Study Title: Abatacept Combined with a Calcineurin Inhibitor and Methotrexate for Graft Versus Host Disease Prophylaxis.

NCT number: NCT01743131

Protocol version date: 1/30/2019

Abatacept Combined with a Calcineurin Inhibitor and Methotrexate for Graft Versus Host Disease Prophylaxis

PBMTC PROTOCOL GVH 1201
Version #9.1.1
Amendment Package #9.1.1

FDA IND#: 111738
SPONSOR: Leslie S. Kean, M.D., PhD
PRODUCT NAME(S): CTLA4lg, abatacept, Orencia

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing Dr. Leslie S. Kean, the Study Sponsor, with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: PBMTC GVH 1201

Protocol Title: Abatacept Combined with a Calcineurin Inhibitor and Methotrexate for Graft Versus Host Disease Prophylaxis: A Randomized Controlled Trial

Protocol Date: 1/30/2019

____ __________________

Investigator Signature *Date*

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Study Synopsis

Principal Investigator: Leslie S. Kean, MD, PhD

Study design: This is a multicenter phase II trial with two strata: a randomized, double blind, placebo-controlled stratum (stratum 1) for patients receiving transplants from 8 of 8 HLA matched unrelated donors and a single arm stratum (stratum 2) for patients receiving transplants from 7 of 8 matched donors.

Primary Objective: To determine the impact that abatacept will make on the incidence of early, severe acute GVHD (aGvHD), when it is added to a standard GvHD prophylaxis regimen during unrelated-donor hematopoietic stem cell transplantation (HSCT) for patients with hematologic malignancies.

Secondary Objective: To characterize the impact of abatacept on the post-transplant reconstitution of anti-viral protective immunity.

Eligibility Criteria: Eligible patients will be at least 6 years old, have high-risk leukemia, adequate organ function, lack an HLA-matched related donor, and have an unrelated bone marrow or peripheral blood stem cell donor who is HLA-matched at no less than seven of eight loci (A, B, C, DRB1). This may be an allele or antigen mismatch.

Treatment Description: Patients will receive intensive pre-transplant conditioning. Those receiving transplants from 8 of 8 matched donors will be randomly assigned to standard aGVHD prophylaxis (placebo, calcineurin inhibitor, and short-course methotrexate) or investigational prophylaxis (abatacept, calcineurin inhibitor and short-course methotrexate), while those receiving transplants from 7 of 8 matched donors will all be assigned to receive the investigational prophylaxis.

Accrual Objective: 40 non-randomized 7/8 HLA matched patients and 140 randomized 8/8 HLA matched patients will be accrued to this study.

Accrual Period: The estimated accrual period is 4 years.

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1. Hypothesis and Aims: This trial is being conducted as a step toward testing the long-term hypothesis that the costimulation blockade agent abatacept can be added to a standard acute graft-versus-host disease (aGvHD) prophylaxis regimen (which includes a calcineurin inhibitor (CNI) and methotrexate), to improve disease-free survival after unrelated donor (URD) hematopoietic stem cell transplantation (HSCT) for patients with hematologic malignancies. As a phase II study, the overall aim of this trial is to make a preliminary assessment of abatacept's clinical safety and efficacy using short-term outcomes. Thus, this trial is designed to test two hypotheses:

(1) A primary hypothesis that the addition of abatacept to calcineurin inhibition + methotrexate can decrease the incidence of early-onset (before day 100 post-transplant) Gr III-IV aGVHD in HLA 8/8 matched-unrelated donor transplants and Gr III-IV aGVHD in HLA 7/8 mismatched-unrelated donor transplants.

(2) A secondary hypothesis that its addition will not hinder post-transplant reconstitution of protective immunity against latent viruses.

To test these two hypotheses, this study will have the following Specific Aims.

Specific Aim #1: To conduct a multicenter phase II trial to assess the impact of abatacept on the incidence of aGVHD and its biology. We will compare investigational GVHD prophylaxis, abatacept combined with a CNI and methotrexate, to standard GVHD prophylaxis, a CNI and methotrexate alone in two patient strata. Patients receiving an 8/8 HLA matched URD HSCT (stratum 1) will be randomly assigned to the investigational regimen or the standard regimen; all patients receiving 7/8 matched URD HSCT (stratum 2) will be assigned to the investigational regimen and be compared to a historical 7/8 matched URD control group drawn from the Center for International Blood and Marrow Research (CIBMTR) registry. In both strata, correlative immunology studies will be performed to elucidate abatacept's effects on the graft-versus-host response.

Specific Aim #2: To assess the impact of abatacept on post-transplant reconstitution of protective immunity against viruses. This will involve monitoring the longitudinal recovery of lymphocyte subsets and virus-specific immunity, using tetramer analysis and viral stimulation assays. It will also involve monitoring viral infection and disease.

2. Background and Rationale:

The Unmet Need: Allogeneic HSCT is an effective treatment for aggressive leukemias and other hematological malignancies, often representing the only option for cure. However, some of its benefit, especially in the case of unrelated donor transplantation, is off-set by a high rate of transplant-related mortality (approximately 30% of recipients of unrelated donor transplantation will die of transplant-related complications) stemming largely from severe aGVHD and infection.[1-7] aGvHD occurs when reconstituting donor T cells[8] become activated against recipient tissues.[9] This activation can result in severe immune-mediated tissue damage to the host, with the skin, liver and GI tract being the most common targets. aGvHD-mediated damage to these vital organs can result in significant morbidity, and in death. While whole-scale T cell depletion of the allograft can successfully reduce rates of aGvHD, patients receiving T cell-depleted grafts exhibit profound defects in protective immunity, and often die of infection or relapse of their primary disease.[10-12] This has created an unmet clinical need for a strategy that more effectively prevents severe aGvHD while preserving the transplant recipient's protective immune response.

Targeting T cell costimulation to prevent aGvHD: The immune activation observed in aGvHD bears close resemblance to the immune activation that occurs during both organ rejection and autoimmunity. Studies in these diseases have led to the development of a new class of agents, called 'costimulation blockade' reagents, which specifically target activated T cells and block their ability to become fully activated effector cells. [13] One of the most studied of the costimulatory pathways is the CD28:CD80/86 receptor:coreceptor interaction.[14] Considerable work on this pathway has been accomplished, and has demonstrated the efficacy of inhibition of CD28:CD80/86 signaling in inhibiting T cell-mediated immune activation. The first CD80/86-directed costimulation blockade agent, CTLA4Ig, or 'abatacept,' is approved for use in rheumatoid arthritis, both in adults and in children older than 6 years.[15-18] The experience with abatacept from 3 large randomized, placebo-controlled clinical trials, two in adults with rheumatoid arthritis and one in children with juvenile idiopathic arthritis (ages 6 and older) indicates that it is a safe agent.[19-21] In these three trials, abatacept was dosed at 10 mg/kg and was administered IV on day 1, 15, 29 (one trial used day 30) and then every 28 days for a total of 6 to 10 months of total treatment. Collectively, most patients also received weekly, oral, low-dose methotrexate and low dose prednisone concurrently. In these trials, abatacept was well tolerated. Acutely, infusional reactions were rare and mild and occurred at rates that did not differ significantly from those with placebo. Abatacept was not associated with any hematologic, renal, cardiac, pulmonary, hepatic or neurologic abnormalities. Similarly, the rates of both total and serious adverse events were low, and did not differ from those with placebo. Abatacept has been shown to be safe, even in extended open label trials,[22,23] not associated with excessive PTLD or other malignancies. [22-25] However, chronically-treated patients did experience a slightly higher risk of infections. [22,24,25]

Phase III studies of a second-generation, higher avidity abatacept analog, belatacept (which is identical to abatacept except for two amino acid substitutions) have demonstrated efficacy in preventing renal transplant rejection. [26,27] Patients who received 10mg/kg of belatacept on days 1, 5, 14, 28, and every 28 days thereafter demonstrated improved renal function compared to those receiving cyclosporine, and similar graft survival.[26,27] These results have led to the FDA approval of belatacept for a renal transplant indication. While overall rates of patient death, infection and serious infection in patients receiving belatacept were not different than in those receiving traditional immunosuppression,[26,28] belatacept was associated with a statistically-significant increased rate of EBV-associated PTLD compared to cyclosporine-based immunosuppression (especially in patients that were EBV sero-negative prior to transplant).[26,28] This observation raises an important question about the negative impact that belatacept and related compounds may have on protective immune responses to latent viruses. Rates of PTLD were much lower in EBV sero-positive patients,[26,28] suggesting that any defect in protective immunity induced by belatacept may be more significant in the setting of primary EBV infection than during EBV reactivation. These observations underscore the critical importance of evaluating novel immunosuppressive strategies for their impact both on alloreactivity and on the post-transplant protective immune response.

Prior to our work, abatacept had not been tested for its ability to prevent GvHD in BMT patients. However, there was considerable evidence from murine models to suggest that it might be an active compound against the immune activation that occurs during GvHD.[29-33] In addition, our research group developed a non-human primate model of GvHD [34] and used this model to demonstrate that an abatacept-containing immunosuppressive regimen could significantly protect against the development of primate GvHD. [34] These results, along with the clinical evidence for efficacy of abatacept and belatacept in both autoimmunity and solid organ transplantation provided the rationale for the development of a first-in-disease feasibility trial of abatacept for GvHD prevention (Clinical Trials.org #NCT01012492). This trial, which has now completed enrollment, has documented encouraging early results with respect to both the safety and efficacy of abatacept for GvHD prevention (Kean et al., ASH 2011, Koura et al., ASH 2012

and [57]). These results have led to the creation of the current Phase II clinical trial of abatacept for prevention of severe aGvHD. The current trial uses the same dosing schedule as was implemented in the Abatacept Feasibility Study[57] with the goal of gaining an initial assessment of relative efficacy, comparing standard tacrolimus/methotrexate GVHD prophylaxis with tacrolimus/methotrexate/abatacept prophylaxis.

3. Research Design and Methods

3.1. Study Design: This is a multicenter phase II trial with two strata: a randomized, double blind, placebo-controlled stratum (stratum 1) for patients receiving transplants from 8 of 8 HLA matched unrelated donors and a single arm stratum (stratum 2) for patients receiving transplants from 7 of 8 matched donors.

3.2. Study Population, Subject Recruitment and Selection: Patients will be recruited from the Children's Healthcare of Atlanta Pediatric Blood and Marrow Transplant Program, the Emory University Adult Blood and Marrow Transplant Program, the University of Florida Adult Blood and Marrow Transplant Program, the Seattle Cancer Care Alliance (SCCA), and from participating centers in the Pediatric Blood and Marrow Transplant Consortium (PBMTC). A total of 40 non-randomized 7/8 HLA matched patients and 140 8/8 HLA matched patients will be enrolled on this study. Patients who are enrolled, but determined to be Assignment Failures before receiving study drug/placebo will be replaced. 7/8 HLA matched patients enrolled prior to Protocol Amendment 4 and who were randomized to the study drug/placebo arm are not counted in either group of 40 or 140.

3.3. Patient Eligibility, Enrollment and Treatment Assignment:

a) **Enrollment:** All forms for this trial will be completed online, using the REDCap system. Secure REDCap access will be established for each site after IRB approval by emailing the study coordinator. Patients will then be enrolled by completing the official online enrollment form using REDCap. If online access is not available, the form may also be submitted by e-mailing or faxing the study coordinator. In addition to the enrollment form, the supporting source documents shall also be sent to the study coordinator via email or fax. She will confirm eligibility and assign a subject number.

b) **Assignment**: After enrollment, patients receiving 8/8 matched URD transplants (stratum 1) will be randomly assigned to the standard GVHD prophylaxis arm or to the investigational abatacept arm of the trial. The study statistician will perform the randomization. He will convey the randomization to the central research pharmacist at Children's Healthcare of Atlanta/Emory University, who, in turn, will convey the information to the treating center's investigational pharmacist. Patients receiving 7/8 matched transplants (stratum 2) will be assigned to receive investigational therapy. Study drug with appropriate labeling for patients assigned to receive abatacept will then be delivered to the treating center's investigational pharmacy.

c) **Blinding:** For the 8/8 matched group (stratum 1) the study statistician, the central research pharmacist, the treating center research pharmacist, the study monitors and members of the PBMTC DSMC will not be blinded**.** All participating patients, their families, all medical providers, and all investigators and study personnel other than

those listed above will be blinded to treatment assignment . Other persons may be unblinded at the discretion of the DSMC.

d) Inclusion Criteria:

(1) Must be at least **6 years old and weigh 20 kg**.

