting on day +5. Stem cell source was peripheral blood. The results of this study showed cumulative incidence of grades II-IV and III-IV acute GVHD of 77% and 0% respectively. Rates of chronic GVHD were 16%. Estimates of the cumulative incidence of non-relapse mortality and relapsed disease at 2 years were 14% and 17% respectively.

Additionally, the initial studies with post-transplant cyclophosphamide as GVHD prophylaxis utilized bone marrow as the stem cell source. However, many institutions have gravitated towards the use of peripheral blood stem cells. Compared to bone marrow, there are several advantages to using peripheral blood as the stem cell source. Procurement of stem cells from growth factor mobilized peripheral blood is generally easier to tolerate and poses less risk to the donor in contrast to a bone marrow harvest. Some randomized trials have shown that use of peripheral blood stem cells from HLA identical siblings is associated with more rapid engraftment. However, this potentially comes with a higher risk of acute and chronic GVHD. Anasetti, et al[9] performed a phase 3, randomized, multicenter trial of alloHCT using either peripheral blood stem cells or bone marrow from unrelated donors. Their results showed similar overall survival rates at 2 years between the two modalities. There was also no difference in rates of relapse or acute GVHD. However, the overall incidence of graft failure was 3% in patients receiving peripheral blood versus 9% in patients receiving bone marrow (P=0.002). The incidence of chronic GVHD at 2 years was 53% with peripheral blood versus 41% with bone marrow (P=0.01).

Reduced intensity conditioning (RIC) during alloHCT reduces transplant related mortality (TRM) by relying more on the graft-versus-malignancy effect rather than the conditioning regimen to eradicate disease. Reduced intensity conditioning regimens can provide a curative option for patients with hematologic malignancies who might otherwise not be candidates for myeloablative (intensive) transplantation because of age or poor performance status. Since the ability to perform transplant safely is the primary measure of efficacy for reduced intensity conditioning, TRM is the appropriate measure of efficacy of the conditioning regimen.

RIC regimens are characterized by: 1) Reversible myelosuppression and autologous hematopoietic recovery without allogeneic stem cell support (usually within 28 days), 2) Mixed chimerism in a proportion of patients early after transplant, 3) Low rates of non-hematologic toxicity. We now have over a decade of data pertaining to reduced intensity conditioning regimens and over 10,000 reduced intensity transplants have been reported worldwide.

There were 2 trials performed at Roswell Park utilizing RIC alloHCT. The first was a pilot trial utilizing FLU/MEL/50TBI as a RIC regimen (protocol number I-118807). Fludarabine was dosed at 40 mg/m2, melphalan at 50 mg/m^2, and TBI at 400cGy. This study did not meet early stopping rules for the planned interim analysis of safety. Based on the safety and efficacy data from this study, a phase II study utilizing FLU/MEL/75TBI was performed. The dosing regimen was Fludarabine at 40 mg/m^2, melphalan at 75 mg/m^2, and TBI at 400cGy. A retrospective analysis comparing the results of these 2 prospective clinical trials with a concurrent cohort of patients who received FLU/MEL was performed. Overall, for all patients, the 1 year cumulative incidence of extensive chronic GVHD were 77% (95% CI 67-84%) for FLU/MEL, 69% (95% CI 52-82%) for FLU/MEL/50TBI, and 66% (95% CI 53-76%) for FLU/MEL/75TBI. The patients were then stratified by age. For patients between the ages of 40-59, the 1 year cumulative incidence of extensive chronic GVHD was 75% (95% CI 61-84%) for FLU/MEL, 64% (95% CI 37-82%) for FLU/MEL/50TBI, and 62% (95% CI 40-78%) for FLU/MEL/75TBI. For patients ages ≥ 60, the 1 year cumulative incidence of extensive chronic GVHD was 81% (95% CI 60-91%) for FLU/MEL, 74% (95% CI 42-90%) for FLU/MEL/50TBI, and 71% (95% CI 52-84%) for FLU/MEL/75TBI.

Given the promising results of these studies, we have designed a phase 1b/2 trial in which patients with high-risk hematologic malignancies will undergo an alloHCT with a conditioning regimen of fludarabine, melphalan, and TBI to test post-transplant cyclophosphamide as GVHD prophylaxis with the goal to lower rates of both TRM and chronic GVHD. As described above, there will be a dose escalation component based on melphalan dosing for early toxicity and stopping points. Given that we will be administering

post-transplant cyclophosphamide, we will be starting with a lower dose of melphalan 25 mg/m^2 administered on day -2 which will be on age stratified. There will be an assessment of toxicities and if acceptable, there will be a dose escalation of melphalan to 50 mg/m^2 followed by a further increased to 75 mg/m^2. The primary objective of this study is determination of the incidence of chronic GVHD at 1 year after allogeneic hematopoietic cell transplantation utilizing this novel conditioning/GVHD prophylaxis regimen. This study will build on our previous experience on Clinical Trial I-118807 and I-I77110, which utilized a novel reduced intensity conditioning regimen of FLU/MEL/TBI for patients undergoing allogeneic hematopoietic cell transplantation.

Our choice of sirolimus is on the basis of recent data from JHH that suggests sirolimus may be less nephrotoxic than tacrolimus in this setting. In the unexpected situation in which a patient is unable to tolerate oral medications, IV tacrolimus will be administered instead of sirolimus (which cannot be administered intravenously). If tacrolimus is utilized in lieu of sirolimus, it will be dosed and levels will be monitored as per physician discretion. [10]

2.2 Indications for Allogenic HCT in Various Disease States

Allogeneic HCT has an established role in therapeutic strategies for acute and chronic leukemias, bone marrow failure states (aplastic anemia, paroxysmal nocturnal hemoglobinuria and, myelodysplastic syndromes), lymphomas, myeloma, second transplant and immunodeficiencies.

2.2.1.1 Acute Myeloid Leukemia

Less than 20% of adults who are ≥ 60 years with acute myeloid leukemia (AML) in first complete remission (CR1) will achieve a 3 year DFS with conventional chemotherapy-based consolidation.[11,12] While conventional risk factors like cytogenetics and antecedent hematological disorder play a role, age does appear to be an independent variable.[13-15] Patients over the age of 60 to 65 and those with poor functional status have unacceptable TRM with myeloablative transplant. RIC offers an opportunity to achieve a cure in a subset of these patients with outcomes comparable to standard myeloablative allogeneic HCT.[16,17]

2.2.1.2 Myelodysplastic Syndrome

Myelodysplastic Syndrome (MDS) is incurable except by alloHCT. The risk-benefit ratio favors alloHCT for patients as soon as they progress to more advanced disease (Int-2 or higher IPSS score).[18] It is estimated that only about 5% of MDS patients are eligible to receive myeloablative alloHCT because of age and other exclusions. RIC has allowed for a reduction in TRM in this group of patients and allowed for wider application of alloHCT as a therapy for MDS.

2.2.1.3 Myeloproliferative Disorder

The only cure for a myeloproliferative disorder (MPD) is an allogeneic HCT, which needs to be balanced against the TRM associated with conventional myeloablative HCT. RIC has been used in an effort to reduce the TRM in this group of patients.[19-21]

2.2.1.4 Non Hodgkin Lymphoma

Reduced intensity allogeneic transplantation has a definite role in the treatment of defined subgroups of patients with high-risk Non Hodgkin Lymphoma (NHL) with or without a prior autologous transplant.[22]

2.2.1.5 Hodgkin Lymphoma

Approximately 50% of patients with relapsed and progressive disease after autologous HCT can be rescued by a RIC.[23]

2.2.1.6 Multiple Myeloma

Durable remissions for multiple myeloma can occur after allogeneic HCT. Previous experience with standard myeloablative alloHCT was disappointing due to a high early TRM despite achieving durable remissions for survivors.[24,25] Reduced intensity transplantation from a suitable related or unrelated donor may permit the development of a curative graft-versus-myeloma effect without high TRM.[26-28] The usual approach is to utilize RIC for selected younger patients with well-matched donors either right after auto transplant ("auto-allo") or after relapse from an auto transplant. A phase III trial has demonstrated improved overall and disease-free survivals in newly diagnosed myeloma for recipients of a hematopoietic stem-cell autograft followed by a stem-cell allograft from an HLA-identical sibling versus a standard tandem autologous transplant.[29]2.2.7 Non-hematologic Malignancy

Patients with solid tumors will be excluded from the current protocol.

2.2.1.7 Non-hematologic Malignancy

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2.2.1.8 Second Allogenic Transplants

Second allogeneic transplantation is often offered for relapse after a first allogeneic or autologous transplant and for graft failure. Higher conditioning intensities when used in a second transplant are associated with high rate of regimen related toxicity (RRT) and TRM. Therefore, a RIC regimen is an appropriate treatment option for these patients.

2.2.1.9 Matched Unrelated Donor (MUD) Transplants

TRM in well matched unrelated donor transplants now approximates that of related transplants because of recent advances in supportive care including the use of fludarabine-based conditioning regimens, tacrolimus-based GVHD prophylaxis, peripheral blood stem cells and high-resolution HLA typing. In one retrospective study of reduced intensity conditioning, overall survival for MUD transplants approximated that of sibling donor transplants.[30]

2.3 Correlative Studies

Correlative studies will include chimerism analysis by molecular analysis and evaluation of immune reconstitution.

2.4 Study Rationale

In this study, we propose to incorporate post-transplant cyclophosphamide into the regimen used for GVHD prophylaxis with the goal to lower rates of chronic GVHD. In combination with this GVHD prophylaxis regimen, we will also utilize a reduced intensity conditioning regimen consisting of fludarabine, melphalan, and TBI. This regimen was developed at Roswell Park (Roswell Park protocol numbers: I 118807 and I 177110). The advantages of using this regimen are that it can provide adequate cytoreduction while minimizing rates of TRM. It also relies on graft-versus-tumor effect to eradicate disease.

Rationale for Regimen Dose Selection:

Fludarabine (FLU), a purine nucleoside analog is a potent immunosuppressant that will help prevent donor cell rejection by the recipient. Doses range from 120-200 mg/m^2 over 4 or 5 days and this protocol will utilize 40 mg/m^2/ per day x 4 days.

Melphalan (MEL) is an alkylating agent and doses above 140 mg/m^2 are considered myeloablative. In our trial of reduced intensity allogeneic hematopoietic cell transplantation, we evaluated a melphalan dose of 75 mg/m^2 in combination with fludarabine (40 mg/m^2/day x 4 days) and lower dose total body irradiation (TBI) (400 cGy) (Roswell Park Protocol No. I-118807 and I-177110) with the intention to improve cytoreduction and improve disease control while preserving the low rate of transplant-related mortality (TRM). The results of this trial showed that the regimen was well tolerated. Final analysis of this study is still pending but thus far, the analysis indicates that FLU/MEL/TBI generates the same overall survival as FLU/MEL. Our results suggest that for high-risk patients (i.e., patients who are not in remission at the time of alloHCT); FLU/MEL/TBI is actually superior. Despite older age and more advanced disease, the patients receiving FLU/MEL/TBI did as well as the patients who received Flu/Mel when retrospectively compared. Preliminary data also show that there are adequate rates of engraftment with this regimen. One year estimate of extensive chronic GVHD for FLU/MEL/TBI was 66% versus 77% for FLU/MEL. In this trial, given that we will be administering post-transplant cyclophosphamide, we will be starting with a lower dose of melphalan: 25mg/m^2 administered on Day -2 to ensure no excess toxicities when used in combination with post-transplant cyclophosphamide. A safety dose run-in will be performed. Dosing of melphalan will be based on age stratification to account for possible comorbidities. There will be an assessment of transplant related mortality and if acceptable, there will be a dose escalation of melphalan to 50 mg/m^2 followed by a further increased to 75 mg/m^2.

Total Body Irradiation (TBI) is considered myeloablative above 500 to 600 centiGray (cGy). There is extensive experience with lower doses of TBI with fludarabine in nonmyeloablative conditioning, usually at 200 cGy. The TBI dose in this study will be 400 cGy administered in 2 doses of 200 cGy. The conditioning intensity, even with 400 cGy TBI, is less than half that of a standard myeloablative Mel 140 mg/m^2/1200 cGy TBI regimen. We therefore consider the dosing range of our regimen to lie within the parameters that define "reduced intensity" and to be a regimen that will not generate excess TRM.

Cyclophosphamide is an alkylating agent which has both antineoplastic and immunosuppressive properties. There is a wide range of dosing but at a dose of 50 mg/kg/day x 2 days, it has been shown to have primarily an immunosuppressive effect and has been safe and effective at reducing rates of chronic GVHD. There has been extensive experience with its use in haploidentical transplants. In our protocol, we propose to apply the same regimen to patients undergoing matched related and matched unrelated allogeneic hematopoietic cell transplantation with the premise that it will have similar effects in reducing rates of GVHD. It will be given again at 50 mg/Kg on days +3 and +4 post-hematopoietic cell infusion.

3 INCLUSION AND EXCLUSION CRITERIA

3.1 Inclusion Criteria

To be included in this study, participants must meet the following criteria (**Appendix A**: INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM: INCLUSION CRITERIA):

1. The patient must have a diagnosis of one of the following (one must be yes):
    - AML
    - ALL
    - CLL
    - CML (chronic phase intolerant or unresponsive to tyrosine kinase inhibitors, history of accelerated phase, or history of blast crisis)
    - MDS
    - NHL
    - HL (received and failed frontline therapy or failed autologous transplantation or inability to collect enough PBSC for auto-HCT)
    - MM
    - Severe aplastic anemia
2. Histocompatible donor identified:
    - Related donor 5/6 or better (A, B, DRB1)
    - Unrelated donor 7/8 or better (A, B, C and DRB1)
3. Age ≥ 18 years.
4. The following are eligible for study inclusion (refer to **Appendix H)**:
    - AML may be in morphologic CR or CRi (MRD positive is allowed)
    - ALL may be in morphologic CR or CRi (MRD positive is allowed)
    - CLL may be in morphologic CR or CRi (MRD positive is allowed)
    - CML must be in chronic phase (MRD positive is allowed)
    - MDS- Patients with MDS only require ≤5% myeloblasts on bone marrow evaluation. There is no requirement for platelet or neutrophil recovery.
    - NHL must be in CR
    - HL must be in CR
    - MM may be in VGPR
    - SAA do not have disease response requirements; however, if the patient has a mismatched donor, the patient must have had prior therapy with ATG.
5. Have a Karnofsky performance status score of ≥ 50% (refer to **Appendix C**)
6. Have the following clinical laboratory values:
    - Diffusing Capacity of the Lung for Carbon Monoxide (DLCO) ≥ 40% predicted, corrected for hemoglobin and/or alveolar ventilation.
    - Cardiac: left ventricular ejection fraction ≥ 40%.
    - Bilirubin, liver alkaline phosphatase, SGOT or SGPT ≤ 3 x upper limit of normal

- Calculated creatinine clearance ≥ 40 cc/min by the modified Cockroft-Gault formula (refer to **Appendix D)**

7. Patient must be cleared pre-transplant by Radiation Oncology to be able to receive 400 cGy.
8. Participants of child-bearing potential must agree to use adequate contraceptive methods (e.g., hormonal or barrier method of birth control; abstinence) prior to study entry. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.
9. Patients who have failed a prior autologous or allogeneic transplant are eligible. However, at least 6 months must have elapsed between the start of this reduced intensity conditioning regimen and the last transplant if patient had a prior autologous or allogeneic BMT.
10. At least 2 weeks since prior radiation treatment and/or surgery. Appropriate washout of prior chemotherapy per BMT standard of care (see Appendix K). If medication is not on the list, go by physician discretion.
11. Participant must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.

3.2 Exclusion Criteria

Participants will be excluded from this study for the following (**Appendix B**: ELIGIBILITY VERIFICATION FORM: EXCLUSION CRITERIA):

1. Moderate to severe myelofibrosis within 60 days prior to transplant.
2. Presence of HLA antibodies to the donor within 60 days prior to transplant.
3. Uncontrolled CNS disease (for hematologic malignancies).
4. Patients who in the opinion of the treating physician are unlikely to comply with the restrictions of allogeneic stem cell transplantation based on formal psychosocial screening. (i.e., serious, uncontrolled psychiatric illness/social situations that would limit compliance with study requirements).
5. Uncontrolled diabetes mellitus, cardiovascular disease, active serious infection or other condition which, in the opinion of treating physician, would make this protocol unreasonably hazardous for the patient.
6. Known HIV positive.
7. Pregnant or nursing female participants.
8. Unwilling or unable to follow protocol requirements.
9. Any condition which in the Investigator's opinion deems the participant an unsuitable candidate to receive study intervention.

3.3 Special Populations

The following special populations are excluded from this study:

- Cognitively impaired adults/adults with impaired decision-making capacity
- Individuals who are not yet adults (infants, children, teenagers)
- Pregnant women
- Prisoners

3.4 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this study.

4 LOCAL AND STUDY-WIDE NUMBER OF SUBJECTS

This is a single-institution study.

5 LOCAL AND STUDY-WIDE RECRUITMENT METHODS

Participants will be identified/recruited/screened from patients at the BMT service at Roswell Park Comprehensive Cancer Center and from multi-disciplinary conference discussion.

6 MULTI-SITE RESEARCH

Not Applicable.

7 STUDY TIMELINES

Projected accrual is approximately to have a maximum of 33 evaluable patients, and therefore recruitment is expected to be complete within 8 years following the study starting point.

Treatment duration is 11 days.

Subjects will be followed for at least 12 months after transplantation and annually thereafter as per BMT clinic standard of care.

Therefore, the study completion is approximately 9 years from study activation.

8 STUDY ENDPOINTS

8.1 Primary Endpoints

- The primary endpoint is extensive chronic GVHD in the first 365 days from Day 0 of the transplant (refer to **Appendix E**).

8.2 Secondary Endpoints

- Clinical response, engraftment rate, progression free survival (PFS) at one year, and overall survival (assessments as per BMT standard of care)
- Cumulative incidence of relapse
- Treatment-related mortality rates

- Cumulative incidence of grade III-IV acute GVHD

Correlative studies will include chimerism analysis by molecular analysis and evaluation of immune reconstitution (refer to Section 11.7).

9 DESIGN

This is a Phase 1b/2 open label, non-randomized, single institution study. The primary objective of this study is determination of the cumulative incidence of chronic graft-vs.-host disease at 1 year after allogeneic hematopoietic cell transplantation utilizing a novel, reduced-intensity conditioning/GVHD prophylaxis regimen (Fludarabine/Melphalan/Total Body Irradiation).

The design of this trial consists of two components: 1) A safety run-in component which will be broken out into two strata (≤50 years of age, > 50 years of age) and 2) A two-stage efficacy analysis of chronic GVHD rates combining subjects from both strata. Prior to the November 16, 2021 revision, each component of this Phase Ib trial was broken out into two strata (≤50 years of age, > 50 years of age). The safety run-in component of the trial precedes the efficacy analysis. As of the November 16, 2021 revision the Phase Ib components are closed to accrual and treatment design.

Phase Ib participants enrolled and treated prior to the November 16, 2021 revision will be analyzed as within their respective age strata and cohort.

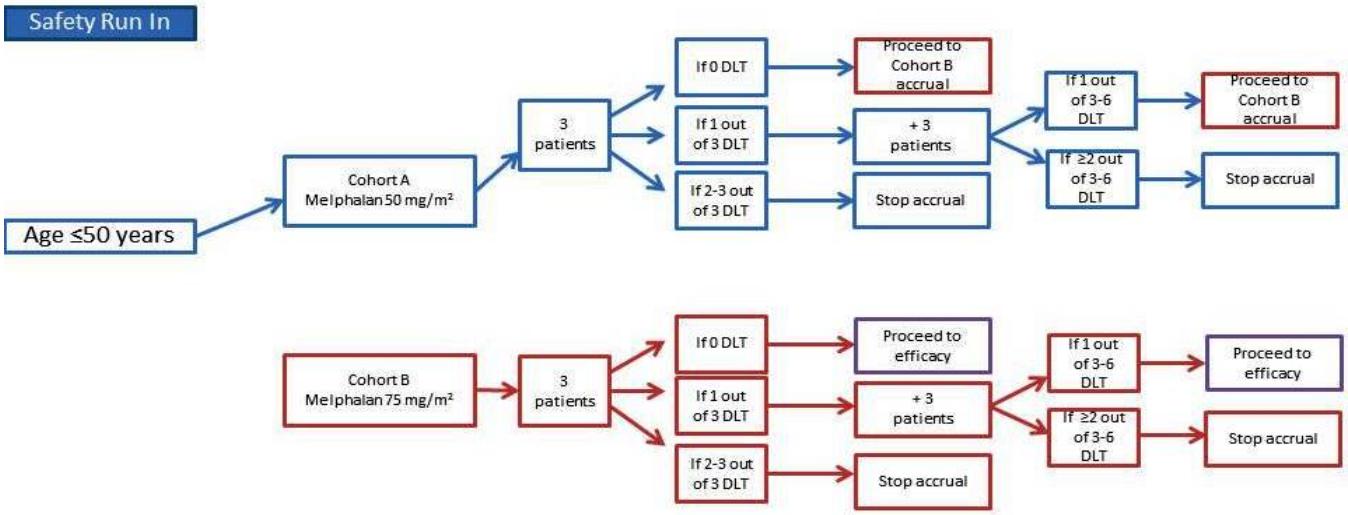
For Phase II: The November 16, 2021 revision removed the Age ≤50 and Age >50 strata as well as closed the previous Age ≤50 and Age >50 groups. Therefore, Phase II participants enrolled and treated after the November 16, 2021 revision will receive 75 mg/m^2 melphalan on Day -2.

Please see Section 19 regarding prior to November 16, 2021 strata analyses.

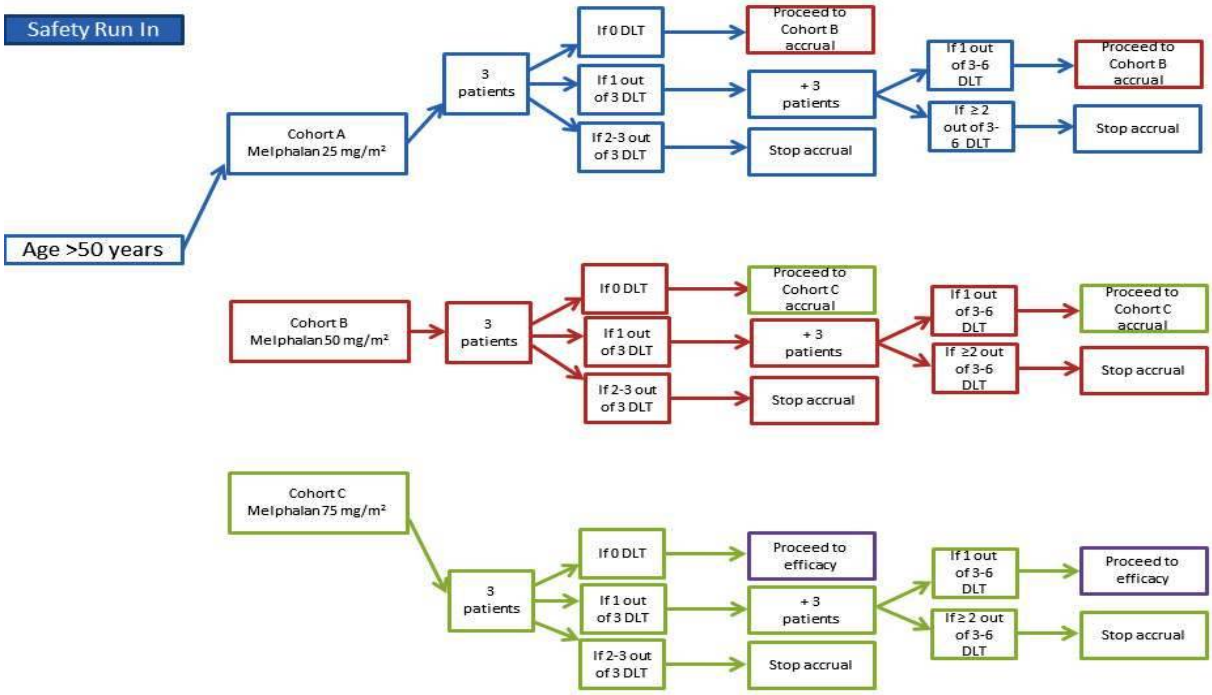
9.1 Cohort Management

9.1.1.1 Safety Run-In Design:

- Age ≤ 50 years (Discontinued to accrual as of November 16, 2021)



- Age > 50 years (Accrual met as of July 2019)

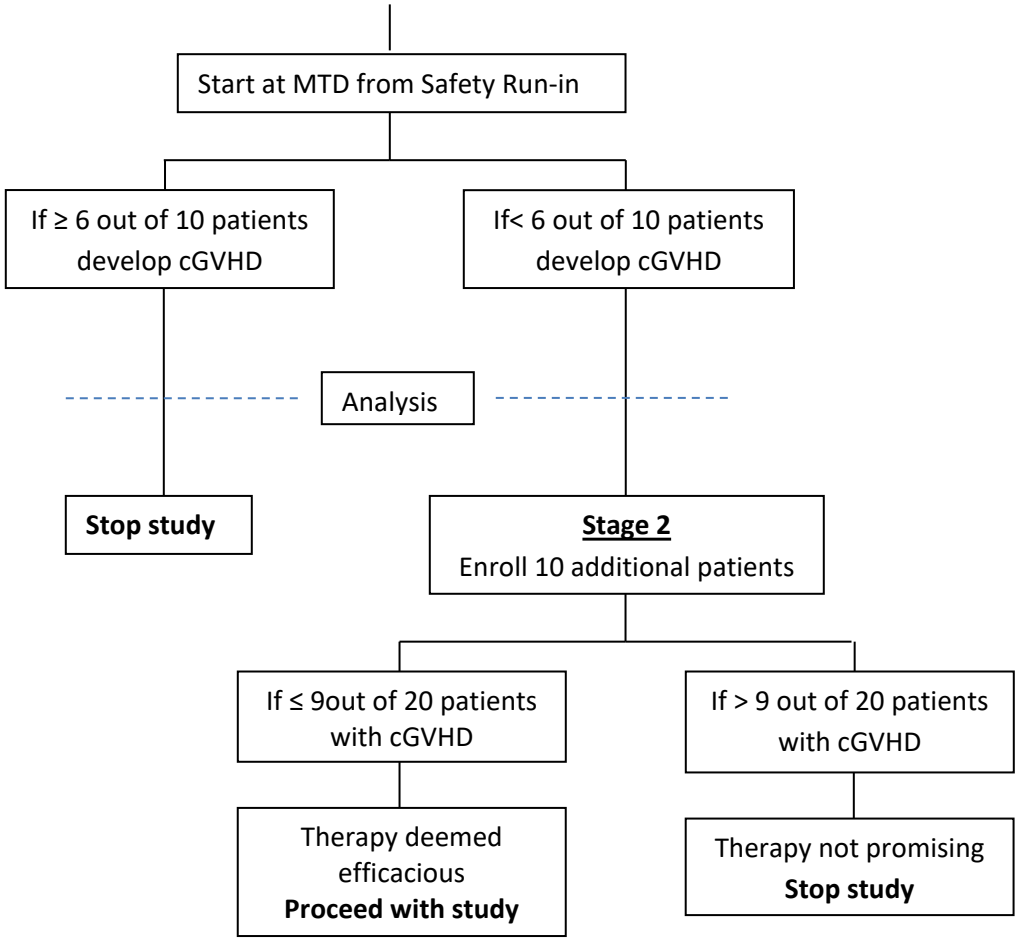


Prior to the November 16, 2021 revision, both strata utilized a 3+3 dose escalation scheme in order to determine the safest dose from which to utilize in the efficacy analysis. This is slightly different than a pure Phase I trial in that the study drug is already in use in the clinic. The goal is to better understand the maximum tolerated dose (MTD) within the age strata in a conservative fashion prior to proceeding on to the efficacy analysis.

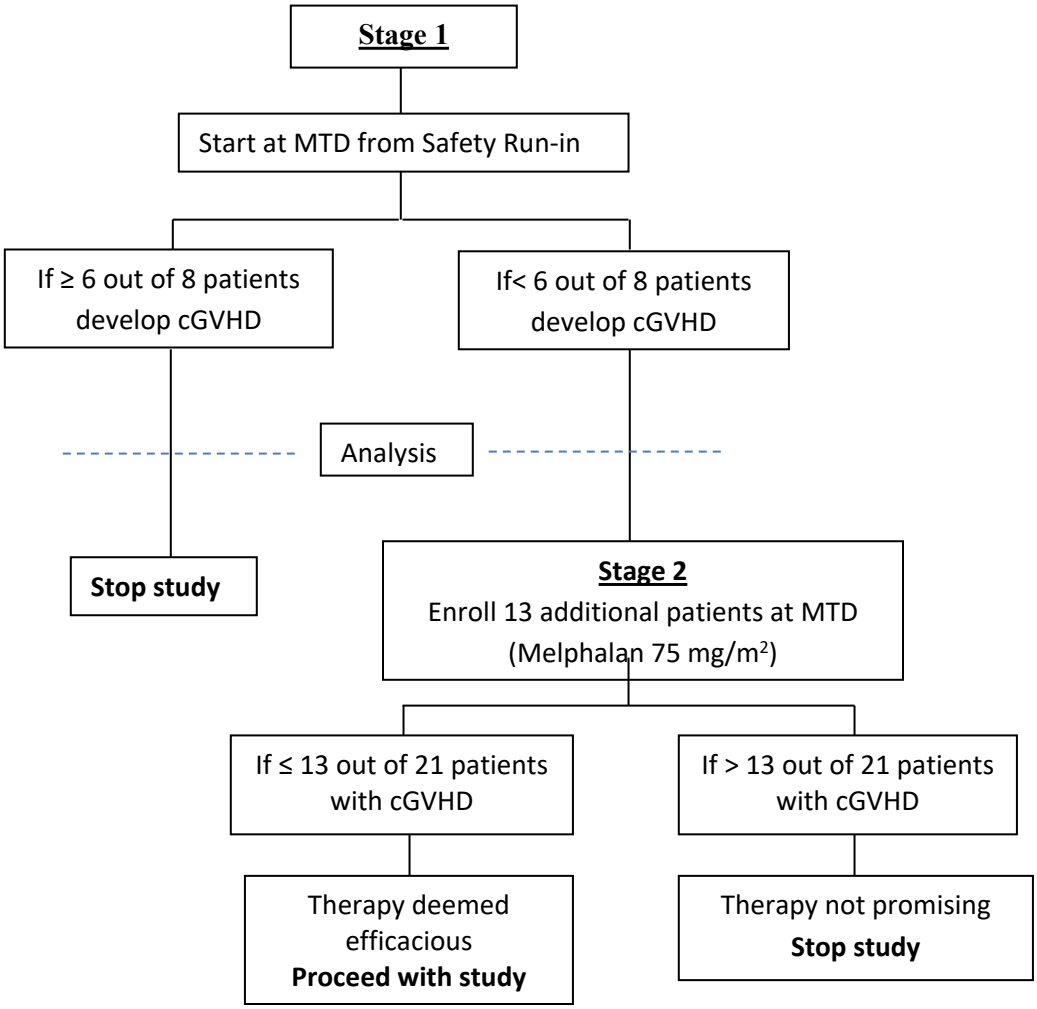
- For the **age ≤50 years strata** we examined two dosing cohorts (A, B) *following the administration of Fludarabine 40 mg/m²/day on Days -5 to -2*.
  - Cohort A: 50 mg/m² Melphalan
  - Cohort B: 75 mg/m² Melphalan
- For the **age >50 years strata** we examined three dosing cohorts (A B C) *following the administration of Fludarabine 40 mg/m²/day on Days -5 to -2.*
  - Cohort A: 25 mg/m² Melphalan
  - Cohort B: 50 mg/m² Melphalan
  - Cohort C: 75 mg/m² Melphalan

9.1.1.2 Efficacy Analysis Design

- Age ≤ 50 years (Discontinued as of November 16, 2021)



- Combined Age Groups (as of November 16, 2021)



9.2 Definition of Dose-Limiting Toxicity

The timeframe for examining the DLT per subject is 28 days from stem cell infusion (Day 0) DLT will be defined as a transplant-related death or, failure to engraft neutrophils by Day 28 post-transplant.