(2) Must have a willing unrelated adult donor (bone marrow or peripheral blood). Donors may have a single mismatch (i.e. be a 7/8) and this mismatch may be at the allele or antigen level; however, donors with allele level disparity should be given preference over those with antigen level disparity. **The use of mismatched donors in which disparity is only in the host versus graft direction (because of recipient homozygosity) is discouraged because of the potentially heightened risk for graft rejection. Centers may perform extended typing (e.g. DQB1 and DPB1) according to institutional practices and use these results in selecting donors; however, it is recommended that this extending typing be used only to select between donors who are equally well matched with the recipient at the A, B, C and DRB1.**

(3) All patients and/or their parents or legal guardians must sign a written informed consent. Assent, when appropriate, will be obtained according to institutional guidelines.

Must have a high risk hematologic malignancy as defined below. If the patient does not meet defined eligibility requirements as stipulated below, the PI must be contacted to determine eligibility.

(a) Acute myeloid leukemia (AML).

(i) Patients with AML in the first complete remission (CR) with intermediate or high risk disease.

(ii) Patients with AML in first CR with high-risk disease as defined by one of the following abnormalities. CR is defined as an M1 marrow (<5% blasts by morphology), no evidence of extramedullary disease, and an absolute neutrophil count ≥ 1.0 x 10^9/L. Cases where the ANC is < 1.0 x 10^9/L and rising will also be considered. The PI will need to approve such cases for enrollment. Complete remissions without platelet recovery (CRp) will be considered complete remissions.

Indicators of High-risk Disease are as follows:

1. Flt3/ITD+(If quantitative testing was performed, the allelic ratio must be >0.4)
2. Residual marrow disease (≥0.1%) detected by multidimensional flow cytometry after completing at least one cycle of induction chemotherapy.
3. Secondary AML. If the AML is secondary to treatment for another malignancy, the first malignancy must be in a complete remission.
4. High-Risk cytogenetic abnormalities: Different high-risk cytogenetic criteria have been defined for adult and pediatric AML. We will, therefore, use two sets of cytogenetic criteria, one based on Children's Oncology Group (COG) criteria for pediatric patients and one based on Southwestern Oncology Group (SWOG)/Eastern Oncology Group (ECOG) or MRC criteria for adult patients.

Examples of high-risk cytogenetics:

a. Adult patients (≥21 years):

i. -5/del (5q)
ii. -7/del (7q)
iii. inv 3q
iv. del (9q)
v. abn 11q
vi. abn 20q
vii. abn 21q

viii. abn 17P
ix. t(6;9)
x. t(9;22)
xi. complex karyotypes (≥ 3 unrelated abnormalities)

b. Pediatric patients (<21 years)
i. -5/del (5q)
ii. -7

5. Other abnormalities associated with a higher risk for AML relapse. There are an increasing number of abnormalities being identified that have been associated with an intermediate or high risk of relapse, but have yet to be incorporated into cooperative group risk classification systems. Patients with AML characterized by these abnormalities will be considered. The PI will need to approve such cases for enrollment.

(ii) Patients with a partial first remission (PR, defined as an M2 marrow (5-19% blasts by morphology), no evidence of extramedullary disease, and an absolute neutrophil count ≥ 1.0 x 10^9/L. Cases where the ANC is < 1.0 x 10^9/L and rising will also be considered. The PI will need to approve such cases for enrollment..
(ii) Patients in 2nd or greater complete or partial remission.

(b) Myelodysplastic syndrome
i. Adult patients (≥21 years) with secondary disease or de novo disease that meets criteria for intermediate, high or very high-risk disease based on the Revised International Prognostic Scoring System.[35]

Intermediate risk (3.1-4.5 points), high risk (4.6-6 points), very high risk (>6 points)

IPSS-R prognostic score values

Prognostic variable	0	0.5	1	1.5	2	3	4
Cytogenetics	Very good*	—	Good**	—	Intermediate***	Poor#	Very poor^
BM blast, %	≤ 2	—	> 2%- < 5%	—	5%-10%	> 10%	—
Hemoglobin	≥ 10	—	8- < 10	< 8	—	—	—
Platelets	≥ 100	50-< 100	< 50	—	—	—	—
ANC	≥ 0.8	< 0.8	—				

*−Y, del(11q); **Normal, del(5q), del(12p), del(20q), double including del(5q); ***del(7q), +8, +19, i(17q), any other single or double independent clones; # −7, inv(3)/t(3q)/del(3q), double including −7/del(7q), complex: 3 abnormalities; ^ Complex: > 3 abnormalities

ii. Pediatric patients with MDS, regardless of subtype, will be eligible.

c) Acute lymphoblastic leukemia (ALL).

Given the poor prognosis of adults (≥21 years) with ALL, adults in 1st or greater complete remission will be eligible.. CR is defined as an M1 marrow (<5% blasts), no evidence of extramedullary disease, and an absolute neutrophil count ≥ 1.0 x 10^9/L. Cases where the ANC is < 1.0 x 10^9/L and rising will also be considered. The PI will need to approve such cases for enrollment.Complete remissions without platelet recovery (CRp) will be considered remissions

Given the generally good prognosis of children (<21 years) with ALL, they will have to meet one of the criteria listed below. Additionally, children who are enrolled on a COG ALL trial for newly diagnosed or relapsed disease will have to meet the criteria for BMT outlined in that trial. CR is defined as an M1 marrow (<5% blasts), no evidence of extramedullary disease, and an absolute neutrophil count (ANC) ≥ 1.0 x 10^9/L. Cases where the ANC is < 1.0 x 10^9/L and rising will also be considered. The PI will need to approve such cases for enrollment. Complete remissions without platelet recovery (CRp) will be considered remissions.

1. In 1st complete remission with a very high risk for relapse.
    a. Hyplodiploidy (<44 chromosomes, as evidenced by the results of routine analysis of G-banded chromosomes, DNA index (<0.81), or other appropriate methodology).
    b. >1% residual marrow blasts by flow cytometry at the end of induction.
    c. >0.01% residual marrow blasts by flow cytometry at the end of consolidation.
    d. Early T-Cell Precursor (ETP) phenotype
2. In 2nd complete remission with B-lineage disease after a marrow relapse occurring less than 36 months from diagnosis.
3. In 2nd complete remission with T-lineage disease or Ph+ disease after a marrow relapse occurring at any time.
4. In a 2nd complete remission with T-lineage disease after an extra-medullary relapse occurring less than 18 months from diagnosis.
5. In 3rd or greater complete remission after a marrow or extramedullary relapse
6. Other indications for transplant in pediatric patients with ALL must be approved by the

Study PI with a note to file reflecting study team discussion and approval.

(d) Patients with acute undifferentiated, biphenotypic, or bilineal leukemia, which is in 1st or greater complete remission (CR) or partial remission (PR). Cr will be defined as an M1 marrow (<5% blasts), no evidence of extramedullary disease, and an absolute neutrophil count ≥ 1.0 x 10^9/L. Cases where the ANC is < 1.0 x 10^9/L and rising will also be considered. The PI will need to approve such cases for enrollment. CR without platelet recovery (CRp) will be considered complete remissions.) PR will be defined as an M2 marrow (5-19% blasts), no evidence of extramedullary disease, and an absolute neutrophil count ≥ 1.0 x 10^9/L.). Cases where the ANC is < 1.0 x 10^9/L and rising will also be considered. The PI will need to approve such cases for enrollment.

(e) Chronic myelogenous leukemia (CML).

(i) Chronic phase with resistance to tyrosine kinase inhibitors.

(ii) accelerated phase (development of cytogenetic abnormality in addition to t(9:22), blood blast percentage ≥10, blood basophil percentage ≥20, platelet count <100,000 X 10^9/L)

iii. blast crisis.

iv. 2nd or greater chronic phase.

(f) Acute Lymphoblastic Lymphoma in 2nd or greater complete remission.
Complete remission includes confirmed complete response (CR) defined as the disappearance of all evidence of disease from all sites for at least 4 weeks. Bone marrow and CSF must be normal and any macroscopic nodules in any organs detectable on imaging techniques shall no longer be present. Imaging should include PET scanning. CR will also include unconfirmed complete responses defined as a residual lymph node mass > 1.5 cm in greatest transverse diameter that has regressed by > 75% in sum of the products of the greatest perpendicular diameters (SPD), or any residual lesions in organs that have decreased by > 75%, with a negative PET scan, negative bone marrow and CSF.

(g) Peripheral T cell lymphoma (PTCL).

(i) In first response (must have at least a partial response)
   1. PTCL, unspecified.
   2. Hepatosplenic gamma-delta T cell lymphoma

(ii) Recurrent PTCL (must be treatment sensitive with at least a partial response). If patient has had a previous autologous transplant, the melphalan and fludarabine conditioning regimen must be utilized.

For these lymphomas a Complete Response (CR) is defined as the disappearance of all detectable clinical evidence of disease and disease-related symptoms present before therapy. Post-treatment residual nodal mass of any size is permitted as long as it is PET negative and the disease is FDG avid. If the lymphoma is variably FDG-avid or PET negative, regression of nodal masses to normal size must be demonstrated by CT scan. Spleen and liver must be normal in size with disappearances of nodules (assessed as described for nodal mass). The bone marrow shall be negative by morphology (or immunohistochemistry if morphology is indeterminate).
For these lymphomas, a partial response (PR) is defined as at least a

50% decrease in sum of the product of the perpendicular diameters (SPPD) of up to 6 of the largest dominant nodes or nodal masses (if FDG-avid, based on PET; if variably FDG-avid or PET negative based on CT scan). There should be no increase in the size of other lymph nodes. Splenic and hepatic nodules must regress by 50% in their SPPD or, for single nodules, in the greatest transverse diameter. There should be no measurable disease involving other organs. No new sites of disease. At sites where the FDG-PET scan was positive before therapy, the post-treatment PET is positive at one or more of the previously involved sites. Bone marrow may be positive or negative for disease.

(h) Chronic myelomonocytic leukemia.

(i) Atypical (BCR-ABL negative) chronic myelogenous leukemia

(j) Hodgkin lymphoma that has recurred or progressed after an autologous BMT.

(i) Newly diagnosed or recurrent disease initially refractory to intensive chemotherapy. A partial response or better must be achieved prior to transplantation. Please see (g) for definitions of response. Eligibility of newly diagnosed cases must be discussed with the study PI.
(ii) Disease that has recurred or progressed after an autologous BMT. Salvage chemotherapy must produce a partial response or better. Please see (g) for definitions of response. The melphalan and fludarabine conditioning-regimen must be used for these patients.

(k) Non-Hodgkin lymphoma (other than lymphoblasitc (f) or peripheral T cell lymphoma (g)).
(i) Newly diagnosed or recurrent disease initially refractory to intensive chemotherapy. A partial response or better must be achieved prior to transplantation. Please see (g) for definitions of response. Eligibility of newly diagnosed cases must be discussed with the study PI.
(ii) Disease that has recurred or progressed after an autologous BMT. Salvage chemotherapy must produce a partial response. Please see (g) for definitions of response. The melphalan and fludarabine conditioning-regimen must be used for these patients.

e)Exclusion Criteria:

1. Prior allogeneic HSCT.
2. The patient is enrolled on a COG trial that uses criteria for unrelated donor HSCT, which conflict with our eligibility criteria.
3. The patient is enrolled on a COG trial that utilizes unrelated donor HSCT and requires that patients be transplanted using an approach specified by the protocol that is in conflict with the approach specified in this protocol.
4. Availability of a willing and suitable HLA identical related donor.
5. Uncontrolled viral, bacterial, fungal or protozoal infection at the time of study enrollment.
6. HIV infection.
7. Serious psychiatric disease including schizophrenia, bipolar disorder and severe depression.