For Phase Ib participants that were treated prior to the November 16, 2021 revision, for each stratum (≤50 years of age, > 50 years of age) the given cohort will start with three patients, with three patients added to any cohort if we observe 1 out of 3 dose limiting toxicities (DLTs). If ≥ 2 out of 3 to 6 subjects have a DLT we will not proceed to the next cohort. Cases will be reviewed by HLA match to determine if change to eligibility criteria is needed. Overall, accrual within the age ≤50 years strata for this component of the trial ranges between 3-12 patients and the accrual within the age >50 years strata for this component of the trial ranges between 3-18 patients. It is anticipated that we will escalate to the highest dose per strata. However, if ≥2/3 DLTs are observed in Cohort A in either stratum, that component of the trial relative to the given strata will be suspended and the dosing scheme will be re-evaluated.

Evaluable patients are defined as patients who do not have disease progression within Day 100 post-transplant. Non-evaluable patients (defined as patients that, prior to Day 100 post-transplant: have withdrawn, have been removed from study for any reason or, have disease progression) will be replaced.

All participants will sign an informed consent prior to any study-related tests. All participants will meet the inclusion and exclusion criteria summarized in **Appendix A** and **Appendix B**.

Failure to engraft platelets will not be considered a dose limiting toxicity for the purposes of this protocol. There was an initial error and subsequent amendment which led to two artificial DLTs involving platelet engraftment in a previous protocol amendment. The IRB determined that we could proceed with enrollment and continue to escalate to subsequent dose levels per protocol.

10 TREATMENT

Eligible patients will receive conditioning regimen, GVHD prophylaxis, and allogeneic hematopoietic stem cell transplant as follows:

For Phase Ib (prior to the November 16, 2021 revision):

Conditioning regimen:

- Fludarabine: 40 mg/m^2/day on Days -5 to -2
- Melphalan: Age > 50 years: 25 mg/m^2 on Day -2*
    - Age ≤ 50 years: 50 mg/m^2 on Day -2**
- Total body irradiation (TBI): 400 cGy (two fractions of 200 cGy each) on Day -1

* Melphalan dosing will be escalated to 50 mg/m2 followed by 75 mg/m2 in subsequent cohorts (Cohort B and Cohort C in age > 50 years strata) of patients based on evaluation of TRM in Phase 1. In Phase 2, the MTD (Melphalan 75 mg/m^2) will be used for dosing patients.

** Melphalan dosing will be escalated to 75 mg/m2 in Cohort B (age ≤ 50 years strata).

Stem cell infusion:

- Hematopoietic stem cell infusion: on Day 0

GVHD prophylaxis regimen:

- Cyclophosphamide: 50mg/kg/day Days +3 and +4
- Mycophenolate mofetil: starts Day +5§
- Sirolimus: starts Day +5

§ Mycophenolate mofetil will start on Day +5 and continue until approximately Day+35 (as per standard of care and, physician's discretion).

Sirolimus

On Day +5, starting dose will be 0.5 mg orally daily. The sirolimus dose will be adjusted to target trough levels between 5-10 ng/mL (as per standard of care, and physician's discretion).

Supportive Care:

- G-CSF (filgrastim-sndz or other approved biosimilar is acceptable) or GM-CSF: starts Day +5 and, per standard of care, continues until ANC > 1500 for 2 consecutive days

For Phase II (after the November 16, 2021 revision):

Conditioning regimen:

- Fludarabine: 40 mg/m^2/day on Days -5 to -2
- Melphalan: 75 mg/m^2 on Day -2
- Total body irradiation (TBI): 400 cGy (two fractions of 200 cGy each) on Day -1

Stem cell infusion:

- Hematopoietic stem cell infusion: on Day 0

GVHD prophylaxis regimen:

- Cyclophosphamide: 50mg/kg/day Days +3 and +4
- Mycophenolate mofetil: starts Day +5[§]
- Sirolimus: starts Day +5

[§] Mycophenolate mofetil will start on Day +5 and continue until approximately Day+35 (as per standard of care and, physician's discretion).

Sirolimus

On Day +5, starting dose will be 0.5 mg orally daily. The sirolimus dose will be adjusted to target trough levels between 5-10 ng/mL (as per standard of care, and physician's discretion).

Supportive Care:

- G-CSF (filgrastim-sndz or other approved biosimilar is acceptable) or GM-CSF: starts Day +5 and, per standard of care, continues until ANC > 1500 for 2 consecutive days

10.1 Treatment Plan

10.1.1.1 Stem Cell Collection

- Per BMT Standard of Care

10.1.1.2 Preparative Regimen

Table 1 400 cGY TBI-based transplant

	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day +1	Day +2	Day +3	Day +4	Day +5	Day +6	Day +7	Day +8*
Fludarabine (total 160mg/m2)	40	40	40	40										
Melphalan				X										
TBI (400 cGy)					200+ 200									
Stem cell infusion						X								
Sirolimus											X	X	X	X
MMF											X	X	X	X
Cyclophosphamide/ Mesna									X	X				
GCSF											X	X	X	X

*After Day +8, the patient will continue with GVHD prophylaxis regimen as per BMT Allo-Treatment Plan standard of care and, physician's discretion.

Fludarabine

- Fludarabine: 40 mg/m^2 (actual body weight) is infused over 30 minutes on Days -5, -4, -3, and -2 (total dose 160 mg/ m^2).

Melphalan

- Phase 1: Melphalan: mg/m^2 per cohort (actual body weight) is infused over 30 minutes following fludarabine on Day -2.
- Phase 2: For all participants, the dose will be Melphalan 75 mg/m^2.

Total Body Irradiation (TBI)

- Schedule- on Day -1: Two fractions of 200 cGy will be given at least 6 hours apart.

Total Body Irradiation will be delivered using a nominal photon beam energy of no less than 4 MV. Dynamic or static fields may be used. However, the patient should be entirely included within the dynamic or static treatment field. Lung shields are to be applied in pairs (both AP & PA) with evenly weighted mid-plane dose from each field. When necessary, lung shields can be combined with open (non-shield) fields during the same fraction to achieve the required mid-plane and lung dose. Dose heterogeneity resulting from lung transmission will be assessed using the AAPM Task Group 29/AAPM-Report Number 17, *The physical aspects of total and half-body photon irradiation*.[31]

TBI, 200 cGy, will be delivered as prescribed to mid-plane at the level of the umbilicus or pelvis; whichever region is thicker. Tissue compensators or other devices will be used to optimize dose homogeneity within ± 5 % of the prescribed dose. Dose homogeneity will be assessed at a minimum of

mid plane points at: a) head, at largest diameter, b) neck, at the level of thyroid notch, c) chest, at the xiphoid process, d) umbilicus or pelvis, whichever is thicker, e) mid-thigh, and, f) mid-calf. Optional dose points will be located at: g) knee, h) ankle and, i) umbilicus or pelvis, whichever is thinner. In addition, a set of lung shields, or an equivalent device, will be used to ensure that the lungs will receive within 90 to 100 % of the prescribed dose per fraction. The dose rate at the prescription point shall be between 10 cGy and 15 cGy per minute.

Allogeneic Stem Cell Reinfusion

- On Day 0 hematopoietic stem cells will be infused per BMT SOP.

Hydration regimens

- Per clinicians' discretion

Supportive medications

- Antiemetics, antimicrobials, etc. will be used according to BMT standard of care.
- Systemic steroids will not be allowed prior to administration of post-transplant cyclophosphamide.

10.1.1.3 GVHD Prophylaxis

GVHD prophylaxis will be post-transplant cyclophosphamide, Sirolimus with mycophenolate mofetil (MMF) as per the BMT SOP. Tacrolimus (IV or oral) or cyclosporine (IV or oral) may be substituted for sirolimus in the case of inability to tolerate oral medications or other intolerance or adverse reaction and dosed per Roswell Park standards. If this is a temporary issue, a transition back to sirolimus will be made once it has resolved; otherwise, the patient may be continued on either tacrolimus or cyclosporine as per physician discretion.

DAY	0	+1	+2	+3	+4	+5	+6	+7	+8*
Sirolimus						X	X	X	X
MMF						X	X	X	X
Cyclophosphamide				X	X				

***** After Day +8, the patient will continue with GVHD prophylaxis regimen as per BMT Allo-Treatment Plan standard of care and, physician's discretion

Sirolimus

- On Day +5, starting dose will be 0.5 mg orally daily. The sirolimus dose will be adjusted to target trough levels between 5-10 ng/mL (as per standard of care, and physician's discretion).

In the absence of GVHD, sirolimus will continue until Day +100 and then taper with a goal to be discontinued at approximately 6 months post-transplant. In the case of disease recurrence, sirolimus may be tapered off more rapidly and/or starting before Day +100 post-transplant, as per the treating clinician's discretion

Mycophenolate mofetil (MMF)/ (Cellcept®) [33]

- MMF starts on Day +5 and, in the absence of GVHD or recurrent disease, will continue as per BMT standard of care until approximately Day+35 (or at physician's discretion).

- The dose will be 1000 milligrams orally every 8 hours or 1500 milligrams intravenously every 6 hours.
- MMF may be withheld at the discretion of the attending physician for patients with cytopenias or other toxicities that are thought to be related to the MMF.

Cyclophosphamide

- Cyclophosphamide will be given as 50 mg/kg IV on Days 3 and 4 for GVHD prophylaxis, as described above.
    - Mesna will be administered with cyclophosphamide and will be based on the same dosing weight as Cyclophosphamide. Dosing of Mesna is 80% of Cyclophosphamide dose every 3 hours x 4 doses on day +3 and Day +4 starting 30 minutes prior to each cyclophosphamide dose.
    - Dosing will be based on ideal body weight or actual weight, whichever is less. If a patient's weight is greater than >125% of their ideal body weight, the adjusted body weight will be used.
        - Adjusted BW= IBW + 0.25 x (ABW-IBW)
        - Ideal Body Weight Dosing:
            - Men= 50 + 0.91 x (height in cm - 152)
            - Women= 45 + 0.91 x (height in cm - 152)

10.2 Supportive Care Recommendations

- Prior to initiating therapy, placement of a multi-lumen indwelling silastic catheter is required, preferably a non-tunneled 5 lumen catheter.
- Patients will receive full supportive care, including transfusions of irradiated blood and blood products, growth factors, antibiotics, anti-emetics, oral care, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded on the flow sheets.
- Mucosal evaluation and care, antifungal prophylaxis, Pneumocystis Pneumonia (PCP), prophylaxis, CMV Infections, anti-bacterial prophylaxis, deep venous thrombosis (DVT) prophylaxis, sinusoidal occlusive syndrome prophylaxis are per BMT standard of care.
- Systemic steroids will not be allowed prior to administration of post-transplant cyclophosphamide.

10.3 Potential Toxicities and their Management

Patients will be managed in the inpatient and outpatient setting per BMT standards of care. Safety will be monitored on an ongoing basis and discussed, graded and recorded in weekly outcome rounds.

10.3.1.1 Management of Graft Versus Host Disease (GVHD)

- BMT standard measures for prevention and treatment of GVHD will be followed.

10.3.1.2 Toxicities

- Infections, hematologic toxicities, organ toxicities, failure to engraft will be managed per BMT standards of care.
- Secondary malignancy

- The cumulative exposure to chemotherapeutic agents and radiation increases the risk of developing leukemia or a second cancer. The incidence of secondary malignancy is approximately 5%.

11 PROCEDURES INVOLVED

Screening: All screening tests must be performed within 60 days of the on-study date (pre-admission clinic visit).

Please contact the Transplant Program Coordinator to enroll patients on the study.

Informed consent: Patient must be aware of the nature of his/her disease and willingly consent after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts. Human protection committee approval of this protocol and of its consent form is required and will have been obtained before this study opens.

Histological review: Submission of appropriate tissue samples or outside histo-pathologic slides to confirm the underlying diagnosis. Remission/relapse status should be confirmed within 30 days and no later than 60 days before transplant conditioning starts.

11.1 Registration

Registration procedures:

- Confirm eligibility criteria for patients (**Appendix A** and **Appendix B**) and donors.
- Complete the transplant registration worksheets for patient and donor. Data pertaining to this protocol will be collected by the BMT APPs, MDs, TCs, data managers and/ or any other persons assigned by the BMT director.

Eligibility of each participant will be established prior to enrollment.

Informed consent MUST be completed prior to receiving any study related procedures.

11.2 Protocol Date Definitions

On-Study Date	Start Treatment Date	Off-Treatment Date	Off-Study Date
Date of pre-admission clinic visit.	Date of conditioning regimen initiation	Date of completion of the last dose of cyclophosphamide	Date of death due to any cause or, until study completion

11.3 Disease Evaluation

- Disease evaluation will be performed pre and post alloHCT as well as once per year clinic visit (in accordance with BMT standard of care evaluation

11.4 Safety Assessments

- Safety will be evaluated on an ongoing basis with weekly outcome assessment and grading for the first 100 days post-transplant (from Day -5 until 100 days after cell infusion).
- Acute GVHD will be graded according to the criteria in **Appendix F.**
- All deaths will be reviewed.

In addition to the adverse events deemed as serious in Section 17.4 and, for the purpose of this study, death due to any transplant-related mortality cause (i.e., infection, GVHD, toxicities, etc.) will be reported to Clinical Research Services.

11.5 Hematology/ Chemistry

- Baseline (Day -5) and daily during hospitalization for transplant (CBC and CMP as per standard of care). Weekly after discharge until day 100 after hematopoietic stem cell infusion.
- Important hematologic recovery endpoints:
    - The first of 3 consecutive days of absolute neutrophil count (ANC) $\geq 0.5 \times 10^9$/L
    - Platelets $\geq 20 \times 10^9$/L after 7 consecutive days with no platelet transfusions
    - Day (post stem cell infusion) of last platelet transfusion

11.6 Follow-up Evaluations

The following BMT standard of care lab assessments will be performed at Day 30 (± 10 days), Day 100 (± 20 days) and at 1 year (± 30 days) following transplant:

- Hematology :CBC with automated differentials
- Chemistry: CMP
- Bone marrow biopsy will be performed for chimerism and engraftment evaluation.