8. Any patient with a known or suspected inherited predisposition to cancer should be discussed with the study team prior to screening for eligibility.
   a. Patients with a known inherited or constitutional predisposition to transplant morbidities, including, but not limited to Fanconi Anemia, Dyskeratosis Congenita, Shwachman-Diamond Syndrome and Down Syndrome will be excluded.
   b. Patients with known inherited or constitutional predisposition to non-hematologic cancers including, but not limited to Li-Fraumeni syndrome, BRCA1 and BRCA2 mutations will be excluded.
   c. Patients with an inherited predisposition to leukemia or otherwise hematologic malignancies that have not been associated with predisposition to transplant morbidities or non-hematologic cancers will not be excluded.
9. Patient with a secondary malignancy who would be otherwise eligible for study, but for whom remission from the primary disease cannot be conclusively confirmed or for whom the chance of relapse of the primary disease is significant.
10. Incompletely treated active tuberculosis Infection.
11. Pregnancy (positive serum b-HCG) or breastfeeding.
12. Estimated GFR of < 50 mL/min/1.73m^2.
13. Cardiac ejection fraction < 50 (using M-Mode if assessment is done by ECHO)
14. T.bilirubin > 2 × upper limit of normal or ALT > 4 × upper limit of normal or unresolved veno-occlusive disease.
15. Pulmonary disease with FVC, FEV1 or DLCO parameters <45% predicted (corrected for hemoglobin) or requiring supplemental oxygen. Children who are developmentally unable to perform pulmonary function testing will be assessed solely on their need for supplemental oxygen.
16. Karnofsky performance score or Lansky Play-Performance Scale score <80
17. Presence of antibodies to a mismatched donor HLA antigen (please refer to Section 3.4.g).

f) Co-enrollment on other studies:

Patients who are currently enrolled and being treated on a cooperative group or local study for newly diagnosed or relapsed hematologic malignancies will be eligible for this phase II trial of abatacept if the patient otherwise meets eligibility criteria for the abatacept study and the patients' participation in the first trial does not in any way interfere with the administration of the therapy, clinical testing or research testing dictated by this abatacept trial. When seeking to enroll patients on this abatacept study, centers must indicate any and all studies that the patients are currently enrolled on. The principal investigator or her designee will review this information before the patient is approved for enrollment.

Patients who are not currently enrolled on another study but who seek to enroll in another trial (either while they enroll on the abatacept trial or after they enroll on the abatacept trial) must be granted permission by the principal investigator or her designee. In general, trials of supportive care interventions, of treatments designed to prevent relapse or of other therapies will be allowed providing they do not interfere with the administration of the therapy, clinical testing or research testing dictated by this trial of abatacept. Participation in trials of agents that are likely to influence risk for GVHD and, therefore, interfere with our ability to assess the primary endpoint of this trial, will not be allowed. Similarly, participation in trials of agents that may increase the risks of participating in this trial of abatacept will not be allowed. In general,

participation in observational studies will be permitted unless they add significantly to research blood draws.

3.4. Routine pre-transplant evaluations (refers to recipient unless otherwise stated)
The following observations are considered standard evaluations for transplant eligibility and are required to be determined ≤ 30 days before initiation of conditioning therapy.

a) History, physical examination, height and weight.
b) Karnofsky/Lansky performance rating.
c) CBC with differential and platelet count, creatinine, bilirubin, alkaline phosphatase, AST, ALT, blood chemistries, ABO/Rh typing.
d) Serum pregnancy test (for women of child-bearing potential) if this testing is a part of institutional pre-transplant standard-of-care. Child-bearing age is determined based on institutional standards.
e) Disease staging (e.g., bone marrow aspiration/biopsy for pathology and cytogenetics, LP, CT scans, etc) specific for underlying disease within four weeks of the start of conditioning. Note that disease staging is disease specific and should align with institutional standards and with the eligibility criteria set forth in Section 3.3d.
f) HLA typing-both the donor and recipient. Participants must have typing sufficiently sensitive to determine whether they are matched at an allele level at the A, B, C and DRB1 loci. Centers may perform extended typing (e.g. DQB1 and DPB1) according to institutional practices and use these results in selecting donors; eligibility for this trial, however, will be based only on typing at the A, B, C and DRB1 loci.
g) For transplants involving HLA mismatched donor-recipient pairs, HLA antibody testing on the recipient (to determine if the recipient has pre-existing anti-donor HLA antibodies). Centers may supplement (but not replace) this testing with other assays, such as lymphocytotoxicity cross-matching.
h) For transplants involving same sex donor-recipient pair baseline variable number tandem repeat (VNTR) testing (or other similar molecular method) shall be performed on both the donor and recipient.
i) Pulmonary function studies including DLCO, FEV1, and FVC. This testing may be omitted in younger children for whom such testing is developmentally inappropriate. In these patients, pulse oximetry testing shall be performed.
j) Echocardiogram, MUGA or thallium scan for ejection fraction.
k) Estimated GFR, performed according to institutional standards.
l) Serology for CMV, EBV, HSV, HIV, HTLV1/2 and hepatitis B and C viruses, as well as syphilis testing by VDRL or RPR in the recipient. Serology for CMV, HIV, HTLV1/2, and hepatitis B and C viruses, as well as syphilis testing by, VDRL or RPR in the donor.
m) Serum for quantification of IgG,
n) Total nucleated cell count, CD3+ and CD34+ counts of the infused product on Day 0. If this test is not performed as part of standard-of-care at a participating institution, it will be included as a specific research test.
o) Patients should continue to meet inclusion criteria at the start of the conditioning regimen.

3.5. Routine post-transplant evaluations. Please see Table 1 (the study visit calendar) and Tables 2 and 3 (which summarize the clinical and research evaluations).

a) Lineage-specific chimerism analysis (CD3+ chimerism and CD33+ chimerism) using VNTR (or other similar molecular assay) shall be obtained at approximately day 28 (+/- 5 days) to confirm engraftment. Any subsequent chimerism analysis should be

performed according to the discretion of the treating physicians and according to institutional practices. However, if any of the lineage-specific chimerism values at day 30 is less than 95%, a repeat lineage-specific chimerism measurement is required to be made between days 60-90 post-transplant. Testing of lineage specific chimerism is a requirement of the study and must be performed on peripheral blood.

b) Complete blood counts with a differential shall be obtained according to institutional practices to monitor myeloid recovery and to determine transfusion needs.

c) Creatinine, bilirubin, alkaline phosphatase, ALT, AST, and other blood chemistries shall be obtained according to institutional practices to monitor for renal and hepatic toxicity as well as electrolyte and metabolic disturbances.

d) Serum IgG, shall be measured at baseline and on day +28 +/- 7 days, +63 +/- 3 days, +100 +/- 7 days, +180 +/- 14 days, +270 +/- 14 days and +365 +/- 30 days. IgG testing done outside the specified window will not be a protocol deviation.

e) Disease re-staging (marrow testing, CSF testing, radiographic assessment, as required for each disease entity, as dictated by institutional standards) should be performed according to the discretion of the treating physicians.

f) Routine clinical monitoring for CMV viremia shall be performed in all patients (regardless of the results of donor and recipient pre-transplant serology) and initiated no later than transplant day +7 and be performed at least once a week through at least transplant day 100, and at least every 4 weeks through day 180 (please see supportive care guidelines for further details). More frequent testing may be indicated for some patients. Either antigen (pp65) or PCR (whole blood, plasma or leukocyte) testing may be used according to institutional practices. Note that baseline CMV testing is based on serology by post-transplant CMV testing is by PCR. Baseline PCR may be performed based in addition to serologic testing, based on institutional standards.

g) Clinical monitoring for EBV PTLD: Clinical monitoring by EBV PCR will be performed in all patients every two weeks (between day +7 and day 100) and once every four weeks through day 180 (please see supportive care guidelines for further detail). If low-level EBV reactivation occurs, treating physicians may choose to monitor for EBV by PCR more frequently than once every 2 weeks, based on institutional standards.

h) A complete history, physical examination, Karnofsky/Lansky score, and assessments for acute and chronic GVHD shall be performed on an ongoing basis according to institutional standards.

i) Acute and Chronic GvHD evaluation shall be reported on Days +30 (+/- 14 days) +60 (+/-14 days), and +100 (+/- 21 days) and then on days +180 +/- 14 days, +270 +/- 14 days, +365 +/- 30 days, Year 2 +/- 90 days, Year 3 +/- 90 days, Year 4 +/- 90 days, and Year 5 +/- 90 days. Reporting shall be performed using the online RedCap GvHD case-report form (CRF) Please see the GvHD evaluation guidelines included with the GvHD Case Report Form (GvHD CRF) to accurately stage and grade GvHD.

j) Patients will be evaluated for infections on an ongoing basis in accordance with the Blood and Marrow Transplant Clinical Trials Network Manual of Procedures (Section 4). These will be reported using the online REDCap Infection CRF (detailed guidance for evaluating infections is included in this form). The Infection CRF will be completed on Days +30, +60, and +100 (+/- 14 days), and then on days +180 +/- 14 days, +270 +/- 14 days, +365 +/- 30 days, Year 2 +/- 90 days, Year 3 +/- 90 days, Year 4 +/- 90 days, and Year 5 +/- 90 days.

k) Patients will be evaluated for disease-free survival and overall survival at the following time-points: On Days +30 +60, and +100 +/- 14 days (and then on days +180 +/- 14 days, +270 +/- 14 days, +365 +/- 30 days, Year 2 +/- 90 days, Year 3 +/- 90 days, Year 4 +/- 90 days, and Year 5 +/- 90 days.

l) Long-term follow-up shall be performed in accordance with institutional practices.

Note: Clinical adenovirus monitoring is not required in this study (based on the fact that retrospective analysis of all patients on the Abatacept Feasibility Study showed no adenovirus reactivation[57]). However, expanded testing, including prospective monitoring for adenovirus reactivation or other viral disease should be performed according to institutional standards.

3.6. Research Pre- and Post-transplant Evaluations (Non-standard testing): Please see Table 1 (the study visit calendar) and Table 2 (which summarizes the clinical and research evaluations). A detailed description of all of the research testing is provided in Appendix 1. Please note that once enrolled in the trial, patients will have research evaluations performed as long as enrollment continues, even in the case where administration of the investigational drug is held or halted (as described in Section 3.83).

3.7. Patient Assessments, Study Blood Draws, and Handling of Samples

3.7.1 Follow-up Schedule:

The follow-up schedule for scheduled study visits is outlined in Table 1.

TABLE 1. Follow-up Schedule

Study Visit[1]	Target Day
Baseline	Within 30 days of the start of conditioning regimen
Day -1	-1
Day 5	5
Day 14	14
Day 21	21 +/-3 days
Day 28	28*
Day 35	35 +/- 3 days
Day 42	42 +/- 3 days
Day 49	49 +/- 3 days
Day 56	56 ± 3 days
Day 63	63 ± 3 days
Day 70	70 ± 3 days
Day 77	77 ± 3 days
Day 84	84 ± 3 days
Day 91	91 ± 3 days
Day 100	100 ± 7 days
Day 180	180 ± 14 days
Day 270	270 ± 14 days
Day 365	365 ± 30 days
Year 2	730 ± 90 days
Year 3	1095 ± 90 days
Year 4	1460 ± 90 days
Year 5	1825 ± 90 days

[1]A summary of the observations required at each visit is detailed in Table 2, and the schedule of blood draws with volumes is detailed in table 3.
*On Day 28, some but not all assessments must be performed exactly on Day 28. See Page 25 for details.

3.7.2 Patient Assessments

Table 2 summarizes the patient assessments that will be performed.

<u>Table 2. Patient Assessments</u>

Please refer to page 15 for the complete list of pre-transplant evaluations.

[1]An extra sample for immune monitoring will be drawn at the time of GVHD diagnosis or viral infection (see Table 3).

[2]A sample will be collected from the donor at the time of donation.

[3]A sample will be collected from the marrow or PBSC graft at the time of harvest.

Table 2.	Days Post-Transplant																					
Assessments and Testing Dates	**Baseline**	**-1**	**5**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**92**	**100**	**180**	**270**	**365**	**Year 2**	**Year 3**	**Year 4**	**Year 5**
H&P, performance status, disease status	Per Institutional Practices																						
CBC and Chemistry Analysis	Per Institutional Practices																						
Serology for CMV, EBV, HSV, HIV, HTLV1/2, hepB, hepC, VDRL or RPR (recipient and donor)	x																						
Serum IgG	x					x					x					x	x	x	x				
Peripheral blood chimerism analysis						x					If necessary					If necessary							
CMV PCR			At least once weekly from Day +7--> +100														At least every four weeks through Day +180						
EBV PCR			At least once every two weeks from Day +7--> +100														At least every four weeks through Day +180						
GVHD assessment				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Infectious Disease Assesment		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Survival (Disese-Free and Overall)		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Blood for Correlative Biology[1,2,3]	x	x	x	x	x	x	x	x			x					x	x	x	x				
Urine sample[1]	x			x		x					x					x	x	x	x				

3.7.3 Blood Draws:

Table 3 summarizes the blood draw schedule and patient age and weight-specific volume specifications.