Patients will be followed for survival for a minimum of 4 years or until the study closure whichever occurs first. Patients will not be taken off protocol for disease progression but will be followed for survival. Subsequent transplant and/or DLI will be captured for all patients.

11.7 Important Immunologic Recovery Endpoints

- Myeloid and lymphoid chimerism expressed as a percentage of donor cells at the following time points: Day 30 (± 10 days), Day 100 (± 10 days), and Day 365 (± 30 days).
- Immune reconstitution is evaluated at the following time points: baseline (prior to conditioning), Day 30 (± 10 days), Day 100 (± 20 days) and Day 365 (± 30 days) by the BMT SOC immunophenotyping panel and by analysis of CMV-specific immunity (CMV dextramer).

11.8 Correlative Studies

11.8.1.1 Chimerism Analysis

Chimerism analysis is clinically useful and important for this study. Samples for chimerism analysis will be obtained on all donor and recipient pairs prior to transplant. After transplant, serial samples of blood and marrow will be analyzed for chimerism analysis. Lineage specific (separate lymphoid and myeloid) chimerism will be analyzed in blood per BMT SOP. Unseparated chimerism may also be analyzed in bone marrow (for leukemia and patients with other malignancies if bone marrow was involved previously) whenever a bone marrow aspirate is performed.

11.8.1.2 Immune Reconstitution

Peripheral blood will be collected per BMT SOP. Immune reconstitution will be measured by flow cytometry using the BMT SOC Flow Panel. Flow cytometry will be performed by the Roswell Park flow cytometry lab.

Refer to **Appendix J** for a complete Schedule of Procedures and Observations.

12 WITHDRAWAL OF SUBJECTS

12.1 Treatment Discontinuation

Upon treatment discontinuation all end of treatment evaluations and tests will be conducted as per BMT standard of care. All participants who discontinue due to an AE must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of the AE will be reported in the participant's medical records and the appropriate eCRF.

Reasons for treatment discontinuation should be classified as follows:

- Death
- Progressive disease
- Toxicity; treatment related or unrelated
- Investigator judgment
- The Investigator may discontinue a participant if, in his/her judgment, it is in the best interest of the participant to do so.
- Noncompliance
- Participant voluntary withdrawal
- A participant may withdraw from the study at any time, for any reason. If a participant discontinues treatment, an attempt should be made to obtain information regarding the reason for withdrawal
- Sponsor decision.

* If the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the patient will be removed from protocol treatment and placed on follow-up. In this event the reason for withdrawal will be documented, the PI will be notified and the patient will be followed for GVHD, disease relapse and survival.

Patients that withdraw or that are taken off-study for any reason will be replaced. These patients do not count toward the accrual target. The accrual is based on the Statistical Objective of evaluable patients. Therefore, if patients are removed because they are not evaluable, they should be replaced.

13 RISKS TO SUBJECTS

13.1 Fludarabine Monophosphate

- 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 25-40 milligrams 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).

13.2 Melphalan

The most frequently reported adverse events include: leukopenia, thrombocytopenia, irreversible bone marrow failure, vasculitis, secondary malignancies, vesiculation of the skin, alopecia, pruritis, rash, SIADH, sterility, amenorrhea, nausea, vomiting, stomatitis, diarrhea, hemorrhagic cystitis, anemia, hemolytic anemia, pulmonary fibrosis, interstitial pneumonitis.

13.3 Cyclophosphamide

The most frequently reported adverse events include: nausea/vomiting, cardiomyopathy, rash, mucositis, stomatitis, sterility, diarrhea, hemorrhagic cystitis, edema, alopecia, anemia, hemolysis, leukopenia, alopecia, appetite loss, amenorrhea, thrombocytopenia, anemia, fatigue, pulmonary fibrosis, secondary malignancies including urological malignancies.

13.4 Tacrolimus

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.

13.5 Sirolimus

In patients receiving sirolimus, 23% to 33% experienced anemia, and 14% to 30% experienced thrombocytopenia. Leukopenia has also been reported. Rare cases of microangiopathic hemolytic anemia have been reported. Cases of lymphoproliferative disorders have been reported. Lymphocele development has been reported.

Hypertension has been reported in 49% of patients receiving sirolimus. Antihypertensive therapy may be required. Edema has been reported in 18% to 20% of patients receiving sirolimus. Chest pain, venous thromboembolic disease, and tachycardia have also been reported.

Headaches have occurred in 20% to 34% of patients on sirolimus, pain has occurred in 29% to 33% of patients, and dizziness has also been reported. Gastrointestinal adverse effects of sirolimus have included constipation (36% to 38%), and stomatitis (3% to >20%).

Hypertriglyceridemia has been reported in 45% to 57% of patients taking sirolimus. Amenorrhea, diabetes mellitus, hypermenorrhea, hypervolemia, hypokalemia, elevated lactate dehydrogenase, menstrual disease and ovarian cyst formation have also been reported.

In patients taking sirolimus, 33% have had urinary tract infections. Herpes simplex infection, herpes zoster, pyelonephritis and sepsis have also been reported.

Arthralgia has been reported in 25% to 31% of patients taking sirolimus. Osteonecrosis has also been reported.

A decline in kidney function has been seen in 39% to 40% of patients taking sirolimus.

Epistaxis and pneumonia have been reported in patients who were taking sirolimus.

Other miscellaneous effects that have been noted to occur in patients taking sirolimus include but are not limited to wound healing impairment, skin cancers, interstitial pulmonary disease (including pneumonitis, pulmonary fibrosis, and bronchiolitis obliterans organizing pneumonia), pulmonary alveolitis, pulmonary hemorrhage, reversible posterior leukoencephalopathy syndrome, liver toxicity, and cardiac tamponade.

13.6 Mycophenolate mofetil

The principal adverse reactions associated with the administration of mycophenolate include diarrhea, leukopenia, sepsis, vomiting, and there is evidence of a higher frequency of certain types of infections. Patients receiving immunosuppressive regimens involving combinations of drugs, including mycophenolate, as part of an immunosuppressive regimen are at increased risk of developing lymphomas

and other malignancies, particularly of the skin. The risk appears to be related to the intensity and duration of immunosuppression rather than to the use of any specific agent. Oversuppression of the immune system can also increase susceptibility to infection, including opportunistic infections, fatal infections, and sepsis.

As usual for patients with increased risk for skin cancer, exposure to sunlight and UV light should be limited by wearing protective clothing and using a sunscreen. Lymphoproliferative disease or lymphoma developed in 0.4% to 1% of patients receiving mycophenolate (2000 to 3000 milligrams daily) with other immunosuppressive agents in controlled clinical trials of renal, cardiac, and hepatic transplant patients.

There are no adequate and well-controlled studies in pregnant women. However, as mycophenolate has been shown to have teratogenic effects in animals, it may cause fetal harm when administered to a pregnant woman. Therefore, mycophenolate should not be used in pregnant women unless the potential benefit justifies the potential risk to the fetus. It is recommended that mycophenolate therapy should not be initiated by the physician until a report of a negative pregnancy test has been obtained.

In patients receiving mycophenolate (2000 or 3000 milligrams) in controlled studies for prevention of renal, cardiac or hepatic rejection, fatal infection/sepsis occurred in approximately 2% of renal and cardiac patients and in 5% of hepatic patients.

Severe neutropenia [absolute neutrophil count (ANC) $<0.5 \times 10^3$ / microliter] developed in up to 2.0% of renal, up to 2.8% of cardiac, and up to 3.6% of hepatic transplant patients receiving mycophenolate 3000 milligrams daily respectively. If neutropenia develops (ANC $<1.3 \times 10^3$ / microliter), dosing with mycophenolate should be interrupted or the dose reduced, appropriate diagnostic tests performed, and the patient managed appropriately. Gastrointestinal bleeding (requiring hospitalization) has been observed in approximately 3% of renal transplant patients treated with mycophenolate 3000 milligrams daily. Gastrointestinal perforations have rarely been observed.

Allergic reactions to mycophenolate have been observed; therefore, mycophenolate is contraindicated in patients with a hypersensitivity to mycophenolate mofetil, mycophenolic acid or any component of the drug product. Mycophenolate intravenous is contraindicated in patients who are allergic to Polysorbate 80 (TWEEN).

13.7 Infusion Risks

Stem Cell reinfusion is associated with the following hazards:

- Temporary shortness of breath due to the lodging of small particles in the blood vessels of the lungs may occur. This is a less likely side effect.
- If the bone marrow or stem cells that are to be used have been cryopreserved (frozen), a chemical called DMSO (dimethylsulfoxide) is used during the freezing process. This chemical is used to protect the cells from damage during freezing. DMSO produces an odor on the breath that lasts 1 to 2 days. In rare instances, severe allergic reaction may occur.
- There is also a chance of developing a blood transfusion reaction during the reinfusion of stem cells. The following less likely side effects may occur: chills, back pain, decreased blood pressure, chest pain, and increased rate of breathing. Wheezing, hives, rash, as well as difficulty breathing, and increased heart rate are considered unlikely. Rarely, temporary life support with artificial ventilation is required.
- Failure to engraft is a rare complication and it is possible that the recipient's own bone marrow might grow back. A second transplant may be needed.

13.8 Total Body Irradiation

During treatment the most common side effects include:

- Headache
- Nausea and vomiting
- Diarrhea
- Fatigue
- Skin reaction

Less common is swelling of the salivary glands, which can produce pain in front of the ear and in the jaw.

The following may occur after receiving TBI (and may be caused, in part, by any subsequent chemotherapy):

- Hair loss.
- Discomfort in the throat and mouth.
- Change in taste.
- Mouth sores.
- Nausea and vomiting.
- Diarrhea.
- Bone marrow suppression (low blood counts).

TBI can cause long-term side effects. They can occur months or years after your transplant.

- Sterility is an expected side effect. Sexual function and pleasure will not be affected. Please talk to your doctor or nurse about any concerns you have.
- About half of the patients will need thyroid supplements.

Other long-term side effects are rare but can occur. They include:

- Inflammation of the sac that surrounds the heart.
- Inflammation of the lungs.
- Cataracts.
- Second malignancies or new cancers

14 POTENTIAL BENEFITS TO SUBJECTS

The addition of post-transplant cyclophosphamide to the regimen used for GVHD prophylaxis may lead to a decrease in incidence of developing chronic GVHD.

15 DATA AND SPECIMEN BANKING

Not Applicable

16 MEASUREMENT OF EFFECT

The following criteria will be used for study evaluation:

16.1 Disease Response Criteria

Refer to **Appendix I** for post-transplant response criteria.

16.2 Study Endpoints

- The primary endpoint is extensive chronic GVHD in the first 365 days from Day 0 of the transplant.

16.3 Engraftment

Neutrophil engraftment is defined as the first day in which ANC is $\geq 0.5x10^9$/ L for three consecutive days. Patient may receive growth factor support. Platelet engraftment is the first day that platelets are ≥ 20 $x10^9$/ L for seven consecutive days without transfusion support.

Chimerism analysis will be performed at regular intervals. Lineage specific (separate lymphoid and myeloid) chimerism will be analyzed in blood at the following time points: day 30 (± 10 days), day 100 (± 20 days) and then at unscheduled intervals until full donor chimerism is attained.

Primary engraftment failure is defined as lack of evidence of neutrophil engraftment at day +28, confirmed by a bone marrow biopsy within 2 weeks showing <5% cellularity in the absence of persistent malignancy. Late graft failure will be defined as initial neutrophil engraftment by day +28 followed by a drop in ANC to <500 for more than three days, independent of myelosuppressive drugs, severe GVHD or infection and unresponsive to growth factor in the absence of malignancy.

Graft rejection is defined as graft failure with documentation of return of recipient hematopoiesis as determined by cytogenetic/chimerism analysis.

17 SAFETY EVALUATION

17.1 Adverse Events

Only grade 3 or greater adverse events will be captured.

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of ‘unrelated’, ‘unlikely’, ‘possible’, ‘probable’, or ‘definite’).

An AE 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, is not consistent with the risk information described in the general investigational plan in other study-related documents.

17.1.1.1 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

17.1.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF. However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

17.1.1.3 Abnormal Laboratory Values

Only clinically significant laboratory abnormalities that are Grade 3-5 and require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated blood potassium level of 7 mEq/L should be recorded as “hyperkalemia”.

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

17.1.1.4 Preexisting Medical Conditions (Baseline Conditions)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens to Grade 3-5 during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

17.2 Grading and Reporting Adverse Events

17.2.1.1 Grading and Relationship to Drug

The descriptions and grading scales found in the CTEP Version 4.0 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 4.0 of the CTCAE is identified and located at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 4.0.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the participant’s clinical state, other therapeutic interventions or concomitant drugs administered to the participant.

- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs.
- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions or concomitant drugs.
- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

17.3 Reporting Adverse Events:

Grade 3-5 AEs occurring between the start date of the conditioning regimen (Day -5) until 30 days after stem cell infusion will be reported.

Guidelines for Routine Adverse Event Reporting for Phase 1 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated	X	X	X	X
Unlikely	X	X	X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Guidelines for Routine Adverse Event Reporting for Phase 2 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

17.4 Serious Adverse Events

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in ANY of the following:

- Death.

- A life-threatening adverse event (experience). Any AE that places a participant or participants, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does NOT include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

17.4.1.1 Reporting Serious Adverse Events

All new SAEs occurring from the date of conditioning regimen (day -5) until 30 days after the stem cell infusion will be reported. The Roswell Park SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

SAEs occurring after the 30 days following stem cell infusion and until completion of the Day 100 follow-up period that the investigator determines to be possibly, probably or definitely related to the study intervention should be reported.

SAEs that are unexpected and possibly, probably or definitely related must be reported as an Unanticipated Problem. Please refer to **Section 17.5.1** for details on reporting Unanticipated Problems.

17.5 Unanticipated Problems

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
    - The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of participant.
    - Privacy or confidentiality of data.
    - The characteristics of the participant population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed **Serious** per **Section 17.4.**

17.5.1.1 Reporting Unanticipated Problems:

Unanticipated problem reporting will begin at the time of participant consent. The Reportable New Information (RNI) Form will be submitted to the CRS Quality Assurance (QA) Office within 1 business day of becoming aware of the Unanticipated Problem. After review, CRS QA Office will submit the RNI to the IRB.

When becoming aware of new information about an Unanticipated Problem, submit the updated information to CRS QA Office with an updated Reportable New Information Form. The site Investigator or designated research personnel will report all unanticipated problems to the IRB in accordance with their local institutional guidelines.

17.6 FDA Reporting

Not Applicable for this study.

18 DATA MANAGEMENT AND CONFIDENTIALITY

18.1 Data Collection

Full build studies are managed by Roswell Park CRS Data Management for analysis by Roswell Park Biostatisticians. All electronic case report form (eCRF) data are captured for these studies.

Data management activities are performed using a CTMS system that enables the collection, cleaning and viewing of clinical trial data. CRS data management designs the study-specific database and facilitates development by the Information Technology team. Once the database design is approved by the Investigator, Statistician, and Clinical Research Coordinator, the database is put into production and data entry can begin. Data can be entered and changed only by those with the rights to do so into the eCRFs.