Table 3 Blood Draw Volumes (Based on Weight)

Table 3A. Blood Draw Volumes (in milliliters (cc) for Patients Weighing 20kg-30kg:[#]

				Table 3A														
Test	Tube	Donor	Product	Recipient														
				Baseline	-1 Pre	-1 Post	5 Pre	14 Pre	21	28 Pre	28 Post	35	42	63	100	180	270	365
PBMC functional and expression studies	Large Citrate CPT (8ml tube)	24		16						16				16	16	16	16	16
Immune Reconstitution	Cyto-chex (5 ml tube)	4		4	4		4	4	4	4				4	4	4	4	4
Plasma Analysis: Cytokines, Viral Load,	PPT (5 ml tube)	5		3	3		3	3	3	3				3	3	3	3	3
Serum Analysis: PK, Ig, Proteomics	BD Red Top Serum Tube (5 ml tube)	5		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Graft Analysis	5ml sterile polyethylene screw top tube, no additive		5															
T cell activation/regulation* *For Patients at Emory University, Children's Healthcare of Atlanta and SCCA	Small Citrate CPT (4 ml tube)			4				4		4				4	4	4	4	4

[#]Note: Additional blood draws for newly diagnosed acute GvHD or BK-virus, adenovirus, EBV infection (including LPD), or CMV infection: For patients weighing between 20-30kg, please draw the following samples before treatment therapy begins, if possible: 4 cc into a cyto-chex tube, 3 cc into a PPT tube, 3cc into a Red-top tube and 16 cc into large (8cc) CPT tubes. If the patient is being treated at either Emory Hospital, Children's Healthcare of Atlanta, or the SCCA, 4 cc into a small CPT tube will also be drawn. For patients who develop more than one of these complications, up to a total of two additional blood draws will be performed. Please also note that for patients weighing 20-30 kg, prior to Day +28, it will not be considered a protocol deviation if 2-4 cc are drawn into the cyto-chex tube rather than 4cc. Once a patient in this weight category reaches day +28, the cyto-chex draws shall all be 4cc.

Table 3B. Blood Draw Volumes (in milliliters (cc) For Patients Weighing 30kg-40Kg:#

Table 3B																		
Test	**Tube**	**Donor**	**Product**	**Recipient**														
				Baseline	**-1 Pre**	**-1 Post**	**5 Pre**	**14 Pre**	**21**	**28 Pre**	**28 Post**	**35**	**42**	**63**	**100**	**180**	**270**	**365**
PBMC functional and expression studies	Large Citrate CPT (8ml tube)	24		16						16				16	16	16	16	16
Immune Reconstitution	Cyto-chex (5 ml tube)	4		4	4		4	4	4	8				8	8	8	8	8
Plasma Analysis: Cytokines, Viral Load,	PPT (5 ml tube)	5		3	3		3	3	3	3				3	3	3	3	3
Serum Analysis: PK, Ig, Proteomics	BD Red Top Serum Tube	5		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Graft Analysis	5ml sterile polyethylene screw top tube, no additive		5															
T cell activation/regulation* *For Patients at Emory University, Children's Healthcare of Atlanta and SCCA	Small Citrate CPT (4 ml tube)			4				4		4				4	4	4	4	4

#Note: Additional blood draws for newly diagnosed acute GvHD or BK-virus, adenovirus, EBV infection (including LPD), or CMV infection: For patients weighing between 30-40kg, please draw the following samples before treatment therapy begins, if possible: 4 cc into a cyto-chex tube, 3 cc into a PPT tube, 3cc into a Red-top tube and 16 cc into large (8cc) CPT tubes. If the patient is being treated at either Emory Hospital, Children's Healthcare of Atlanta, or the SCCA 4 cc into a small CPT tube will also be drawn. For patients who develop more than one of these complications, up to a total of two additional blood draws will be performed.

Table 3C. Blood Draw Volumes (in milliliters (cc) For Patients Weighing 40-50Kg:#

Table 3C

Test	Tube	Donor	Product	Recipient														
				Baseline	-1 Pre	-1 Post	5 Pre	14 Pre	21	28 Pre	28 Post	35	42	63	100	180	270	365
PBMC functional and expression studies	Large Citrate CPT (8ml tube)	24		24						24				24	24	24	24	24
Immune Reconstitution	Cyto-chex (5 ml tube)	4		8	4		4	8	4	8				8	8	8	8	8
Plasma Analysis: Cytokines, Viral Load,	PPT (5 ml tube)	5		4	4		4	4	4	4				4	4	4	4	4
Serum Analysis: PK, Ig, Proteomics	BD Red Top Serum Tube	5		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Graft Analysis	5ml sterile polyethylene screw top tube, no additive		5															
T cell activation/regulation* *For Patients at Emory University, Children's Healthcare of Atlanta and SCCA	Small Citrate CPT (4 ml tube)			4				4		4				4	4	4	4	4

#Note: Additional blood draws for newly diagnosed acute GvHD or BK-virus, adenovirus, EBV infection (including LPD), or CMV infection: For patients weighing between 40-50kg, please draw the following samples before treatment therapy begins, if possible: 4 cc into a cyto-chex tube, 3 cc into a PPT tube, 3cc into a Red-top tube and 16 cc into large (8cc) CPT tubes. If the patient is being treated at either Emory Hospital, Children's Healthcare of Atlanta, or the SCCA 4 cc into a small CPT tube will also be drawn. For patients who develop more than one of these complications, up to a total of two additional blood draws will be performed.

Table 3D. Blood Draw Volumes (in milliliters (cc) For Patients Weighing >50Kg:[#]

Table 3D																		
Test	**Tube**	**Donor**	**Product**	**Recipient**														
				Baseline	**-1 Pre**	**-1 Post**	**5 Pre**	**14 Pre**	**21**	**28 Pre**	**28 Post**	**35**	**42**	**63**	**100**	**180**	**270**	**365**
PBMC functional and expression studies	Large Citrate CPT (8ml tube)	24		32					16	32				32	32	32	32	32
Immune Reconstitution	Cyto-chex (5 ml tube)	4		8	8		8	8	8	8				8	8	8	8	8
Plasma Analysis: Cytokines, Viral Load,	PPT (5 ml tube)	5		4	4		4	4	4	4				4	4	4	4	4
Serum Analysis: PK, Ig, Proteomics	BD Red Top Serum Tube	5		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Gene Array Analysis	PAXgene Tube	2.5		2.5	2.5		2.5	2.5	2.5	2.5				2.5	2.5	2.5	2.5	2.5
Graft Analysis	5ml sterile polyethylene screw top tube, no additive		5															
Costmulation Pathway Analysis* *For Patients at Emory University , Children's Healthcare of Atlanta and SCCA	EDTA Tube (Purple Top)			4				4		4				4	4	4	4	4
T cell activation/regulation* *For Patients at Emory University, Children's Healthcare of Atlanta and SCCA	Small Citrate CPT (4 ml tube)			4				4	4	4				4	4	4	4	4
T Cell Soring for TREC and Gene Array Analysis *For Patients over 18 years old at Emory University, Children's Healthcare of Atlanta and SCCA weighing >60 Kg	Large Citrate CPT (8ml tube)														60			60

[#]Note: Additional blood draws for newly diagnosed acute GvHD or BK-virus, adenovirus, EBV infection (including LPD), or CMV infection: For patients weighing >50kg, please draw the following samples before treatment therapy begins, if possible: 8 cc into a cyto-chex tube, 4 cc into a PPT tube, 4cc into a Red-top tube ,2.5cc PAXgene tube, and 32 cc into large (8cc) CPT tubes. If the patient is being treated at either Emory Hospital, Children's Healthcare of Atlanta, or the SCCA 4 cc into a small CPT tube will also be drawn. For patients who develop more than one of these complications, up to a total of two additional blood draws will be performed.

Sample Acquisition: Considering all of the research lab assays together, samples will need to be obtained on the following days as described below. Please note that if, in addition to study-specific blood draws, a CBC with differential, and a comprehensive chemistry panel will be drawn, these routine, standard-of-care labs should be drawn at the same time as the study-specific blood draws when possible.

Hematopoietic Stem Cell Product (at the time of harvest/collection): For all enrolled patients, the empty bag or syringe in which the stem cell product was infused will be retrieved by the study team and sent to the Central Reference Laboratory at ambient temperature where it will be washed to obtain donor cells for analysis. In addition, if the donor has previously given consent, 5cc of the harvested marrow or PBSC graft will be withdrawn from the bone marrow or PBSC graft at the recipient center (prior to infusion of the stem cell product into the recipient) and sent to the Central Reference Laboratory for analysis of graft content.

Donors (drawn within 60 days of harvest/collection): Please note that donors have the option of consenting to a blood draw at the time of stem cell harvest. However, the participation of the donor in this blood draw or in the donation of the 5cc of harvested marrow or PBSC graft is not a requirement for recipient enrollment on this study. Total blood draw: $\leq$ 40.5 cc (less than 3 tablespoons of blood). The total blood draw shall be collected as follows: 24cc into large (8cc) CPT tubes, 4cc into a cyto-chex tube, 5cc into a plasma preparation tube, and 5cc into a Red top serum tube. If the donor is harvested for a recipient that weighs >50 Kg, then an additional 2.5cc will be drawn into a PAXgene tube. If, in the opinion of the treating physician, it is not in the patient's best interest to remove the 5cc of harvested marrow or PBSC, they may choose not to remove this part of the product.

Recipients: Please note that while there is some latitude in the timing for many of the blood draws, the timing for the majority of the blood draws on days of abatacept administration is strict. The blood draws on recipients are based on weight such that all draws are $\leq$1.95cc/kg for any 28-day period.

Baseline (to be drawn within 30 days of start of prep regimen): Please see Table 3 for exact blood volumes based on patient weight.

Day -1: To be drawn exactly on that day (two blood draws on this day: pre-infusion and post-infusion, (the pre-infusion blood draw occurs within 30 minutes of the start of the infusion. The post-infusion blood draw occurs within 10 minutes of the end of the infusion). Please see Table 3 for exact blood volumes based on patient weight. Note: While every effort should be made to draw the blood within 30 minutes of the start of the infusion and within 10 minutes of the end of the infusion, it will not be considered a deviation from the protocol as long as the pre-infusion blood draw occurs within 60 minutes of the start of the infusion and the post-infusion blood draw occurs within 30 minutes of the end of the infusion.

Day +5: To be drawn exactly on that day. The blood draw occurs within 30 minutes prior to the start of the infusion. Please see Table 3 for exact blood volumes based on patient weight. Note: While every effort should be made to draw the blood within 30 minutes of the start of the infusion it will not be considered a deviation from the protocol as long as the pre-infusion blood draw occurs within 60 minutes of the start of the infusion.

Day +14: To be drawn exactly on that day. The blood draw occurs within 30 minutes prior to the start of the infusion. Please see Table 3 for exact blood volumes based on patient weight. Note: While every effort should be made to draw the blood within 30 minutes of the start of the

infusion, it will not be considered a deviation from the protocol as long as the pre-infusion blood draw occurs within 60 minutes of the start of the infusion.

<u>Day +21 +/- 3 days:</u> Please see Table 3 for exact blood volumes based on patient age and weight.

<u>Day +28:</u> To be drawn exactly on that day (two blood draws on this day: pre-infusion and post-infusion, (the pre-infusion blood draw occurs within 30 minutes of the start of the infusion. The post-infusion blood draw occurs within 10 minutes of the end of the infusion). Please see Table 3 for exact blood volumes based on patient weight. Note: While every effort should be made to draw the blood within 30 minutes of the start of the infusion and within 10 minutes of the end of the infusion, it will not be considered a deviation from the protocol as long as the pre-infusion blood draw occurs within 60 minutes of the start of the infusion and the post-infusion blood draw occurs within 30 minutes of the end of the infusion. * Please note that some, but not all of the Day +28 labs may be drawn with a grace period of +/- 3 days. If Day +28 falls on either a Saturday or Sunday, please discuss moving some of the blood draws with the study team. A decision to move any of the Day +28 blood draws requires a direct email or phone discussion with the study team.

<u>Day +35 ±3 days:</u> Please see Table 3 for exact blood volumes based on patient weight.

<u>Day +42 ±3 days:</u> Please see Table 3 for exact blood volumes based on patient weight.

<u>Day +63 ±3 days:</u> Please see Table 3 for exact blood volumes based on patient weight.

<u>Day +100 ± 7 days:</u> Please see Table 3 for exact blood volumes based on patient weight.