18.2 Confidentiality

All information provided to the Investigator by Roswell Park including preclinical data, protocols, CRFs, and verbal and written information, will be kept strictly confidential and confined to the clinical personnel involved in conducting this study, and no disclosure shall be made except in accordance with any right of publication granted to the Investigator. This information may be related in confidence to the IRB/ERC or other committee functioning in a similar capacity. No report or information about the study will be provided to anyone not involved in the study without consent of Roswell Park except if required by law.

18.3 Maintenance of Study Documents

Essential documents will be retained per Roswell Park's policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with Roswell Park

18.4 Revisions to the Protocol

Roswell Park may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the IRB/ERC for written approval before implementation.

18.5 Termination of the Study

It is agreed that, for reasonable cause, either the Roswell Park Investigators or the Sponsor, may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, Roswell Park may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of participants enrolled in the study.

19 STATISTICAL PLAN

This trial is designed as an open label, non-randomized, single institution study. The primary objective of this study is determination of the cumulative incidence of chronic graft-vs.-host disease (in patients who do not progress before Day 100) at 1 year after allogeneic hematopoietic cell transplantation utilizing this novel conditioning/GVHD prophylaxis regimen. In addition to the sample estimate of this probability, an exact 95% confidence interval will also be provided.

The design of this trial consists of two components: 1) A safety run-in component, which will be broken out into two strata (≤50 years of age, > 50 years of age) and 2) A two-stage efficacy analysis of chronic GVHD rates combining subjects from both strata. The safety run-in component of the trial will precede the efficacy analysis.

19.1 Descriptive Analyses

Descriptive statistics such as frequencies and relative frequencies will be computed for all categorical variables. Numeric variables will be summarized using simple descriptive statistics such as the mean, standard deviation and range. A variety of graphical techniques will also be used to display data, ex. histograms, boxplots, scatterplots, etc.

19.2 Efficacy Analysis

PHASE 1 b SAFETY RUN-IN DESIGN

For both strata we will utilize a 3+3 dose escalation scheme in order to determine the safest dose from which to utilize in our efficacy analysis. This is slightly different than a pure phase I trial in that the study drug is already in use in the clinic. The goal is to better understand the maximum tolerated dose (MTD) within the age strata in a conservative fashion prior to proceeding on to the efficacy analysis.

For the age ≤50 years strata we will examine two dosing cohorts (A,B) following the administration of Fludarabine 40 mg/m2/day on Days -5 to -2. The two cohorts are Cohort A: 50 mg/m^2 Melphalan and Cohort B: 75 mg/m^2 Melphalan. For the age >50 strata we will examine three dosing cohorts (A, B, C) following the administration of Fludarabine 40 mg/m2/day on Days -5 to -2. The three cohorts are Cohort A: 25 mg/m^2 Melphalan, Cohort B: 50 mg/m^2 Melphalan and, Cohort C: 75 mg/m^2 Melphalan.

Overall, accrual within the age ≤50 years strata for this component of the trial ranges between 3-12 patients and the accrual within the age >50 years strata for this component of the trial ranges between 3-18 patients. It is anticipated that we will escalate to the highest dose per strata. However, if ≥2/3 DLTs are observed in Cohort A in either stratum that component of the trial relative to the given strata will be suspended and the dosing scheme will be re-evaluated.

Note: Due to accrual rate differences between the strata, stoppage for interim analysis of one stratum will not necessitate that the other stratum be closed to accrual.

PHASE II ANALYSIS OF EFFICACY

Using the dose per strata deemed to be the MTD from the safety run-in design we will carry forth a one-arm Simon two-stage design combining all eligible age groups. The primary endpoint is the development of extensive chronic GVHD (yes/no) at 1 year after transplantation. Let *p* represent the proportion of the evaluable population of interest who develop chronic GVHD (yes/no) prior to 1 year after transplantation. The evaluable population is defined as patients who meet eligibility requirements. Note that the subjects enrolled in the safety run-in analysis at the MTD will be included in the stage one portions of the respective

two-stage designs in the efficacy analysis presented below. In terms of the Simon two-stage design a true response of less than p_0 is considered acceptable and evidence of such will deem the treatment worthy of further study. The null and alternative hypotheses to be tested are H_0:$p = p_0$ versusH_0:$p < p_0$. The study design stops early for futility only and will proceed in two stages.

For the Phase II portion of the trial we set $p_0 = 0.75$, which leads to the following decision rules:

- Stage 1: If 6 or more of the first $n_1 = 8$ evaluable patients develop chronic GVHD, it will be concluded that the therapy is not promising, and the study will end. Otherwise, the study will progress to the second stage.
- Stage 2: We will accrue 13 additional evaluable patients. If 13 or less of the total of $n_1 + n_2 = 21$ evaluable patients do not develop GVHD, it will be concluded that p is less than p_0 and that the therapy is efficacious; otherwise, it will be concluded that the therapy is not promising.

The nominal significance level of this design is α = 0.10. The sample size calculation is based on testing the hypotheses concerning the proportion of the population with a response to the treatment. For the ≤50 years of age strata this two-stage design requires a potential total of $n_1 + n_2$=8+13 patients in order to achieve approximately 1-β = 0.80 power to detect differences of Δ =0.25 percentage points (p_0 versus$p_0 - \Delta$).

Secondary efficacy endpoints for each stratum will include the following:

- Clinical response, engraftment rate, progression free survival (PFS) at one year, and overall survival
- Cumulative incidence of relapse
- Treatment-related mortality rates
- Cumulative incidence of grade III-IV acute GVHD
- Correlative studies will include chimerism analysis by molecular analysis and evaluation of immune reconstitution

For the time-to-event endpoints we will utilize either straight Kaplan-Meier based estimators or extensions of nonparametric survival models to account for competing risks. Other endpoints will be analyzed in a descriptive fashion with means +/- standard deviations or frequency counts. In addition, we will examine in a post-hoc analysis potential chronic GVHD rates of response by HLA matching status.

20 PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF SUBJECTS

Data and Safety Monitoring Plan

The Principal Investigator (PI) will be responsible for continuous monitoring of the safety of the study.

Safety lead-in patients: Study data from the safety lead-in patients (Phase 1 3x3 design) will be reviewed at the scheduled Roswell Park Early Phase Clinical Trials Committee meetings and the minutes are forwarded to the IRB for review.

Once the MTD from the safety lead-in has been determined, additional participants will be accrued for the Phase II portion of the study. Once the Phase II study is initiated, annual review of the study data will be assumed by the Roswell Park Data and Safety Monitoring and Accrual Committee (DSMAC). The

Roswell Park DSMAC will assess the progress of the study, the safety data, and critical efficacy endpoints. The DSMAC will review the study annually and will make recommendations that include but not limited to; (a) continuation of the study, (b) modifications to the design, (c) suspension of, or (d) termination of the study.

Patient Outcome Rounds are held weekly on the transplant unit at which time active BMT inpatients and outpatients are reviewed, including:

- **Medications**: Chemotherapy for conditioning regimens; graft-versus-host disease prophylactic and therapeutic medications.
- **Adverse events** related to the hematopoietic stem cell infusion; reports are filed according to Roswell Park policy and procedure.
- **Regimen-related toxicity** based on Bearman toxicity grading[34] (**Appendix G**)
- **Graft-versus-Host Disease** based on standard grading and staging of both acute and chronic GVHD
- **Consent**: properly signed and dated transplant consent.
- **Patient Psychosocial Status:**
    - Compliance issues that could compromise patient safety.
    - At pretransplant, a conference is held by the BMT for all BMT patients for the purpose of describing the need for allogeneic patients to obtain lodging within a 30 mile radius of the hospital and to have a caregiver present at all times while the patient is an outpatient up to 100 days following transplant.
    - In addition, psychosocial evaluations are completed on all transplant patients by one of the BMT social worker prior to transplant, to identify any compliance issues.
- **Safety Monitoring** mandated by the BMT Standards of Care and common clinical practice. These include daily physical examinations, clinical laboratory testing, routine surveillance cultures, therapeutic drug level monitoring (i.e., Vancomycin, Tacrolimus, Tobramycin, Cyclosporine, Sirolimus, Mycophenolate), as an inpatient. They are found in the BMT SOPs.
- Patients who have been discharged from the hospital are monitored in the BMT Clinic until all transplant-related issues are resolved and they are returned to the care of their referring physicians.
- **Performance Status**: assignment of Karnofsky Performance Status Score

In addition to weekly monitoring, the BMT **Quality Assurance** plan requires quarterly reporting to the BMT Quality Assurance Committee, which in turn reports to the Roswell Park Quality Assurance Committee. Indicators for BMT patient safety monitoring include:

- Reportable serious adverse events
- Variances in the delivery of standard care that result in a change in practice
- Readmissions prior to day +100 post-transplant
- Deaths occurring prior to day +100 post-transplant
- Engraftment of neutrophils and platelets

BMT Patient Outcomes are reported to the Center for International Blood and Marrow Transplant Research (CIBMTR), and/or the National Marrow Donor Program (NMDP). Registry reports are reviewed internally prior to submission to the respective registry. These data are also entered into the Roswell Park BMT Database, from which patient outcomes are assessed and reviewed on an annual basis. The patients' medical records serve as original source documents for all reporting. Audits are conducted every 4 years by the CIBMTR.

21 VULNERABLE POPULATIONS

Not Applicable.

22 COMMUNITY-BASED PARTICIPATORY RESEARCH

Not Applicable.

23 SHARING OF RESULTS WITH SUBJECTS

Individual response data is shared with the participant as a part of their clinical care.

24 SETTING

Treatment will be conducted in the inpatient and outpatient setting per BMT standards of care at Roswell Park's BMT Center within Roswell Park Cancer Institute.

25 PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF SUBJECTS

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

26 RESOURCES AVAILABLE

Not Applicable

27 PRIOR APPROVALS

Not applicable

28 COMPENSATION FOR RESEARCH-RELATED INJURY

Please refer to the informed consent (Section 13) related to this study.

29 ECONOMIC BURDEN TO SUBJECTS

The participants will not be subject to any economic burden.

30 CONSENT PROCESS

This study will not be initiated until the protocol and informed consent document(s) have been reviewed and approved by a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Each participant (or legal guardian) shall read, understand, and sign an instrument of informed consent prior to performance of any study-specific procedure. It is the responsibility of the

investigator to ensure that the participant is made aware of the investigational nature of the treatment and that informed consent is given.

The Investigator is responsible for the retention of the participant log and participant records; although personal information may be reviewed by authorized persons, that information will be treated as strictly confidential and will not be made publicly available. The investigator is also responsible for obtaining participant authorization to access medical records and other applicable study specific information according to Health Insurance Portability and Accountability Act regulations (where applicable).

This study will be conducted in compliance with all applicable laws and regulations of the state and/or country and institution where the participant is treated. The clinical trial should be conducted in accordance with the ethical principles embodied in the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, consistent with good clinical practice and the applicable regulatory requirements and according to the guidelines in this protocol, including attached appendices.

The Roswell Park SOP: Informed Consent Process for Research (HRP-090) will be followed.

31 PROCESS TO DOCUMENT CONSENT IN WRITING

The Investigator (or IRB specified designee) is responsible for obtaining written consent from each participant in accordance with GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the participant according to applicable GCP guidelines, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional information that is applicable to the study must also be included. Additional national or institutionally mandated requirements for informed consent must also be adhered to. The participant should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator shall provide a copy of the signed consent form to the participant and the signed original shall be maintained in the Investigator File. A copy of the signed consent form must be filed in the participant file. At any stage, the participant may withdraw from the study and such a decision will not affect any further treatment options.

The Roswell Park "SOP: Written Documentation of Consent (HRP-091)" will be followed.

32 DRUGS OR DEVICES

32.1 Fludarabine Monophosphate

32.1.1.1 Availability

Fludarabine monophosphate (Fludara®), a purine nucleoside analog, is commercially available as a clear, sterile solution. Each 2 milliliter vial contains 50 milligrams of fludarabine phosphate, 50 milligrams of mannitol, water for injection, and sodium hydroxide to adjust pH to 6.8. Store at 2 to 8°C (36 to 46°F).

32.1.1.2 Storage and Stability

Fludarabine phosphate 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.

32.1.1.3 Preparation

Fludarabine phosphate vials containing 50 milligrams of fludarabine phosphate, 50 milligrams of mannitol, water for injection and sodium hydroxide to adjust the pH to 6.8. The product may be further diluted for intravenous administration in 100 milliliters or 125 milliliters of 5% Dextrose for Injection USP or 0.9% Sodium Chloride, USP.

32.1.1.4 Administration

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Fludarabine will be delivered as a piggy-bag via an ongoing IV line, over a period of 30 minutes.

32.2 Melphalan

32.2.1.1 Availability

Melphalan (Alkeran®), an alkylating agent, is commercially available as a powder for injection in 50 milligrams vials.

32.2.1.2 Storage and Stability

Intact vials should be stored between 20 to 25°C (68 to 77°F) and protected from light. Following reconstitution with sterile diluent, melphalan hydrochloride solution containing 5 milligrams of melphalan per milliliters is stable for up to 60 minutes at room temperature; this reconstituted solution should not be refrigerated since a precipitate may form at 5°C.

32.2.1.3 Preparation

Reconstitute by adding 10 milliliters of diluent provided by the manufacturer to a vial labeled as containing 50 milligrams of melphalan to provide a solution with a concentration of 5 milligrams/milliliters. The diluent should be added rapidly and the vial should be shaken vigorously until a clear solution is obtained. Reconstituted solutions of melphalan should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

Administration should occur within one hour of drug dissolution.

32.2.1.4 Administration

Administered IV, over 30 minutes.

32.3 Cyclophosphamide

32.3.1.1 Availability

Cyclophosphamide (Cytoxan®), an alkylating agent for injection, is commercially available as a powder for injection in 500 milligram, 1 gram, and 2 gram vials.

32.3.1.2 Storage and Stability

Intact vials should be stored at or below 25°C (77°F). Following reconstitution with sterile diluent, cyclophosphamide is stable for up to 24 hours at room temperature and for up to 36 hours if refrigerated.

32.3.1.3 Preparation

Reconstitute Cyclophosphamide using 0.9% Sodium Chloride Injection, USP or Sterile Water for Injection, USP, with 25 mL for a 500 mg vial, 50 mL for a 1 g vial, and 100 mL for a 2 g vial, for a total concentration of 20 mg/mL.

32.3.1.4 Administration

Administered IV, over 2 hours.

32.4 Sirolimus

32.4.1.1 Availability

Sirolimus is an agent that inhibits the activation of the mammalian Target of Rapamycin (mTOR). It is available for administration as an oral solution containing 1 mg/mL of sirolimus or as a white, triangular-shaped tablet containing 1 mg of sirolimus, or as a yellow triangular-shaped tablet containing 2 mg of sirolimus. Sirolimus is rapidly absorbed. The systemic availability of sirolimus is 14% after administration of the solution and 27% after administration of tablets. Sirolimus is extensively metabolized by the CYP3A4 isozyme in the intestinal wall and liver. The majority of sirolimus is ultimately excreted into the stool, with a small amount excreted in the urine. Due to the reduced intensity nature of the conditioning regimen, an oral regimen should be tolerated.

32.4.1.2 Storage and Stability

Sirolimus solution should be stored, protected from light, and refrigerated at 2°C to 8°C (36°F to 46°F). Once the bottle is opened, the contents should be used within one month. If necessary, the patient may store the bottles at room temperatures up to 25°C (77°F) for a short period of time (e.g., not more than 15 days for the bottles). A dose of sirolimus solution may be kept in an oral syringe at room temperature for up to 24 hours. Sirolimus tablets should be stored at 20°C to 25°C (USP Controlled Room Temperature) (68° to 77°F). Use cartons to protect blister cards and strips from light.