<u>Day +180 ±14 days:</u> Please see Table 3 for exact blood volumes based on patient weight.

<u>Day +270 ±14 days:</u> Please see Table 3 for exact blood volumes based on patient weight.

<u>Day +365 ±30 days:</u> Please see Table 3 for exact blood volumes based on patient weight.

<u>Additional blood draws for newly diagnosed acute GvHD or BK-virus, adenovirus, EBV infection (including LPD), or CMV infection.</u> For patients who are diagnosed with one of these complications, who have not had a research blood sample drawn in the preceding 5 days and are not scheduled to have blood drawn in the coming 5 days, additional blood will be drawn, before treatment begins, if possible. Please see Table 3 for exact blood volumes based on patient age and weight. For patients who develop more than one of these complications, up to a total of two additional blood draws will be performed.

<u>Urine Samples</u> (for viral monitoring): 10cc of urine will be collected at baseline from the recipient pre-transplant, and on Day +14, Day +28, Day +63 +/- 7 days, Day +100 +/- 7 days, Day +180 +/- 14 days, Day +270+/- 14 days, Day +365 +/- 30 days.

3.7.4 Handling and Delivering Samples

<u>For patients enrolled at: Emory University and Children's Healthcare of Atlanta:</u> Please contact Dr. Yvonne Suessmuth at least one day in advance of sample acquisition. She will be available for the collection of all study samples and will be coordinating the biologic correlative studies at the Emory Transplant Center. Her email is: yvonne.suessmuth@emory.edu Her Cell

phone number is: 404-821-3351.

<u>For patients enrolled at the SCCA:</u> Please contact Carol Dean RN, at least one day in advance of sample acquisition. She will be available for the collection of all study samples and will be coordinating the delivery of the samples for biologic correlative studies in Seattle. Her email is: carol.dean@seattlechildrens.org Her Pager number is: 206-469-0780.

<u>For patients enrolled at other centers:</u> Other centers will follow the sample collection and processing instructions given below.

<u>Sample Collection and Processing Instructions</u>

<u>A. Donor Blood Samples:</u>

All donor samples (collected as stipulated above**:** 24cc into large (8cc) CPT tubes, 4cc into a cyto-chex tube, 5cc into a plasma preparation tube, 5cc into a Red top serum tube and 2.5cc into a PAXgene tube) will be shipped without further processing at ambient temperature to the Central Laboratory on the same day that they are drawn.

B. Recipient Samples:

1. Collection and shipment of Whole Blood using Cyto-Chex BCT Tubes:

Cyto-Chex BCT is a blood collection tube for the preservation of whole blood samples for immunophenotyping by flow cytometry. Cell morphology and surface markers are maintained in these tubes for up to 5 days allowing for blood to be sent to a central location for processing.

1. Fill the Cyto-Chex tube with the indicated amount of whole blood using standard venipuncture technique (See Section 3.7.3 for Blood Draw Volumes based on weight)..

2. Immediately mix the collected tube by gentle inversion 8-10 times.

3. Label the tube .

4. Collected blood shall be shipped WITHOUT CENTRIFUGATION by priority overnight express to the Central laboratory on the same day as collection **at ambient temperature**.

2 .Collection and Processing of PAXgene Tubes:

1.Ensure that the PAXgene RNA tube is at 18-25 ºC prior to use.
2.The PAXgene tube shall be the **last** tube drawn in the phlebotomy
3.The PAXgene tube shall be kept upright during the blood draw if blood is being drawn straight into PAXgene tube (using a butterfly needle). If blood is being drawn into a syringe first, transfer the blood into an upright PAXgene tube.
4. Immediately after the blood draw, the PAXgene tube shall be gently inverted 10 times, and then kept upright.
5. The PAXgene tube shall be shipped by priority overnight express WITHOUT CENTRIFUGATION to the central laboratory on the same day as collection **at ambient temperature**.

3. Collection and Centrifugation of CPT tubes prior to shipment to the Central Reference

Laboratory.

Equipment, Supplies and Reagents Needed:

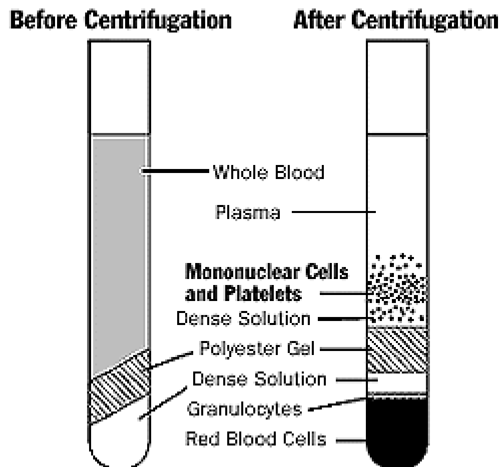
1. BD Vacutainer® CPT™ Tube with Sodium Citrate at room temperature.
2. Centrifuge with a horizontal rotor (swing-out head).

Special Precautions and notes:

1. Make sure tubes are balanced & secured in centrifuge
2. The CPT tube and centrifuge must be at room temperature.

Centrifuging CPT tubes prior to shipment:

1. Completely fill the 8 mL CPT tubes with blood using standard venipuncture technique.
2. Gently invert tube 8-10 times immediately after the blood draw and place tubes UPRIGHT at room temperature until centrifugation.
   Blood samples are required to be centrifuged within two hours of blood collection for best results.
3. If the tubes have been stored upright for any period of time, remix the blood sample immediately prior to centrifugation by gently inverting the tube 8-10 times.
4. Centrifuge tube/blood sample at room temperature (18-25° C) in a horizontal rotor (swing out head) for 30 minutes at 1800 G **with the brake off.** Note that excessive centrifuge speed (over 2000 RCF) may cause tube breakage.
   a. *To calculate centrifuge speed for a given RCF, use the following formula: RPM Speed Setting = (RCF) x (100.000)/ (1.12) x (r)) where r = radius in centimeters, rpm is the rotations per minute and RCF is gravities.*



5. If the samples are collected at the Emory/CHOA or SCCA sites, proceed to PBMC purification (see Appendix 1) immediately after centrifugation. If the samples will be shipped to the Central Laboratory, after centrifugation, invert the centrifuged tubes gently 8-10 times to mix the PBMCs with the plasma. This will not cause re-mixing with the lower layer, because the gel will form a barrier.
6. The centrifuged CPT tubes shall be shipped by priority overnight express to The Central Reference Laboratory on the same day as collection **at ambient temperature.**

4. Collection and Cryopreservation of Serum from BD Red Top Serum (Clot) Tubes:

1. Collect blood into labeled BD Vacutainer® Plus Serum Tubes.

2. Fill the tube with the indicated amount of whole blood using standard venipuncture technique.

3.Immediately mix the collected tube by gentle inversion 8-10 times.

4. Allow the clot to form by keeping the tube upright at room temperature for 60 minutes.

5. Centrifuge the Red top tube at 1800 G for 30 minutes (handle with care after centrifuging). **Centrifugation, aliquoting, and cyropreservation shall occur within 4 hours of sample acquisition.**

6. Aliquot 0.5mL of serum into six labeled and barcoded 1 mL Nunc tubes (note: the desired aliquots are not always obtained).

7. Discard clot.

8. Store serum in a -80°C Freezer. These will be batch-shipped to the Central Laboratory ON DRY ICE on Day +30, Day +100, Day +365 according to the instructions below.

5. Collection and Cryopreservation of Plasma using PPT Plasma Tubes

1. The BD PPT™ Tube needs to be at room temperature (18-25°C) and properly labeled for patient identification.
2. Collect blood into the BD PPT™ Tube using your institution's recommended procedure for standard venipuncture technique and sample collection.
3. After collection of whole blood in the BD PPT™ Tube, gently invert the BD PPT™ Tube 8 - 10 times.
4. After mixing, store the BD PPT™ Tube upright at room temperature until centrifugation. Blood samples need to be centrifuged within two (2) hours of blood collection for best results. Centrifuge the BD PPT™ Tube/blood specimen at room temperature (18-25°C) for a minimum of 20 minutes at 1,100 RCF (Relative Centrifugal Force). WARNING: Excessive centrifuge speed (over 10,000 RCF) may cause tube breakage and exposure to blood and possible injury. To calculate the correct centrifuge speed for a given RCF, use the following calculator. Simply enter two of the three values in the calculator and press the calculate button. http://www.bd.com/vacutainer/s/molecular/ppt/procedure.asp#forcecalc
5. Remove the plasma layer being careful not to disrupt cell layer. Plasma will be saved.
6. Aliquot 0.5ml of plasma into five labeled and barcoded 1mL Nunc tubes. Be careful not to disrupt cell layer.
7. Store plasma in -80 Freezer. These will be batch-shipped to the Central Laboratory ON DRY ICE on Day +30, Day +100, Day +365 according to the instructions below.

6. Collection and Cryopreservation of Urine: Pour urine into a sterile 50mL Falcon tube.

1. Centrifuge the specimen for 30mins at 1800 G.
2. Aliquot 1mL of urine supernatant into labeled and barcoded 1mL NUNC tubes
    a. Repeat this step until 5 aliquots have been collected
2. Store the urine supernatant aliquots at -80ºC prior to shipment to the Central Reference Biorepository. These will be batch-shipped to the Central Biorepository ON DRY ICE on Day +30, Day +100, Day +365 according to the instructions below.

7. Sample of harvested hematopoietic stem cells (bone marrow or leukopheresis products): The 5cc sample of marrow of PBSC graft will be withdrawn from the donor product into a 15mL sterile polyethylene Falcon tube (or the equivalent). It will then be sent to the Central Reference Laboratory for flow cytometric analysis of the graft content. This sample will be drawn after arrival at the recipient center and shipped to the Central Reference Laboratory at ambient temperature on the day of the graft infusion (Day 0).

8. **The empty bag or syringe in which the stem cell product was infused:** This will be retrieved by the study team and sent to the Central Reference Laboratory at ambient temperature, without further processing, on the day of the graft infusion (Day 0).

All samples are shipped to one of two Central Reference Biorepositories:
Samples collected at the University of Florida/Shands site: Shipped to The Emory Transplant Center Biorepository, (address below).

Samples collected at all other sites: Shipped to the Ben Towne Center for Childhood Cancer Research, Attention Kayla Betz (address below).

1. The following samples are to be shipped at AMBIENT TEMPERATURE, the same day as the blood draw, by FEDEX overnight express:
- **a) Cytochex tubes (NOT Centrifuged)**
- **b) PAXGene Tubes (NOT Centrifuged)**
- **c) CPT Tubes (These ARE centrifuged)**

2. As noted above, the marrow or PBSC graft sample will be shipped from the recipient center to one of the Central Reference Biorepositories on Day 0.

3. As noted above, the serum, plasma urine will be cryopreserved and stored at -80°C at the participating center, with shipment to one of the Central Reference Biorepositories ON DRY ICE on Day +30, Day +100, Day +365. Details of shipment are given detailed below.

<u>Shipping Restrictions</u>
<u>Ambient</u> Shipments: The uncentrifuged stem cell product, uncentrifued cyto-chex tubes, uncentrifuged PAXgene Tubes, and centrifuged CPT tubes drawn between Monday-Friday are required to be shipped the same day they are drawn for next day delivery (including Saturday delivery). Samples drawn on a Saturday or Sunday shall be shipped on Monday for Tuesday delivery. An email to appropriate biorepository contact confirming the shipment (with the tracking number) must also be sent.

<u>Shipments of Frozen Serum, Plasma and Urine Samples</u>: Frozen samples can be sent on Monday, Tuesday or Wednesday. These samples are required be shipped ON **PLENTY OF** DRY ICE to the appropriate Central Reference Biorepository. These batch-shipments will be made when the patient has reached Day +30, Day +100 and Day +365 post-transplant.

All shipments will be sent FedEx Priority Overnight (International shipments: FedEx International First).

<u>For University of Florida/Shands:</u>

Prior to shipping, send an email to Yvonne Suessmuth (yvonne.suessmuth@emory.edu) notifying her of an impending shipment. Include the Fed Ex tracking number in the email. Yvonne's phone #'s: Cell: 404-821-3351.

Samples are to be shipped to the Emory Central Reference Biorepository at:

Emory Transplant Center Biorepository
Aba 2 Study
101 Woodruff Circle, #5014-WMB
Emory University
Atlanta, GA 30322
Office Phone: 404-727-8355
Cell: 404-821-3351

FOR ALL OTHER SITES, Prior to shipping, send an email to Kayla Betz (Kayla.betz@seattlechildrens.org) notifying her of an impending shipment. Include the Fed Ex tracking number in the email. Kayla's phone #'s: Cell: 206-459-9659 Office: 206-884-4024.

Samples are to be shipped to the Seattle Children's Central Reference Biorepository at:

Ben Towne Center for Childhood Cancer Research
Attn: Kayla Betz
Aba 2 Study
1100 Olive Way, Suite 100
Seattle, Washington, 98101
Phone number: 206-459-9659 (cell)
Office Phone number: 206-884-4024

3.7.5 Samples from Healthy Volunteers: Blood samples will be obtained from healthy volunteers at Emory University and the Seattle sites, including University of Washington/Seattle Children's Hospital/Seattle Cancer Care Alliance/Seattle Children's Research Institute, to be used for quality control for the flow cytometry analysis. Further detail is provided in Appendix 3.

3.8. Treatment

3.8.1. Donor Selection and Hematopoietic Stem Cell Grafts:

In selecting a donor, priority should be given to donors who are most closely matched at the A, B, C and DRB1 loci. Donors may have a single mismatch (i.e. be a 7/8) and this mismatch may be at the allele or antigen level; however, donors with allele level disparity should be given preference over those with antigen level disparity. The use of mismatched donors in which disparity is only in the host versus graft direction (because of recipient homozygosity) is discouraged because of the potentially heightened risk for graft rejection. Centers may perform extended typing (e.g. DQB1 and DPB1) according to institutional practices and use these results in selecting donors; however, it is recommended that this extending typing be used only to select between donors who are equally well matched with the recipient at the A, B, C and DRB1.

Either bone marrow or peripheral blood grafts may be employed. A minimum cell dose of $2 * 10^8$ nucleated cells/kg (recipient weight) is recommended for transplant. In planning marrow harvests in the setting of donor-recipient ABO incompatibility, the loss of cells due to erythrocyte or plasma depletion should be taken into consideration.

3.8.2. Pre-transplant conditioning:

Treating physicians may select one of four conditioning approaches (outlined below) for use and should choose the regimen that he or she thinks is best suited for the patient and the patient's disease. Patients who have had prior autologous HSCT, however, must be conditioned with melphalan and fludarabine.

1) Total body irradiation (TBI) based conditioning:

Centers may employ the myeloablative TBI-based regimens they ordinarily employ for patients with hematologic malignancies as long as these regimens meet the following criteria:

a) The total dose of TBI is at least 1200 cGy and not more than 1400 cGy.
b) It is administered in no less than 6 fractions.
c) It includes one or two chemotherapy agents (e.g. cyclophosphamide, etoposide, thiotepa)
d) It does not include a lymphocyte depleting antibody (e.g. antithymocyte globulin or alemtuzumab)

Some degree of lung shielding during TBI administration is encouraged, using an electron boost to the chest wall to preserve full dosing to the ribs.

Cranial and testicular boosts may be administered prior to the start of conditioning at the discretion of the treating physician.

2) Busulfan and Cyclophosphamide (recommended for myeloid malignancies only):

In recognition of the fact that varying doses of cyclophosphamide are used in combination with high dose busulfan, centers may use the regimen they ordinarily employ for patients with myeloid malignancies as long as the regimen meets the following criteria:

a) The initial regimen (i.e. before adjustments are made for pharmacokinetic testing results) includes the equivalent* of sixteen, 0.8 mg/kg IV/PO doses (q 6 hours) of busulfan, administered q 6 hours.
b) The regimen uses two daily 60 mg/kg doses or four daily 50 mg/kg doses of cyclophosphamide.
c) It does not include a lymphocyte depleting antibody (e.g. antithymocyte globulin or alemtuzumab)

Adjustments in dosing of busulfan and cyclophosphamide for obesity may be made according to institutional standards.

Busulfan pharmacokinetic testing: Pharmacokinetic testing is recommended, but should be performed according to institutional standards. Pre-transplant test dosing may be used.

***Modifications in busulfan administration**: The busulfan administration may be modified according to institutional practices as follows:

1) Initial doses as high as 1.2 mg/kg q 6 hr (or 4.8 mg/kg/day see #2) may be used for younger children.
2) Daily doses (e.g. 3.2 mg/kg/day rather than 0.8 mg/kg/day) may be used in place of q 6hr dosing.
3) The dosing may be adjusted based on the results of pharmacokinetic testing. This can involve a change in the size of the individual doses or for patients who are getting q 6hr dosing where the pharmacokinetic testing result exceeds the upper limit of the targeted range, the targeted total dose (e.g. 16 * targeted per dose AUC) may be apportioned into 14 or 15 doses rather than 16 as part of the adjustment.

Hydration and MESNA administration: The administration of hydration and cyclophosphamide to prevent hemorrhagic cystitis from cyclophosphamide will follow institutional practices.

Seizure prophylaxis: An anti-convulsant will be administered according to institutional guidelines to prevent busulfan-induced seizures.

3) Melphalan and Fludarabine:

Fludarabine	25 mg/m2/dose IV qd (5 doses)	Days -6 to -2
Melphalan*	70 mg/m2/dose IV qd (2 doses)*	Day -3 and -2

Fludarabine and Melphalan should each be infused over 30 minutes.

Adjustments in dosing of melphalan and fludarabine for obesity may be made according to institutional standards.

*Melphalan dosing as high as 90 mg/m^2/dose IV qd (2 doses) may be used at the discretion of the treating center.

Melphalan and Fludarabine regimens shall not include a lymphocyte depleting antibody (e.g. antithymocyte globulin or alemtuzumab).

4) Busulfan and Fludarabine (recommended for myeloid malignancies):

Fludarabine	40 mg/m^2/dose IV qd-4 doses	Days -9 to –6
Busulfan	0.8 mg/kg/dose IV/PO q6H-16 doses or the equivalent*	Days -5 to –2

Fludarabine should each be infused over 30 minutes and busulfan over two hours.

Adjustments in dosing of busulfan and fludarabine for obesity may be made according to institutional standards.

Busulfan and Fludarabine regimens shall not include a lymphocyte depleting antibody (e.g. antithymocyte globulin or alemtuzumab).

Busulfan pharmacokinetic testing: Pharmacokinetic testing is recommended, but should be performed according to institutional standards. Pre-transplant test dosing may be used.

***Modifications in busulfan administration**: The busulfan administration may be modified according to institutional practices as follows:

1) Initial doses as high as 1.2 mg/kg q 6 hr (or 4.8 mg/kg/day see #2) may be used.If initial dosing is greater than 1.2 mg/kg q 6 hr, contact the PI for allowance.
2) Daily doses (e.g. 3.2 mg/kg/day rather than 0.8 mg/kg/day) may be used in place of q 6hr dosing.
3) The dosing may be adjusted based on the results of pharmacokinetic testing. This can involve a change in the size of the individual doses or for patients who are getting q 6hr dosing where the pharmacokinetic testing result exceeds the upper limit of the targeted range, the targeted total dose (e.g. 16 * targeted per dose AUC) may be apportioned into 14 or 15 doses rather than 16 as part of the adjustment.

Seizure prophylaxis: An anti-convulsant will be administered according to institutional guidelines to prevent busulfan-induced seizures.

c. Allogeneic Stem Cell Infusion:
The donor graft will be infused on day 0. Institutional guidelines should be followed for preparation and infusion of grafts.

3.8.3. Graft Versus Host Disease Prophylaxis and Treatment (with guidelines for dose modifications)

Patients assigned to the standard arm and those assigned to the investigational arm will receive a calcineurin inhibitor (either cyclosporine or tacrolimus) and methotrexate for GVHD prophylaxis. In addition, patients assigned to the investigational arm will receive abatacept, while those assigned to the standard arm will receive a placebo consisting of an equal volume of normal saline solution.

Calcineurin inhibitor (cyclosporine or tacrolimus) administration will commence no later than day –2 (at least 36 hours before the stem cell infusion); cyclosporine doses will be adjusted to maintain a level of 100-300 ng/ml. This range assumes monitoring by mass spectrometry. If an immunoassay is used instead, the equivalent range for that immunoassay should be used ; tacrolimus doses will be adjusted to maintain a level of 5-15 ng/ml. This range should be used regardless of the type of assay used to monitor levels. Centers are encouraged to administer cyclosporine by continuous infusion, but intermittent infusion will be permitted. Once the patient can tolerate oral medications, the CNI should be converted to an oral formulation. Patients shall receive full dose CNI therapy through at least day 100 as tolerated. Tapering off the dose may be initiated between days 100 to 180 at the discretion of the treating physician. Once the taper has been initiated, it will be gradually tapered (25-40% per month). It can be discontinued once the dose is 25% or less of the starting dose.

The CNI may be interrupted or dose reduced at the discretion of the treating physician for renal toxicity, poorly controlled hypertension, neurotoxicity and other serious toxicities.

Methotrexate: Methotrexate will be given at a dose of 15 mg/m^2 IV on day 1, and a dose of 10 mg/m^2 IV on days 3 and 6 and 11. Dosing shall be based on actual weight. For patients with a body mass index of 35 or higher, however, the dose may be adjusted for obesity according to institutional practices. The day 1 dose shall not be administered until 24 hours following

completion of the stem cell infusion. Monitoring of drug levels and leucovorin are permitted according to local institutional guidelines.

As with cyclosporine and tacrolimus, methotrexate dose modifications will be made at the discretion of the treating physician. The following guidelines may be used to guide the treating physician.

***The methotrexate dose may be reduced by 50% in the following situations**:*

1. *Serum creatinine 2-3 times baseline.*
2. *Serum ALT 5-10 times the upper limit of normal or **direct** bilirubin 2.1-4.0 mg/dL.*
3. *Oropharyngeal mucositis that is causing symptomatic, but non-life threatening airway obstruction*

***The methotrexate dose may be held in the following situations**:*

1. *Serum creatinine >3 times baseline.*
2. *Serum ALT >10 times the upper limit of normal or **direct** bilirubin >4.0 mg/dL.*
3. *Oropharyngeal mucositis that is causing life threatening airway obstruction.*
4. *Significant third-spacing of fluid (i.e. pleural effusion, pericardial effusion or ascites).*

***If, however, more than one dose of methotrexate is held, mycophenolate mofetil shall be started in place of methotrexate**:* 15 mg/kg IV or PO (to maximum of 1000 mg) q 8hr through day +30. It may be continued longer at the discretion of the treating physician, if there is evidence of GVHD.

Distribution and Administration of Study Drug:

Abatacept will be distributed by the Emory University Investigational Drug Service Pharmacy, which will serve as the central pharmacy for the trial. After an initial patient is enrolled at a site, a starter supply of abatacept sufficient for all four doses for one patient will be shipped overnight to the treating center. A re-order form will be included with the starter supply. It will be each site's responsibility to order re-supplies of abatacept using the provided re-order form. The placebo, normal saline, will not be delivered to treating sites. Each site will use their local supply of normal saline to prepare placebo doses.

Dosing of Abatacept: Abatacept will be administered to patients assigned to the investigational arm at a dose of 10mg/kg based on dose calculation weight with a maximum of 1000 mg on days -1, +5, +14, +28. In cases where the calculated dose is less than or equal to 110% of a simple multiple of a 250 mg vial: 250 mg (275mg), 500 mg (550 mg) or 750 mg (825 mg), the dose may be rounded down to the nearest multiple. No rounding up of the abatacept dose is permitted.

Abatacept will be supplied as a lyophilized powder in single use 250 vials. Each vial must be reconstituted with 10 mL of Sterile Water for Injection, USP, using only the silicone-free disposable syringe provided. The concentration of abatacept following this step will be 25 mg/mL. If the powder is accidentally reconstituted using a siliconized syringe, the solution may

develop a few translucent particles. Discard any solutions prepared using siliconized syringes. The vial should then be vented with a needle to dissipate any foam that may be present. The solution should be clear and colorless to pale yellow. The reconstituted solution should then be further diluted in 0.9% Sodium Chloride USP (Normal Saline) to a final volume of 100 mL, with a concentration not to exceed 10mg/mL. Slowly add the reconstituted solution into the infusion bag or bottle using the same silicone-free disposable syringe provided with each vial. Gently mix. The final concentration of abatacept in the bag or bottle will depend upon the amount of drug added, but will be no more than 10 mg/ml.

The placebo will consist of 100 ml of 0.9% Sodium Chloride. Normal saline is being used as a placebo, since abatacept is reconstituted in normal saline, since it is clear and colorless after reconstitution and since it has been used as the placebo in previous randomized controlled trials of abatacept. Since abatacept has not been associated with high rates of infusional reactions and is a clear, colorless liquid, saline should be an effective placebo.