32.4.1.3 Preparation

For Oral Use

For sirolimus solution, a syringe and cap are provided for dosing and the product may be kept in the syringe for a maximum of 24 hours at room temperatures up to 25°C (77°F) or refrigerated at 2°C to 8°C (36°F to 46°F). The syringe should be discarded after one use. After dilution, the preparation should be used immediately.

Sirolimus solution provided in bottles may develop a slight haze when refrigerated. If such a haze occurs allow the product to stand at room temperature and shake gently until the haze disappears. The presence of this haze does not affect the quality of the product.

Sirolimus tablets should be dispensed in a tight, light-resistant container.

32.4.1.4 Administration

On Day +5, starting dose will be 0.5 mg orally daily. The sirolimus dose will be adjusted to target trough levels between 5-10 ng/mL (as per standard of care, and physician's discretion). Either sirolimus solution or tablets may be utilized, as per discretion of the treating physician. Co-administration with azole anti-

fungal therapy will be avoided whenever possible in order to mitigate the risk of drug-drug interactions (for example, if posaconazole is given between 6 – 8 AM then sirolimus will be given between 1 - 3 PM).

32.5 Tacrolimus

32.5.1.1 Availability

Tacrolimus (Prograf®), a macrolide compound with potent immunosuppressant properties, is available for oral administration as capsules, containing the equivalent of 0.5, 1, or 5 milligrams of anhydrous tacrolimus and compounded oral suspension containing the equivalent of 1 milligram/milliliter of anhydrous tacrolimus. For IV use, tacrolimus is available as a sterile solution in 1 milliliter ampules containing the equivalent of 5 milligrams of anhydrous tacrolimus per mL.

The oral absorption of tacrolimus is erratic and incomplete; absolute bio-availability is approximately 25%. Peak serum levels are seen 1 to 3 hours after an oral dose. Therapeutic trough blood concentrations have ranged from 5-15 nanograms/milliliter. 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.

32.5.1.2 Storage and Stability

Store tacrolimus capsules at controlled room temperature, 15-30°C (59-86°F). An extemporaneous suspension of tacrolimus with a final concentration of 1 milligram/milliliter was stable for 56 days when it was stored at 24-26°C in glass or plastic amber prescription bottles.

32.5.1.3 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 micrograms/milliliter. 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.

For Oral use

Tacrolimus 1 milligram/milliliter suspension is compounded using twenty-four 5 milligram capsules, 60 milliliters of simple syrup, and 60 milliliters of Oraplus ® suspension. Capsules are emptied into an 8 ounce amber bottle and dissolved in 8 milliliters of sterile water. Simple syrup and Oraplus® are added and shaken vigorously. Storage is at room temperature for 56 days.

32.5.1.4 Administration

Oral therapy should be started as soon as possible after transplantation at a dose of 0.015 milligrams/kilogram (ideal body weight or actual if less than ideal) every 12 hours. In patients unable to tolerate oral therapy, the recommended intravenous dose is one-third of the oral dose administered every 12 hours.

32.6 Mycophenolate

32.6.1.1 Availability

Mycophenolate (CellCept®), a 2-morpholinoethyl ester of mycophenolic acid (MPA), inosine monophosphate dehydrogenase (IMPDH) inhibitor, is available for oral administration as capsules containing 250 milligrams of mycophenolate mofetil, tablets containing 500 milligrams of mycophenolate mofetil, and as a powder for oral suspension, which when constituted contains 200 milligrams/milliliter mycophenolate mofetil.

Each vial of mycophenolate intravenous contains the equivalent of 500 milligrams mycophenolate mofetil as the hydrochloride salt. Reconstitution and dilution with 5% Dextrose Injection USP yields a solution of mycophenolate mofetil, 6 milligrams/milliliter.

32.6.1.2 Storage and Stability

The mycophenolate mofetil powder for reconstitution and reconstituted solution should be stored at 25°C (77°F); however, temperature excursions between 15°C to 30°C (59°F to 86°F) are permitted.

32.6.1.3 Preparation and Administration

Mycophenolate mofetil hydrochloride for injection (CellCept Intravenous) must be reconstituted and diluted to a concentration of 6 mg/mL using 5% Dextrose Injection USP. CellCept Intravenous is incompatible with other intravenous infusion solutions. Following reconstitution, CellCept Intravenous must be administered by slow intravenous infusion over a period of NO LESS THAN 2 HOURS by either peripheral or central vein.

CAUTION: CELLCEPT INTRAVENOUS SOLUTION SHOULD NEVER BE ADMINISTERED BY RAPID OR BOLUS INTRAVENOUS INJECTION

Refer to package insert for detailed instructions.

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34 APPENDICES/SUPPLEMENTS

Appendix A ELIGIBILITY VERIFICATION FORM: INCLUSION CRITERIA

Participant Name: _____

Medical Record No.: _____

Title: A Phase 1b/2 Trial of Fludarabine/Melphalan/Total Body Irradiation with Post Transplant Cyclophosphamide as Graft versus Host Disease Prophylaxis in Matched-Related and Matched-Unrelated Allogeneic Hematopoietic Cell Transplantation

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. The patient must have a diagnosis of one of the following (one must be yes): • AML • ALL • CLL • CML (chronic phase intolerant or unresponsive to tyrosine kinase inhibitors, history of accelerated phase or history of blast crisis) • MDS • NHL • HL (received and failed frontline therapy or failed autologous transplantation or inability to collect enough PBSC for auto-HCT) • MM • Severe aplastic anemia	
☐	☐	☐	2. Histocompatible donor identified • Related donor 5/6 or better (A, B, DRB1) • Unrelated donor 7/8 or better (A, B, C and DRB1)	
☐	☐	☐	3. Age ≥ 18 years.	
☐	☐	☐	4. The following are eligible for study inclusion (refer to **Appendix H**): • AML may be in morphologic CR or CRi (MRD positive is allowed) • ALL may be in morphologic CR or CRi (MRD positive is allowed) • CLL may be in morphologic CR or CRi (MRD positive is allowed) • CML must be in chronic phase (MRD positive is allowed) • MDS- Patients with MDS only require ≤5% myeloblasts on bone marrow evaluation. There is no requirement for platelet or neutrophil recovery. • NHL must be in CR • HL must be in CR • MM may be in VGPR • SAA do not have disease response requirements; however, if the patient has a mismatched donor, the patient must have had prior therapy with ATG.	
☐	☐	☐	5. Have a Karnofsky performance status score of ≥ 50 (refer to **Appendix C**).	
☐	☐	☐	6. Have the following clinical laboratory values: • Diffusing Capacity of the Lung for Carbon Monoxide (DLCO) ≥ 40% predicted, corrected for hemoglobin and/or alveolar ventilation. • Cardiac: left ventricular ejection fraction ≥ 40%.	

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for participant enrollment.**	**Date**
			• Bilirubin, liver alkaline phosphatase, SGOT or SGPT ≤ 3 x upper limit of normal. • Calculated creatinine clearance ≥ 40 cc/min by the modified Cockroft-Gault formula (refer to **Appendix D**)	
☐	☐	☐	7. Patient must be cleared pre-transplant by Radiation Oncology to be able to receive 400cGy.	
☐	☐	☐	8. Participants of child-bearing potential must agree to use adequate contraceptive methods (e.g., hormonal or barrier method of birth control; abstinence) prior to study entry. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.	
☐	☐	☐	9. Patients who have failed a prior autologous or allogeneic transplant are eligible. However, at least 6 months must have elapsed between the start of this reduced intensity conditioning regimen and the last transplant if patient had a prior autologous or allogeneic BMT.	
☐	☐	☐	10. At least 2 weeks since prior radiation treatment and/or surgery. Appropriate washout of prior chemotherapy per BMT standard of care (see Appendix K). If medication is not on the list, go by physician discretion.	
☐	☐	☐	11. Participant must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.	

Investigator Signature: _____ **Date:** _____

Printed Name of Investigator: _____

Appendix B ELIGIBILITY VERIFICATION FORM: EXCLUSION CRITERIA

Participant Name: _____

Medical Record No.: _____

Title: A Phase 1b/2Trial of Fludarabine/Melphalan/Total Body Irradiation with Post Transplant Cyclophosphamide as Graft versus Host Disease Prophylaxis in Matched-Related and Matched-Unrelated Allogeneic Hematopoietic Cell Transplantation

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. Moderate to severe myelofibrosis within 60 days prior to transplant.	
☐	☐	☐	2. Presence of HLA antibodies to the donor within 60 days prior to transplant.	
☐	☐	☐	3. Uncontrolled CNS disease (for hematologic malignancies).	
☐	☐	☐	4. Patients who in the opinion of the treating physician are unlikely to comply with the restrictions of allogeneic stem cell transplantation based on formal psychosocial screening. (i.e., serious, uncontrolled psychiatric illness/social situations that would limit compliance with study requirements)	
☐	☐	☐	5. Uncontrolled diabetes mellitus, cardiovascular disease, active serious infection or other condition which, in the opinion of treating physician, would make this protocol unreasonably hazardous for the patient	
☐	☐	☐	6. Known HIV positive	
☐	☐	☐	7. Pregnant or nursing female participants	
☐	☐	☐	8. Unwilling or unable to follow protocol requirements	
☐	☐	☐	9. Any condition which in the Investigator's opinion deems the participant an unsuitable candidate to receive study intervention.	

Participant meets all entry criteria: ☐ **Yes** ☐ **No**
If "NO", do not enroll participant in study.

Investigator Signature: _____ **Date:** _____

Printed Name of Investigator: _____

Appendix C Karnofsky Performance Scale

Karnofsky Performance Scale *	
Percent	**Description**
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.
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 needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization is indicated although death not imminent.
20	Hospitalization necessary, very sick, active supportive treatment necessary.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

* Karnofsky, D.A., and Burchenal, J.H. (1949). The Clinical Evaluation of Chemotherapeutic Agents in Cancer. In Evaluation of Chemotherapeutic Agents: Symposium Held at the New York Academy of Medicine, C.M. MacLeod, ed. (New York, Columbia University Press), pp. 191-205.

Appendix D Calculation for Creatinine Clearance

Cockroft-Gault Equation*

Men: CrCl = [(140-YR) x IBW] / (SCr x 72)

Women: CrCl = 0.85 x [(140-YR) x IBW] / (SCr x 72)

Where:

CrCl is creatine clearance (mL/min)

IBW is ideal body weight (kg)

SCr is serum creatinine (mg/dL)

YR is age (years)

*Cockcroft D, W, Gault M, H, Prediction of Creatinine Clearance from Serum Creatinine. Nephron. 1976; 16 (1):31-41).

Appendix E Clinical Grading of Chronic GVHD

Chronic Graft Versus Host Disease*

Involvement	Description
Limited	Localized skin involvement and/or hepatic dysfunction due to chronic GVHD.
Extensive	One or more of the following: 1) Generalized skin involvement; or 2) Liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis, or 3) Involvement of the eye, or 4) Involvement of minor salivary glands or oral mucosa, or 5) Involvement of any other target organ (lung, GI, GU, musculoskeletal, serositis, etc…).
Severity	**Description**
Mild	Signs and symptoms of chronic GVHD do not interfere substantially with function and do not progress once appropriately treated with local therapy or standard systemic therapy (i.e., corticosteroids and/or CSA/Tacro).
Moderate	Signs and symptoms of chronic GVHD interfere somewhat with function despite appropriate therapy or are progressive through first line systemic therapy (i.e., corticosteroids and/or CSA/Tacro).
Severe	Signs and symptoms of chronic GVHD limit function substantially despite appropriate therapy or are progressive through second line therapy.
GVHD type	**Description**
De novo	No prior acute GVHD.
Progressive	Acute GVHD that progressed directly to chronic GVHD.
Interrupted	Acute GVHD that resolved symptoms (may still be on tapering treatment), then redeveloped later as chronic GVHD.

*Modified from CIBMTR Form 2100, revision 4.[35]

Appendix F Criteria for Acute Graft-vs-Host Disease

Clinical staging of acute graft-vs.-host disease according to organ involvement

STAGE	SKIN	LIVER	LOWER INTESTINAL TRACT	UPPER INTESTINAL TRACT
0	No rash	Bilirubin < 2.0 mg/dL < 34 µmol/L	Diarrhea < 500 mL/day Peds: < 10 ml/kg/day	No persistent anorexia, nausea, or vomiting
+	Maculopapular rash < 25% of body surface	Bilirubin 2.0-3.0 mg/dL 34-52 µmol/L	Diarrhea 500-1000 mL/day Peds: 10-19.9 ml/kg/day	Persistent anorexia, nausea, or vomiting
++	Maculopapular rash 25-50% of body surface	Bilirubin 3.1-6.0 mg/dL 53-103 µmol/L	Diarrhea > 1000-1500 mL/day Peds: 20-30 ml/kg/day	
+++	> 50% body surface	Bilirubin 6.1-15.0 mg/dL 104-256 µmol/L	Diarrhea > 1500 mL/day Peds: > 30 ml/kg/day	
++++	Generalized erythroderma with bullous formation and/or desquamation	Bilirubin > 15.0 mg/dL > 256 µmol/L	Severe abdominal pain with or without ileus	

(https://www.cibmtr.org/DataManagement/DataCollectionForms/Documents/2450/Rev4.0/2450R4.0.pdf)

Glucksberg et al. Transplantation 1974;18:295-304
Thomas et al. New Engl J Med 1975;292:895-902
Przepiorka et al. Bone Marrow Transplant 1995;15:825-828

Clinical (Glucksberg) grading of severity of acute graft-vs-host disease

GRADE	DEGREE OF ORGAN INVOLVEMENT
I	1 to 2 skin rash; no gut involvement; no liver involvement; no decrease in clinical performance
II	1 to 3 skin rash; 1 gut involvement or 1 liver involvement (or both); mild decrease in clinical performance
III	2 to 3 skin rash; 2 to 3 gut involvement or 2 to 4 liver involvement (or both) marked decrease in clinical performance
IV	Similar to Grade II with 2 to 4 organ involvement and extreme decrease in clinical performance

Source: Thomas et al, N Engl. J Med. 1975; 292, 832

IBMTR Severity Index
A – Stage 1 skin involvement, no liver or gut involvement
B – Stage 2 skin involvement; Stage 1 to 2 gut or liver involvement
C – Stage 3 skin, liver, or gut involvement
D – Stage 4 skin, liver or gut involvement

Source: Rowlings et al., Br J Haematol 1997; 97:855

Appendix G Bearman Scale

BEARMAN TOXICITY GRADING SCALES

Regimen-Related Toxicity According to Organ System

	Grade I	Grade II	Grade III
Cardiac toxicity	Mild EKG abnormality, not requiring medical intervention; or noted heart enlargement on chest x-ray with no clinical symptoms	Moderate EKG abnormalities requiring and responding to medical intervention; or requiring continuous monitoring without treatment; or congestive heart failure responsive to digitalis or diuretics	Severe EKG abnormalities with no or only partial response to medical intervention; or heart failure with no or only minor response to medical intervention; or decrease in voltage by more than 50%
Bladder toxicity	Macroscopic hematuria after 2 days from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection	Macroscopic hematuria after 7 days from last chemotherapy dose not caused by infection; or hematuria after 2 days with subjective symptoms of cystitis not caused by infection	Hemorrhagic cystitis with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomy or other surgical procedure
Renal toxicity	Increase in creatinine up to twice the baseline value (usually the last recorded before start of conditioning)	Increase in creatinine above twice baseline but not requiring dialysis	Requirement of dialysis
Pulmonary toxicity	Dyspnea without chest x-ray changes not caused by infection or congestive heart failure; or chest x-ray showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure	Chest x-ray with extensive localized infiltrate or moderate interstitial changes combined with dyspnea and not caused by infection or CHF; or decrease of PO_2 (> 10% from baseline) but not requiring mechanical ventilation or > 50% O_2 on mask and not caused by infection or CHF	Interstitial changes requiring mechanical ventilatory support or > 50% oxygen on mask and not caused by infection or CHF
Hepatic toxicity	Mild hepatic dysfunction with bilirubin ≥ 2.0 mg/dL and ≤ 6.0 mg/dL or weight gain > 2.5% and < 5% from baseline, of non-cardiac origin; or SGOT increase more than 2- fold but less than 5-fold from lowest preconditioning	Moderate hepatic dysfunction with bilirubin > 6.0 mg/dL and < 20 mg/dL; or SGOT increase > 5-fold from preconditioning; or clinical ascites or image documented ascites > 100 mL; or weight gain > 5% from baseline of non-cardiac origin	Severe hepatic dysfunction with bilirubin > 20 mg/dL; or hepatic encephalopathy; or ascites compromising respiratory function
CNS toxicity	Somnolence but the patient is easily aroused and oriented after arousal	Somnolence with confusion after arousal; or other new objective CNS symptoms with no loss of consciousness not more easily explained by other medication, bleeding or CNS infection	Seizures or coma not explained (documented) by other medication, CNS infection, or bleeding

	Grade I	Grade II	Grade III
Stomatitis	Pain and/or ulceration not requiring a continuous IV narcotic drug	Pain and/or ulceration requiring a continuous IV narcotic drug (morphine drip)	Severe ulceration and/or mucositis requiring preventive intubation; or resulting in documented aspiration pneumonia with or without intubation
GI toxicity	Watery stools > 500 mL but < 2,000 mL every day not related to infection	Watery stools > 2,000 mL every day not related to infection; or macroscopic hemorrhagic stools with no effect on cardiovascular status not caused by infection; or subileus not related to infection	Ileus requiring nasogastric suction and/or surgery and not related to infection; or hemorrhagic enterocolitis affecting cardiovascular status and requiring

Note: Grade IV regimen-related toxicity is defined as fatal toxicity
Abbreviation: IV = intravenous

Appendix H Disease Status Requirements to Determine Eligibility

Acute Myeloid Leukemia

Complete Remission (CR)

Hematologic complete remission is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- Evidence of trilineage hematopoiesis in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)
- Neutrophils ≥ 1,000/μL
- Platelets ≥ 100,000/μL
- Transfusion independent

Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- Evidence of trilineage hematopoiesis in the bone marrow
- Normal maturation of all cellular components in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)

Acute Lymphoblastic Leukemia

Complete Remission (CR)

Hematologic complete remission is defined as meeting **all** of the following response criteria:

- < 5% blasts in the bone marrow
- Evidence of trilineage hematopoiesis in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)
- ANC (absolute neutrophil count) ≥ 1,000/μL
- Platelets ≥ 100,000/μL

Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- Evidence of trilineage hematopoiesis in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)

Multiple Myeloma* / Plasma Cell Disorder

Stringent Complete Remission (sCR)

Follows criteria for CR as defined below, **plus all of the following**:

- Normal free light chain ratio
- Absence of clonal cells in the bone marrow by immunohistochemistry, immunofluorescence, flow cytometry (MRD testing)

Complete Remission (CR)

A treatment response where all of the following criteria are met:

- Negative immunoelectrophoresis (immunofixation) on serum and urine samples
- Disappearance of any soft tissue plasmacytomas
- < 5% plasma cells in the bone marrow (confirmation with repeat bone marrow biopsy not needed)
- No known evidence of new or progressive bone lesions on radiographic studies.

For recipients with **non-secretory myeloma**, all of the following criteria must be met:

- Disappearance of all soft tissue plasmacytomas
- < 5% plasma cells in the bone marrow (confirmation with repeat bone marrow biopsy not needed)
- No known evidence of new or progressive bone lesions on radiographic studies.

Very Good Partial Response (VGPR)

- Absence of soft tissue plasmacytoma

One or more of the following must be present:

- ≥ 90% reduction in serum M-protein and urine M-protein level < 100 mg/24 hours

OR

- Serum and urine M-protein detectable by immunofixation but not on electrophoresis

  At diagnosis, if:

  - M protein is < 1 g/dL, you must use serum free light chain or urine measurement)
  - IgA monoclonal protein, you must use an estimate by total IgA or HevyLite®
  - Urine M-protein is < 200 mg/24 hours, use serum free light chains or if serum free light chains are not elevated, then use nonsecretory myeloma criteria

*Modified based on the International Myeloma Working Group (IMWG) Uniform Response Criteria for Multiple Myeloma[36].

Chronic Myeloid Leukemia

- Chronic Phase (<10% blasts in the blood and marrow)
- If present, splenomegaly must be < 20 cm in the widest dimension on radiographic imaging

Hodgkin and Non-Hodgkin Lymphoma

Complete Remission (CR)

- Complete disappearance of all known disease. For typically PET-avid lymphoma, a post-treatment residual mass of any size is permitted as long as it is PET negative. For variably, PET-avid lymphoma, all lymph nodes and nodal masses must have regressed as measured by CT to < 1.5 cm (for nodes > 1.5 cm before therapy) or < 1 cm (for nodes 1.1 cm to 1.5 cm before therapy)

CRu

- All CR criteria are met except, there may be CT scan abnormalities of uncertain significance. PET scan must be negative.

Chronic Lymphocytic Leukemia

Complete Response (CR)

All of the following:

- Any lymphadenopathy must be ≤ 1.5 cm in the widest dimension
- If present, splenomegaly must be < 20 cm in the widest dimension on radiographic imaging
- Neutrophils ≥ 1.5×10^9/L
- Platelets > 100×10^9/L
- Hemoglobin > 11 g/dL
- Lymphocytes < 4×10^9/L
- Bone marrow < 30% lymphocytes
- Absence of constitutional symptoms (including weight loss, fever, and night sweats)

CRi

All of the following:

- No evidence of lymphadenopathy
- No organomegaly
- Absence of constitutional symptoms (including weight loss, fever, and night sweats)

Appendix I Post-Transplant Response Criteria[37]

Acute Myeloid Leukemia

Continued Complete Remission (CCR)

- For patients transplanted in CR, the best response post BMT is CCR as long as there is no documented disease progression

Complete Remission (CR)

Hematologic complete remission is defined as meeting all of the following response criteria for at least four weeks.

- < 5% blasts in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)

Relapse (REL)

Relapse is defined as the recurrence of disease after CR, meeting one or more of the following criteria:

- ≥ 5% blasts in the marrow or peripheral blood
- New or recurrence of extramedullary disease

Acute Lymphoblastic Leukemia

Continued Complete Remission (CCR)

- For patients transplanted in CR, the best response post BMT is CCR as long as there is no documented disease progression

Complete Remission (CR)

Hematologic complete remission is defined as meeting all of the following response criteria for at least four weeks.

- < 5% blasts in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)

Relapse (REL)

Relapse is defined as the recurrence of disease after CR, meeting one or more of the following criteria:

- ≥ 5% blasts in the marrow or peripheral blood
- New or recurrence of extramedullary disease

Myelodysplastic Syndrome (MDS)

Continued Complete Remission (CCR)

- For patients transplanted in CR, the best response post BMT is CCR as long as there is no documented disease progression

Complete Remission (CR)

Bone marrow evaluation:

- < 5% myeloblasts with normal maturation of all cell lines

Peripheral blood evaluation:

- Hemoglobin ≥ 11 g/dL un-transfused within 7 days without erythropoietic support
- ANC ≥ 1000/mm^3 without myeloid growth factor support
- Platelets ≥ 100,000/mm^3 without thrombopoietic support
- 0% blasts in blood

Hematologic Improvement (HI)

- <5% blasts in the bone marrow

Hematologic improvement – erythropoietic (HI-E)

- Hemoglobin increase of ≥ 1.5 g/dL un-transfused for 7 days

Hematologic improvement – platelets (HI-P)

- For pre-treatment platelet count of > 20 ×10^9, platelet absolute increase of ≥ 30 ×10^9
- For pre-treatment platelet count of < 20 ×10^9, platelet absolute increase of ≥ 20 ×10^9 and ≥ 100% increase from pre-treatment level

Hematologic improvement – neutrophils (HI-N)

- Neutrophil count increase of ≥ 100% from pre-transplant level and an absolute increase of ≥ 500/mm^3

No Response (NR)/Stable Disease (SD)

- Does not meet the criteria for at least HI; but no evidence of disease progression to AML.

Relapse/Progression from Complete Remission (Rel from CR or HI)

Requires at least one of the following:

- Bone marrow blasts >5% and clonal
- At least a 50% decrease in platelets or granulocytes that is related to the malignant clonal population of cells (not due to ABO incompatibility, medication, angiopathy, or other nonmalignant causes)
- The hemoglobin level must decrease by at least 1.5 g/dL from pre-transplant level.

Progression to AML

- ≥ 20% blasts in the blood or bone marrow

Multiple Myeloma / Plasma Cell Disorder

Continued Complete Remission (CCR)

- For patients transplanted in CR, the best response post BMT is CCR as long as there is no documented disease progression

Stringent Complete Remission (sCR)

Follows criteria for CR as defined below, plus all of the following:

- Normal free light chain ratio
- Absence of clonal cells in the bone marrow by immunohistochemistry, immunofluorescence, flow cytometry (MRD testing)

Complete Remission (CR)

- A treatment response where all of the following criteria are met:
- Negative immunoelectrophoresis (immunofixation) on serum and urine samples
- Disappearance of any soft tissue plasmacytomas
- < 5% plasma cells in the bone marrow (confirmation with repeat bone marrow biopsy not needed)
- No known evidence of new or progressive bone lesions on radiographic studies.

For recipients with non-secretory myeloma, all of the following criteria must be met:

- Disappearance of all soft tissue plasmacytomas
- < 5% plasma cells in the bone marrow (confirmation with repeat bone marrow biopsy not needed)
- No known evidence of new or progressive bone lesions on radiographic studies.

Very Good Partial Response (VGPR)

- Absence of soft tissue plasmacytoma

One or more of the following must be present:

- ≥ 90% reduction in serum M-protein and urine M-protein level < 100 mg/24 hours

OR

- Serum and urine M-protein detectable by immunofixation but not on electrophoresis

At diagnosis, if:

- M protein is < 1 g/dL, you must use serum free light chain or urine measurement)
- IgA monoclonal protein, you must use an estimate by total IgA or HevyLite®
- Urine M-protein is < 200 mg/24 hours, use serum free light chains or if serum free light chains are not elevated, then use nonsecretory myeloma criteria

Partial Response (PR)

Both of the following must be present:

- ≥ 50% reduction in serum M-protein
- Reduction in 24-hour urinary M-protein by ≥ 90% or to < 200 mg/24 hours.

At diagnosis, if:

- M protein is < 1 g/dL, you must use serum free light chain or urine measurement)
- IgA monoclonal protein, you must use an estimate by total IgA or HevyLite®
- Urine M-protein is < 200 mg/24 hours, use serum free light chains or if serum free light chains are not elevated, then use nonsecretory myeloma criteria

In addition to the above-listed criteria, if soft tissue plasmacytomas were present at baseline, a ≥ 50% reduction in their size is also required.

Stable Disease (SD)

- Does not meet the criteria for CR, VGPR, PR, or PD.

Progressive Disease (PD)

Requires **one or more** of the following:

- Increase of ≥ 25% from the lowest post-transplant response value achieved in:
- Serum M-component with an absolute increase ≥ 0.5 g/dL
- Urine M-component with an absolute increase ≥ 200 mg/24 hours;
- For recipients without measurable serum and urine M-protein levels, the difference between involved and uninvolved free light chain levels with an absolute increase > 10 mg/dL;
- Bone marrow plasma cell percentage with absolute percentage increase of ≥ 10%;
- Definite development of new bone lesions or soft tissue plasmacytomas, or definite increase in the size of any existing bone lesions or soft tissue plasmacytomas;

Chronic Myeloid Leukemia

Continued Complete Remission (CCR)

- For patients transplanted in CR, the best response post BMT is CCR as long as there is no documented disease progression

Complete Hematologic Remission (CR)

A treatment response where all of the following criteria are met:

- White blood count is < 10×10^9/L, without immature granulocytes and with < 5% basophils
- Platelet count < 450×10^9/L
- Non-palpable spleen

Complete Molecular Remission[38]

- 0% BCR/ABL transcripts detected in peripheral blood or bone marrow

Major Molecular Remission

- >0-0.1% BCR/ABL transcripts detected in peripheral blood or bone marrow

Complete Cytogenetic Response

- 0% Ph+ cells detected in bone marrow

Partial Cytogenetic Response

- >0-35% Ph+ cells in bone marrow

Minor Cytogenetic Response

- >35-65% Ph+ cells in bone marrow

Minimal Cytogenetic Response

- >65-95% Ph+ cells in bone marrow

No Cytogenetic Response

- >95% Ph+ cells in bone marrow

Chronic Phase

- Characterized by relatively few blasts (<10%) present in the blood and bone marrow. Symptoms are often not present. The chronic phase may last several months to years, depending on the recipient and the treatment they receive.

Accelerated Phase

One or more of the following must be present:

- 10%-19% blasts in blood or marrow
- ≥ 20% basophils in peripheral blood
- Clonal marrow cytogenetic abnormalities in addition to the single Philadelphia chromosome (clonal evolution)
- Increasing spleen size, unresponsive to therapy
- Increasing WBC, unresponsive to therapy
- Thrombocytopenia (platelets < 100,000), unrelated to therapy
- Thrombocytosis (platelets > 1,000,000), unresponsive to therapy

Blast Phase

Characterized by having ≥ 20% blasts (formerly ≥ 30%) in the peripheral blood or bone marrow. Having extramedullary blastic infiltrates (i.e., myeloid sarcoma, granulocytic sarcoma, or chloroma) also qualifies as blast phase. The red cell, platelet, and neutrophil counts may decrease and episodes of infection and bleeding may result. Symptoms such as fatigue, shortness of breath, abdominal pain, bone pain, and spleen enlargement may occur.

Relapse

For reporting purposes, relapse is defined as recurrence of disease after complete hematologic remission. In general, relapse should be confirmed by a clinical / hematologic assessment (e.g., pathology, CBC, or clinical exam).

Progression

For reporting purposes, progression is defined as any of the following changes in disease status:

- Advancement from chronic phase to accelerated phase.
- Advancement from chronic phase to blast phase.
- Advancement from accelerated phase to blast phase.

Hodgkin and Non-Hodgkin Lymphoma

Continued Complete Remission (CCR)

- For patients transplanted in CR, the best response post BMT is CCR as long as there is no documented disease progression

Complete Remission (CR)

Complete disappearance of all known disease. For typically PET-avid lymphoma, a post-treatment residual mass of any size is permitted as long as it is PET negative. For variably PET-avid lymphoma, all lymph nodes and nodal masses must have regressed as measured by CT to ≤ 1.5 cm (for nodes > 1.5 cm before therapy) in the longest diameter or ≤ 1 cm (for nodes 1.1 cm to 1.5 cm before therapy).