For patients in Stratum #1 (the randomized, double-blind stratum for those receiving an 8/8 HLA matched transplant), the study drug (abatacept or placebo) shall be labeled in such a manner as to maintain the study blind. For patients in Stratum #2 (the single-arm stratum for those receiving a 7/8 HLA matched transplant), all patients will be receiving abatacept, and thus the study drug will be labeled as “abatacept”.

The study drug (abatacept or placebo) shall be infused intravenously over 1 hour. Abatacept must be administered with an infusion set and a sterile, non-pyrogenic, low-protein-binding filter (pore size of 0.2 um to 1.2 um)

Premedication with IV diphenhydramine shall be performed 30 minutes prior to each study drug dose. (It will not be considered a protocol deviation if patient receives premedication up to 60 minutes prior to each study drug dose) Given the rare but serious side effect of anaphylaxis to abatacept, epinephrine and other drugs to treat anaphylaxis shall be readily available to patients when they are receiving study drug. (It will not be a protocol deviation if patient receives PO diphenhydramine)

The study drug must be held for the following infections:

1) *Active serious invasive viral disease (e.g. CMV pneumonitis or enterocolitis).*
2) *Active invasive fungal infections, including candidal infections, mold infections and pneumocystis.*
3) *Active opportunistic protozoal and helminthic infections, such as toxoplasmosis.*
4) *Imminently life threatening bacterial infections, such as septic shock.*

In general, all remaining doses must be withheld for patients with imminently life threatening bacterial infections, however, if the infection responds promptly to treatment, the study drug may be resumed and subsequent doses administered. Also, the day +28 dose may be delayed up until day +42 to allow bacterial infections to clear. The day -1, +5 and +14 doses, however, may only be withheld-they may not be given after a delay.

It is recommended that the study drug ***not*** *be held for less severe infections, including the following:*

1) CMV or EBV reactivation.
2) HSV stomatitis
3) Bacteremia when the patient is clinically stable.
4) Superficial fungal infections.
5) Fever without documented infection.

*If **lymphoproliferative disease** is diagnosed prior to day 28, all remaining doses must be held.*

If a patient has an anaphylactic reaction, other systemic allergic reaction (e.g. urticarial rash), or other severe infusional reaction to any study drug dose, all remaining study drug doses must be held.

If the study drug is held as stipulated above, the patient's treatment shall continue to adhere to the treatment protocol, including scheduled research blood draws, unless the patient's physician determines that it is in the patient's best interest to discontinue protocol treatment or the patient, the patient's parent or the patient's guardian refuses further protocol therapy (please see section 3.8.6).

2) **Treatment of acute and chronic graft versus host disease**: Treatment of patients who develop graft versus host disease, either acute or chronic, will be prescribed by the treating physician. **For patients still on prophylaxis when their GVHD develops, the administration of prophylactic agents, including abatacept, may be discontinued at the discretion of the treating physician**.

3.8.4 Post-transplant FLT3 inhibitors: For patients who are treated for a leukemia with FLT3-ITD, post-transplant FLT3 inhibitors may be administered at the discretion of the treating physician. It should, however, not be started prior to post-transplant day 50.

3.8.5 Post-transplant tyrosine kinase inhibitors (TKI) in patients with BCR-ABL positive leukemias and other post-transplant treatments to decrease the risk of relapse: TKI may be administered post-transplant to patients with BCR-ABL positive leukemias at the discretion of the treating physicians. Other agents that are used post-transplant to decrease the risk of relapse must be approved by the Study PI, given the impact that some of these agents may have on immune activation.

3.8.6 Taking patients off protocol and study:

Patients who meet any of the following criteria will be taken off treatment protocol.

a) Relapse.
   i. Malignancy Relapse is defined by either morphological or standard cytogenetic evidence of acute leukemia or MDS consistent with pre-transplant features, or radiologic evidence of lymphoma, documented or not by biopsy.
   ii. Minimal Residual Disease (MRD) in the absence of morphologic or standard cytogenetic evidence is not defined as relapse in this study.
   iii. Progression of Disease applies to patients with lymphoproliferative diseases (lymphoma or chronic lymphocytic leukemia) not in remission prior to transplantation. The event is defined as increase in size of prior

sites of disease or evidence of new sites of disease, documented or not by biopsy.

b) Refusal of further protocol therapy by patient/parent/guardian.
c) Completion of planned therapy.
d) Physician determines it is in patient's best interest.

Patients will be left on study, meaning that laboratory samples and their data will continue to be collected.

(2) Patients who meet any of the following criteria will be taken off study.

a) Death.
b) Completion of follow-up.
c) Lost to follow-up.
d) Withdrawal of consent for any further data submission

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3.8.7 Protocol Deviations:

Circumstances may arise, where it is necessary for patient safety to deviate from the study protocol. When feasible, treating centers should contact the protocol chair or clinical vice chair beforehand to discuss the intended deviations. All deviations, whether approved beforehand or not, are required to be reported within 7 working days by completing and submitting the protocol deviation form electronically. Centers are also required to note deviations in patient medical records and report them to their local IRB in accordance with local policies. All protocol deviations will be compiled centrally and reported to the Dana-Farber Cancer Institute IRB and the DSMC.

3.8.8 Drug Information (drugs listed alphabetically):

ABATACEPT: (Orencia, CTLA4-Ig)

Pharmacology: Abatacept is a soluble fusion protein that consists of the extracellular domain of human cytotoxic T-lymphocyte antigen 4 (CLTA-4) linked to the modified Fc (hinge, CH2 and CH3 domains) portion of human immunoglobulin G1 (IgG1). Abatacept is produced by recombinant DNA technology in a mammalian cell expression system. The apparent molecular weight of abatacept is 92 kilodaltons. Abatacept, a selective costimulation modulator, inhibits T cell (T lymphocyte) activation by binding to CD80 and CD86, thereby blocking interaction with CD28. In vitro, abatacept decreases T cell proliferation and inhibits the production of the cytokines tumor necrosis factor (TNF) and iterleukin-2. In other settings the terminal half-life is approximately 14 days.

Formulation and Stability: Abatacept is supplied as a sterile, white, preservative-free, lyophilized powder for parenteral administration. Following reconstitution with 10mL of Sterile Water for Injection, USP, the solution of abatacept is clear, colorless to pale yellow, with a pH range of 7.0-8.0. Each single-use vial of abatacept provides 250mg abatacept, 500mg maltose, 17.2 mg monobasic sodium

phosphate, and 14.6 mg sodium chloride for administration. Abatacept must be administered with an infusion set and a sterile, non-pyrogenic, low-protein-binding filter (pore size of 0.2 um to 1.2 um)

Supplier: Abatacept, who's trade name is Orencia, will be supplied by Bristol Myers Squibb and distributed through the Investigational Pharmacy at Emory University.

Toxicity: As an immunosuppressant its most serious side-effect is infection. The closely related agent, belatacept, which is being developed for use in solid organ transplantation, has been associated with post-transplant lymphoproliferative disorder. When used for patients with rheumatoid arthritis the incidence of serious infection is relatively low (<5%). The intravenous infusion is generally well tolerated. It has been associated with a low incidence headache and nausea. It is associated with a rare but serious side effect of hypersensitivity reactions, which has also included anaphylaxis.

BUSULFAN (Busulfex®, Myleran®, BU)

Pharmacology: Busulfan is a polyfunctional alkylating agent. It interferes with the normal function of DNA by alkylating intracellular nucleophiles and cross linking DNA strands. Busulfan is cell cycle phase non-specific. It is well absorbed orally and is metabolized by the liver. Drugs that induce hepatic metabolism (e.g., phenytoin) increase clearance and those that inhibit hepatic metabolism (e.g., itraconazole) may decrease clearance. The plasma half life is $\approx$ 2.5 hours in adults, but children may have higher clearances.

Formulation and Stability: Busulfan is available as a solution for injection as 6 mg/ml in 10 ml ampoules of 60 mg each and available as 2 mg oral tablets

Supplier: Solution for injection (ESP Pharma, Edison, NJ) is commercially available. Oral tablets (GlaxoSmithKline, Research Triangle Park, NC) are commercially available

Toxicity: Acute dose limiting toxicity is myelosuppression including leukopenia, thrombocytopenia and anemia. This effect is delayed with a nadir of 14-21 days. Some patients may develop bone marrow fibrosis. Nausea and vomiting are generally mild. Other GI symptoms include diarrhea, anorexia and associated weight loss. Seizures are associated with high doses, including those used in transplantation. Other side effects include liver dysfunction, skin hyperpigmentation (Addisonian-like syndrome, often unassociated with impaired corticosteroid production), skin rash, gynecomastia, sterility, cataracts and alopecia. Secondary cancers have occurred. "Busulfan lung", manifested by diffuse interstitial pulmonary fibrosis, persistent cough, fever, rales and dyspnea may occur, most commonly after high doses or prolonged therapy.

CYCLOPHOSPHAMIDE (Cytoxan®, CTX)

Source and Pharmacology: Cyclophosphamide is a nitrogen mustard derivative. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. Cyclophosphamide is cell cycle, phase non-specific. Cyclophosphamide is well absorbed from the GI tract with a bioavailability of > 75%. Cyclophosphamide is a prodrug that requires activation. It is metabolized by mixed-function oxidases in the liver to 4-hydroxycyclophosphamide, which is in equilibrium with aldofosfamide. Aldofosfamide

spontaneously splits into cyclophosphamide mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxycyclophosphamide may be enzymatically metabolized to 4-ketocyclophosphamide and aldofosfamide may be enzymatically metabolized to carboxyphosphamide, which are generally considered to be inactive. Cyclophosphamide and its metabolites are excreted mainly in the urine. Dosage adjustments should be made in patients with a creatinine clearance of < 50 ml/min.

Formulation and Stability: Cyclophosphamide is available in vials containing 100, 200, 500, 1000 and 2000 mg of lyophilized drug and 75 mg mannitol per 100 mg of cyclophosphamide. Cyclophosphamide is available in 25 and 50 mg tablets. Both forms of the drug can be stored at room temperature. The vials are reconstituted with 5, 10, 25, 50 or 100 ml of sterile water for injection respectively to yield a final maximum concentration of 20 mg/ml. Reconstituted solutions may be further diluted in either 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically stable for 24 hours at room temperate but contain no preservative, so they should be used within 24 hours of preparation.

Supplier: Commercially available (Bristol-Myers Squibb, Princeton, NJ)

Toxicity: Dose limiting toxicities of cyclophosphamide are bone marrow suppression (nadir 8-14 days) and cardiac toxicity. Cardiac toxicity is typically manifested as arrhythmias, congestive heart failure, cardiac necrosis or hemorrhagic myocarditis and can be fatal. Hemorrhagic cystitis may occur and necessitates withholding therapy. The incidence of hemorrhagic cystitis is related to cyclophosphamide dose and duration of therapy. Forced fluid intake and/or the administration of MESNA decreases the incidence and severity of hemorrhagic cystitis. Fluid retention due to SIADH may occur. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on dosage), diarrhea, anorexia, alopecia, immunosuppression and sterility. Pulmonary fibrosis, transiently blurred vision, anaphylaxis and secondary neoplasms have been reported rarely. Gonadal dysfunction is dose-related. The prepubertal gonad is less susceptible. Hormonal function is generally preserved, especially in the male; fertility is mainly affected (spermatogenesis in the male, follicle formation in the female). Sterility may be partially reversible. Gonadal effects of administration during puberty are uncertain; the risk of sterility may be increased.

<u>CYCLOSPORINE</u> (Gengraf, Neoral, CsA)

Source and Pharmacology: Cyclosporine is an immunosuppressive agent produced by Tolypocladium inflatum Gams or Cylindrocarpon lucidum Booth. Cyclosporine is a nonpolar, cyclic polypeptide antibiotic consisting of 11 amino acids. It is thought that cytotoxic T-lymphocytes (CTLs) together with helper T-cells, natural killer cells and the release of lymphokines, including interleukin-2 (IL-2), play a major role in graft rejection and graft versus host disease. The exact mechanism(s) of the immunosuppressive action of cyclosporine has not been fully elucidated. Experimental studies suggest that the primary actions in preventing graft rejection and in establishing immunologic unresponsiveness to allografts are (1) the prevention of precursor CTL from acquiring responsiveness to IL-2, and thereby inhibiting the activation of CTLs; (2) the inhibition of production of IL-2 and other cytokines, the signals that lead to proliferation and activation of effector cells. In contrast, cyclosporine apparently leaves B cells and macrophage function essentially unimpaired. The selective effect on the immune system is largely unexplained. Studies seem to indicate

that at the intracellular level cyclosporine interferes with the calcium-dependent activation of various enzymes systems including the enzymatic cascades accounting for IL-2 production and IL-2 receptor formation.