Bone Marrow (optional as part of work-up unless it was positive for disease immediately prior to transplant): If bone marrow was positive any time prior to transplant, it must be repeated and negative to confirm CR.

Partial Remission (PR)

- ≥ 50% reductions in the greatest diameter of up to six of the largest dominant nodes or nodal masses, extranodal sites and no new sites of lymphoma

Stable Disease

- Does not meet criteria for CR, PR, or PD

Progressive Disease

- Any new lesion and/or > 50% increase in the least diameter of previously involved sites
- New or recurrent involvement of bone marrow
- New or recurrent extranodal disease

Chronic Lymphocytic Leukemia

Continued Complete Remission (CCR)

- For patients transplanted in CR, the best response post BMT is CCR as long as there is no documented disease progression

Complete Response (CR)

All of the following:

- Any lymphadenopathy must be ≤ 1.5 cm in the widest dimension
- If present, splenomegaly must be < 20 cm in the widest dimension on radiographic imaging

- Neutrophils ≥ 1.5 × 10^9/L
- Platelets > 100 × 10^9/L
- Hemoglobin > 11 g/dL
- Lymphocytes < 4 × 10^9/L
- Bone marrow < 30% lymphocytes
- Absence of constitutional symptoms (including weight loss, fever, and night sweats)

Partial Response (PR)

- ≥ 50% decrease in peripheral blood lymphocyte count from pretreatment value
- ≥ 50% reduction in lymphadenopathy if present pretreatment
- ≥ 50% reduction in liver and/or spleen size if enlarged pretreatment

One or more of the following:

- Neutrophils ≥ 1.5 ×109/L or 50% improvement over baseline
- Platelets > 100 ×109/L or 50% improvement over baseline
- Hemoglobin > 11 g/dL or 50% improvement over baseline

Stable Disease (SD)

Not meeting the definition of complete response, partial response, or progressive disease.

Progressive Disease (PROG)/Relapse

One or more of the following:

- ≥ 50% increase in the sum of the products of ≥ 2 lymph nodes (≥ 1 lymph node must be ≥ 2 cm) or new nodes
- ≥ 50% increase in liver or spleen size, or new hepatomegaly or splenomegaly
- ≥ 50% increase in absolute lymphocyte count to ≥ 5 ×109/L
- Transformation to a more aggressive histology

Aplastic Anemia

Complete Response:

- At least 20% cellularity in the bone marrow with neutrophil and platelet engraftment (ANC >500 and Platelets <20)

No Response:

- Does not meet definition of CR

Appendix J Schedule of Procedures and Observations

Tests and Observations	Prior to Study[1]	Day 30 (± 10 days)	Day 100 (± 20 days)	1 Year (± 30 days)	Annual Survival Follow up[7]
History and Physical Exam	X				
Fulfill Criteria for Standard BMT workup per SOP	X				
HLA Antibodies Class I/II	X				
Physical Exam and Updated History		X	X	X	X
Height/Weight/Body Surface Area	X				
Performance Status (KPS)	X			X	
CBC w/diff[4]	X	X	X	X	
CMP[4]	X	X	X	X	
MUGA or ECHO	X				
Splenomegaly Assessment	X[6]				
PFT	X				
Radiation Oncology Clearance	X				
Chimerism Peripheral Blood	X	X	X	X	
Chimerism Bone Marrow		X	X	X	
Disease Assessment per SOP	X	X	X	X	X
Bone Marrow Biopsy	X[5]	X	X	X	
Immune Reconstitution/Flow[3]	X	X	X	X	
Adverse Events[2]		X	X		
Capture GVHD medication		X	X	X	X
GVHD Assessment		X	X	X	X
Survival Status		X	X	X	X

1 Screening work up must be completed within 60 days of pre admit visit. Exceptions per PI discretion.

2 Grade 3-5 adverse events are collected until Day +30, Serious Adverse Events that are considered to be possibly, probably, or definitely related to study intervention are collected until Day +100. SAEs that are ongoing at 1 year follow up will be considered ongoing and will not continue to be followed past this timepoint.

3 BMT SOC flow panel (peripheral blood) should be collected for Immune Reconstitution.

4 CBC and CMP should be completed weekly after hospital discharge until Day 100.

5 Only patients with prior bone marrow involvement are required to have a bone marrow biopsy at baseline.

6 Required for CML patients per SOC.

7 Annual follow up will be completed per treating physician discretion and TCT clinic standard of care.

Appendix K Chemotherapy Discontinuation Prior to Bone Marrow Transplant

Alkylating Agents:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Bendamustine	Treanda	Alkylating Agent	3 hours	Hepatic/ Renal + Fecal	7 Days
Carboplatin	Paraplatin	Alkylating Agent	1-6 hours	Renal	7 Days
Carmustine	BNCU	Alkylating Agent	22 minutes	Hepatic/ Renal	7 Days
Chlorambucil	Leukeran	Alkylating Agent	1.5–2 hours	Hepatic/ Renal	7 Days
Cisplatin	Platinol	Alkylating Agent	16-53 hours	Renal	7 Days
Cyclophosphamide	Cytoxan	Alkylating Agent	4-16 hours	Hepatic (CYP450)/ Renal	7 Days
Ifosfamide	Ifex	Alkylating Agent	7-15 hours	Hepatic (CYP3A4/5)/ Renal	7 Days
Melphalan	Alkeran, Evomela	Alkylating Agent	0.5–2.5 hours	Blood/ Fecal + Renal	7 Days
Procarbazine	Matulane	Alkylating Agent	~10 minutes	Hepatic + Renal/ Renal + Fecal	7 Days

Anthracyclines:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Daunorubicin	Cerubidine	Anthracycline	23-40 hours	Hepatic/ Biliary + Renal	7 Days
Doxorubicin	Adriamycin	Anthracycline	20-48 hours	Hepatic (CYP3a4/2D6)/ Fecal + Biliary + Renal	7 Days
Idarubicin	Idamycin	Anthracycline	~45 hours	Hepatic/ Renal + Biliary	7 Days

Antimetabolites:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Cladribine	Leustatin	Antimetabolite	6-7 hours	Renal	7 Days
Cytarabine	Cytosar	Antimetabolite	1-3 hours	Hepatic/ Renal	7 Days
Fludarabine	Fludara	Antimetabolite	10-20 hours	Hepatic/ Renal	7 Days
Hydroxyurea	Hydrea	Antimetabolite	2 – 4.5 hours	Hepatic/ Renal	1 Day
Mercaptopurine	Purinethol	Antimetabolite	~2 hours	Hepatic/ Renal	7 Days
Methotrexate	Trexall	Antimetabolite	8–15 hours	Hepatic/ Renal	7 Days

BCL-2 Inhibitor:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Venetoclax	Venclexta	BCL-2 Inhibitor	~26 hours	Hepatic (CYP3A4)/ Fecal + Renal	7 Days

Calcineurin/ mTOR Kinase Inhibitors:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT

Cyclosporine	Gengraf, Neoral	Calcineurin Inhibitor	10-27 hours	Hepatic (CYP3A4)/ Biliary	7 Days + Levels < 30
Sirolimus	Rapamune	mTOR Kinase inhibitor	~62 hours	Hepatic (CYP3A4)/ Fecal	7 days + Level < 2
Tacrolimus	Prograf	Calcineurin Inhibitor	8.7-11.3 hours	Hepatic (CYP3A4)/ Biliary	7 Days + Level < 2

DNA Fragmentation Agent:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Arsenic Trioxide	Trisenox	DNA Fragmentation	10-14 hours	Hepatic/Renal	2 Days

FLT-3/Multi-Kinase/Janus Kinase Inhibitors:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Gilteritinib	ASP2215	Tyrosine Kinase Inhibitor + FLT-3 Inhibitor	45-159 hours	Hepatic/ Renal + Fecal + Biliary	7 Days
Ibrutinib	Imbruvica	Bruton's Tyrosine Kinase Inhibitor	4 – 6 hours	Hepatic (CYP3A4/2D6)/ Fecal + Renal	1 Day
Midostaurin	Rydapt	Tyrosine Kinase Inhibitor +FLT-3 Inhibitor	~20 days	Hepatic (CYP3A4)/ Fecal + Renal	2 Days
Quizartinib	AC220	Tyrosine Kinase Inhibitor +FLT-3 Inhibitor	~3 Days	Hepatic (CYP3A4)/ Fecal	7 Days
Ruxolitinib	Jakafi	Janus Kinase Inhibitor	~5.8 hours	Hepatic (CYP3A4)/ Renal + Fecal	1 Day
Sorafenib	Nexavar	Multi-kinase Inhibitor	25–48 hours	Hepatic (CYP3A4)/ Fecal+ Renal	7 Days
Tofacitinib	Xeljanz	Janus Kinase Inhibitor	3-6 hours	Hepatic (CYP3A4)/ Renal	1 Day

HDAC Inhibitors:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Panobinostat	Farydak	HDAC Inhibitor	~37 hours	Hepatic (CYP3A4)/ Fecal + Renal	7 Days
Vorinostat	Zolinza	HDAC Inhibitor	~2 hours	Hepatic/ Renal	1 Day

Hydrolysis Catalyzer:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Asparaginase	Erwinase	Hydrolysis Catalyzer	15.6 hours	Blood/ Renal	7 Days
Pegaspargase	Oncaspar	Hydrolysis Catalyzer	3.2-5.7 days	Blood/ Renal	14 Days

Hypomethylating Agents:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Azacitadine	Vidaza	Hypomethylating Agent	4 hours	Renal	7 Days

Decitabine	Dacogen	Hypomethylating Agent	~0.5 hours	Hepatic	7 Days

Immunomodulatory Agents:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Lenalidomide	Revlimid	Immunomodulatory Agent	3–5 hours	Limited Metabolism/ Renal	2 Days
Pomalidomide	Pomalyst	Immunomodulatory Agent	~7.5 hours	Hepatic (CYP3A4/1A2)/ Renal + Fecal	2 Days
Thalidomide	Thalomid	Immunomodulatory Agent	5-7 hours	Hepatic/ Renal	2 Days

Isocitrate Dehydrogenase 2 (IDH2).Inhibitor:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Enasidenib	Idhifa	Inhibitor of the enzyme isocitrate dehydrogenase 2 (IDH2).	137 hours	AGI-16903 is the N-dealkylated metabolite of enasidenib and represents 10% of radioactivity in circulation.	1 Day

Kinase Inhibitors:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Bosutinib	Bosulif	Tyrosine Kinase Inhibitor	22–27 hours	Hepatic (CYP3A4)/ Fecal	5 days
Dasatinib	Sprycel	Tyrosine Kinase Inhibitor	3–5 hours	Hepatic (CYP3A4)/ Fecal + Renal	1 Day
Gilteritinib	ASP2215	Tyrosine Kinase Inhibitor + FLT-3 Inhibitor	45-159 hours	Hepatic/ Renal + Fecal + Biliary	7 Days
Ibrutinib	Imbruvica	Bruton's Tyrosine Kinase Inhibitor	4 – 6 hours	Hepatic (CYP3A4/2D6)/ Fecal + Renal	1 Day (Assess Bleeding Risk)
Imatinib	Gleevec	Tyrosine Kinase Inhibitor	~40 hours	Hepatic (CYP3A4)/ Fecal + Renal	7 Days
Midostaurin	Rydapt	Tyrosine Kinase Inhibitor +FLT-3 Inhibitor	~20 days	Hepatic (CYP3A4)/ Fecal + Renal	2 Days
Nilotinib	Tasigna	Tyrosine Kinase Inhibitor	15–17 hours	Hepatic (CYP3A4)/ Fecal	3 Days
Ponatinib	Iclusig	Tyrosine Kinase Inhibitor	~24 hours	Hepatic (CYP3A4)/ Fecal + Renal	7 Days
Quizartinib	AC220	Tyrosine Kinase Inhibitor +FLT-3 Inhibitor	~3 Days	Hepatic (CYP3A4)/ Fecal	7 Days
Ruxolitinib	Jakafi	Janus Kinase Inhibitor	~5.8 hours	Hepatic (CYP3A4)/ Renal + Fecal	1 Day
Sorafenib	Nexavar	Multi-kinase Inhibitor	25–48 hours	Hepatic (CYP3A4)/ Fecal+ Renal	7 Days

Microtubule Inhibitors:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Vincristine	Oncovin	Vinca Alkaloid	~85 hours	Hepatic (CYP3A4/5)/ Fecal + Biliary + Renal	7 Days

Monoclonal Antibodies:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Adalimumab	Humira	Monoclonal Antibody	2 weeks	N/A	30 Days
Blinatumomab	Blincyto	Monoclonal Antibody	~2 hours	Renal	7 Days
Brentuximab Vedotin	Adcetris	Monoclonal Antibody	4-6 days	Hepatic (CYP3A4/5)/ Renal + Fecal	24 days
Etanercept	Enbrel	Monoclonal Antibody	102 hours	N/A	30 Days
Infliximab	Remicade	Monoclonal Antibody	8-14.7 days	N/A	30 Days
Ipilimumab	Yervoy	Anti-CTLA-4 Antibody	~15 days	N/A	30 Days
Nivolumab	Opdivo	PD-1 Inhibitor	26.7 days	N/A	30 Days
Ofatumumuab	Arzerra	Monoclonal Antibody	~17.6 days	N/A	30 Days
Pembrolizumab	Keytruda	PD-1 Inhibitor	23 days	N/A	30 Days
Tocilizumab	Actemra	Monoclonal Antibody	4.2-7.9 days	N/A	30 Days
Rituximab	Rituxin	Monoclonal Antibody	18-32 days	N/A	30 Days
Vedolizumab	Entyvio	Monoclonal Antibody	25 days	N/A	30 Days

PI3K (delta) Inhibitor:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Idelalisib	Zydelig	PI3K (delta) Inhibitor	~ 8 hours	Hepatic (CYP3A4)/ Fecal + Renal	1 Day

Proteasome Inhibitors:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Bortezomib	Velcade	Proteasome Inhibitor	9-15 hours	Hepatic (CYP450)/ Renal	3 Days
Carfilzomib	Kyprolis	Proteasome Inhibitor	1 hour	Hepatic (CYP450)	1 Day
Ixazomib	Ninlaro	Proteasome Inhibitor	~9.5 days	Hepatic (CYP450)/ Renal + Fecal	14 Days

Protein Synthesis Inhibitor:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Omacetaxine	Synribo	Protein Synthesis Inhibitor	~14.6 hours	Blood +Hepatic/ Fecal + Renal	7 Days

Retinoic Acid Derivative:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Tretinoin (ATRA)	Vesanoid	Retinoic Acid Derivative	0.5–2 hours	Hepatic (CYP450)/ Renal + Fecal	7 Days

TPO Receptor Agonist:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Eltrombopag	Promacta	TPO Receptor Agonist	26-35 hours	Hepatic (CYP1A2/2C8)/ Renal + Fecal	7 Days

Topoisomerase II Inhibitor:

Generic	Brand	Class/MOA	Half-life	Metabolism/ Excretion	Discontinuation Prior to BMT
Etoposide	Vepesid	Topoisomerase II Inhibitor	4-11 hours	Hepatic/ Renal + Biliary	7 Days
Mitoxantrone	Novantrone	Topoisomerase II Inhibitor	23-215 hours	Hepatic/ Fecal +Biliary + Renal	7 Days