Formulation and Stability: Cyclosporine oral solution is supplied in 50 ml bottles. It has a clear, yellow, oily appearance. Each ml contains 100 mg of cyclosporine and ethanol 12.5% by volume dissolved in an oil compound as the vehicle. Prior to oral administration, the appropriate dose of the CsA solution must be further diluted with milk, chocolate milk, or orange juice, stirred well, and taken by the patient at once. To ensure adequate dose administration, the glass should be rinsed with more milk or orange juice and given to the patient. It should not be allowed to stand for any length of time.

Cyclosporine is also supplied as soft gelatin capsules (Neoral, Gengraf) in a 25 mg and 100 mg strength. Both capsules contain a maximum of 12.7% ethanol, USP dehydrated.

Intravenous CsA is supplied in a 5 ml sterile ampule for intravenous administration. Each ml contains 50 mg of CsA, 650 Cremophor IL (polyoxethylated castor oil) and ethanol 32.9% by volume. Cyclosporine concentrate for injection diluted to a final volume of 2- 2.5 mg/ml is stable for 24 hours in 5% dextrose. Dilutions of the drug in dextrose or sodium chloride should not require protection from light. The solution should be checked for particulate matter and discoloration. The solution may be administered as a continuous infusion over 24 hours, or divided as short-term infusions (2 hours) every 12 hours.

Supplier: Commercially available (Novartis and Abbott)

Toxicity: The most frequent and clinically important adverse effect of cyclosporine is nephrotoxicity. Elevation of BUN and serum creatinine concentrations resulting from cyclosporine therapy appear to be dose-related, may be associated with high trough concentrations of the drug, and are usually reversible with discontinuation of the drug. Clinical manifestations may include fluid retention, edema, and in some cases, hyperchloremic, hyperkalemic metabolic acidosis. The risk of renal toxicity increases with administration of other potentially nephrotoxic agents. Hypertension may develop, generally within the first few weeks of therapy and affects both systolic and diastolic blood pressure. Some evidence suggests that the hypertension results from the renal vasoconstrictive effects of the drug. Hypertension may respond to dosage reduction and/or antihypertensive therapy. Adverse nervous system effects occur frequently, with tremor occurring in a large percentage of patients. Tremor may be manifested as fine hand tremor, usually is mild in severity, may improve despite continued therapy, and/or may be alleviated with dosage reduction. Seizures, headache, paresthesia, hyperesthesia, flushing and confusion have also been reported occasionally. Other adverse effects include hirsutism, gingival hyperplasia, and hepatotoxicity. Adverse GI effects occur frequently during cyclosporine therapy, including diarrhea, nausea, vomiting, anorexia and abdominal discomfort. Infectious complications have occurred frequently. Adverse hematologic effects have occurred occasionally during cyclosporine therapy.

FLUDARABINE (FLU, Fludara®)

Source and Pharmacology Fludarabine phosphate is a synthetic purine antimetabolite, a fluorinated nucleotide analog of the antiviral agent adenine arabinoside (Ara-A). Fludarabine undergoes rapid dephosphorylation to 2-fluoro-ara-A and then is phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate metabolite, 2-fluoro-ara-ATP. Through its inhibition of ribonucleotide reductase and DNA

polymerase, this active metabolite ultimately inhibits DNA synthesis. The mean distribution and terminal half-lives of 2-fluoro-ara-A are approximately 0.6 and 10 hours, respectively. The mean total plasma clearance is 8.9 L/h/m^2 and the mean volume of distribution is 98L/m^2. Approximately 23% of a dose is excreted in the urine as unchanged 2-fluoro-ara A (with dosages of 18-25 mg/m^2/day for 5 days).. Total body clearance of 2-fluoro-ara-A has been shown to be inversely correlated with serum creatinine. Renal clearance appears to become more important at higher doses, with approximately 41-60% of the dose being excreted as the active metabolite in the urine with dosages of 80-260 mg/m^2. An inverse correlation also exists between absolute granulocyte count and the area under the concentration x time curve (AUC).

Formulation and stability: Fludarabine is supplied as a white, lyophilized solid phase in single-dose vials containing 50 mg of fludarabine phosphate, 50 mg of mannitol and sodium hydroxide (to adjust the pH to 7.7). The intact vials should be stored under refrigeration. This vial should be reconstituted with 2mL of Sterile Water for Injection USP, resulting in a final concentration of 25 mg/ml. The solution can then be further diluted in 250 mL 5% Dextrose Injection USP.

Supplier: Commercially available (Berlex**,** Richmond, CA)

Toxicity : The major dose-limiting toxicity of fludarabine is myelosuppression. Nausea and vomiting are usually mild. Side effects reported commonly include, anorexia, fever and chills, alopecia and rash. Neurotoxicity has been observed at high doses and can be manifested by somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness and coma; neurotoxicity is very rare in the doses used for hematopoeitic cell transplantation. Neurotoxicity is usually delayed, occurring 21-60 days after the completion of a course of therapy and may be irreversible. Side effects reported less commonly include diarrhea, stomatitis, increased liver function tests, liver failure, chest pain, arrhythmias and seizures. Pulmonary toxicity includes allergic pneumonitis characterized by cough, dyspnea, hypoxia and pulmonary infiltrates. Drug induced pneumonitis is a delayed effect, occurring 3-28 days after the administration of the third or later course of therapy. Administration of corticosteroids usually results in resolution of these symptoms.

MELPHALAN HYDROCHLORIDE (Alkeran®L-PAM)

Source and Pharmacology: Melphalan hydrochloride is a chemotherapy drug belonging to the class of nitrogen mustard alkylating agents. Otherwise known as L-Phenylalanine Mustard, or L-PAM, melphalan is a phenylalanine derivative of mechlorethamine. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. Melphalan is cell cycle phase non-specific. Melphalan hydrochloride is given intravenously and is eliminated rapidly (half life of less than two hours) primarily by hydrolysis (the metabolites are inactive) in the blood.

Formulation and Stability: Melphalan is available in vials containing lyophilized drug, which can be stored at room temperature. The drug is reconstituted in 0.9% NaCl solution. Once reconstituted, melphalan should be administered within 60 minutes, since it is unstable in solution.

Supplier: Commercially available (GlaxoSmithKline)

Toxicity: Dose limiting toxicities of melphalan are bone marrow suppression.. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on dosage), diarrhea, anorexia, alopecia, immunosuppression, lung injury, acute and chronic, hypogonadism and infertility. Secondary neoplasms and anaphylaxis have been reported. Gonadal dysfunction is dose-related. The prepubertal gonad is less susceptible. .

<u>MESNA</u> (Sodium 2-Mercaptoethane Sulfonate, Mesnex)

Source and Pharmacology: MESNA is a synthetic sulfhydryl (thiol) compound. It contains free sulfhydryl groups that interact chemically with urotoxic metabolites of the oxazaphosphorines, ifosfamide and cyclophosphamide. Within 1 hour of administration, MESNA is completely oxidized to DiMESNA, a totally inert compound. There is little or no tissue penetration. Following glomerular filtration DiMESNA is rapidly reduced in the renal tubules back to MESNA, which inactivates the oxazaphosphamides, thus preventing bladder toxicity. Oral bioavailability is ≈50%. Mesna and mesna disulfide are excreted primarily via the urine.

Formulation and Stability: Available in ampules containing 100 mg/ml of solution containing disodium edentate 0.25 mg and sodium hydroxide to adjust pH 6.5 to 8.5 in water for injection. Supplied on 200, 400 and 1000 mg ampules. Store intact ampules at controlled room temperature (15º-30ºC). MESNA is not light-sensitive, and intact ampules are stable for five years from manufacture. For this protocol, further dilution is not required. If further dilution is desired, MESNA can be mixed in the following intravenous infusion solutions with less than 5% decomposition over 24 hours: 5% dextrose, 5% dextrose in 0.45 sodium chloride, 0.9% sodium chloride and lactated Ringer's.

Supplier: Commercially available (Bristol-Meyers Squibb, Princeton, NJ)

Toxicity: Mesna is generally well tolerated. It may cause nausea and vomiting, and a bitter taste during IV administration. At 60-70 mg/kg IV daily x 4, abdominal pain, rash, headache, limb and joint pain, lethargy, diarrhea, and transient hypotension have been encountered. Mesna may cause false positive urine dipstick readings for ketones.

<u>METHOTREXATE</u> (MTX, amethopterin, Trexall®)

Source and Pharmacology: A folate analogue, which reversibly inhibits dihydrofolate reductase, the enzyme that reduces folic acid to tetrahydrofolic acid. Inhibition of tetrahydrofolate formation limits the availability of one carbon fragments necessary for the synthesis of purines and the conversion of deoxyuridylate to thymidylate in the synthesis of DNA and cell reproduction. The polyglutamated metabolites of MTX also contribute to the cytotoxic effect of MTX on DNA repair and/or strand breaks. MTX cytotoxicity is highly dependent on the absolute drug concentration and the duration of drug exposure. MTX is actively transported across cell membranes. At serum methotrexate concentrations exceeding 0.1µmol/mL, passive diffusion becomes a major means of intracellular transport of MTX. The drug is widely distributed throughout the body with the highest concentration in the kidney, liver, spleen, gallbladder and skin. Plasma concentrations following high dose IV MTX decline in a biphasic manner with an initial half-life of 1.5-3.5 hours, and a terminal half life of 8-15 hours. About 50% is bound to protein. Small amounts are excreted in the feces. There is significant entero-hepatic circulation of MTX. The distribution of MTX into third-space fluid collections, such as

pleural effusions and ascitic fluid, can substantially alter MTX pharmacokinetics. The slow release of accumulated MTX from these third spaces over time prolongs the terminal half-life of the drug, leading to potentially increased clinical toxicity.

Formulation & Stability: Methotrexate for Injection is available as a lyophilized powder for injection in 20 mg and 1 gm vials. The powder for injection contains approximately 0.14 mEq sodium in the 20 mg vial; 7 mEq sodium in the 1 g vial. Methotrexate for Injection is also available as a 25 mg/mL solution in 2, 4, 8, 10, 20 and 40 ml preservative free vials and 2 and 10 ml vials with preservative. The 2, 4, 8, 10, 20, and 40 mL solutions contain approximately 0.43, 0.86, 1.72, 2.15, 4.3, and 8.6 mEq sodium per vial, respectively. The preserved vials contain 0.9% benzyl alcohol as a preservative and must not be used for intrathecal or high dose therapy. Sterile methotrexate powder or solution is stable at 20 to 25 Cº (68 to 77 Fº); excursions permitted to 15 to 30 Cº (59 to 86 Fº). Protect from light.

Supplier: Commercially available from various manufacturers.

Toxicity: In the low doses administered for GVHD prophylaxis can contribute to transplant related hepatoxicity, contribute to oropharyngeal and gastrointestinal mucositis, and delay hematopoeitic recovery.

<u>Tacrolimus</u> (FK-506, Prograf®)

Source and Pharmacology: A macrolide antibiotic derived from the soil fungus Streptomyces tsukubaensis, it inhibits activation of T lymphocytes. Its major mechanism of action is blockade of the signal stemming from T cell receptor binding of an antigenic peptide complexed with an HLA molecule. Tacrolimus binds the intracellular protein FKBP-12 and this complex, in turn, inhibits calcineurin. In its oral formulation, tacrolimus' bioavailability is approximately 15-30%. Once absorbed it is highly protein bound. It is cleared primarily by hepatic metabolism (cytochrome P-450 system) with biliary excretion of these metabolites. Its elimination half-life is approximately 9-11 hours.

Formulation & Stability: Tacrolimus for intravenous administration is available in ampules of concentrated solution. It must be diluted in D5W or NS to a final concentration ranging from 0.004 mg/ml to 0.02 mg/ml. The undiluted solution should be stored at 5 to 25 degrees Celsius. Tacrolimus is also available in oral capsules, which should be stored at 25 degrees Celsius. A suspension can be prepared from the capsules. .

Supplier: Commercially available from various manufacturers.

Toxicity: The most frequent and clinically important adverse effect of tacrolimus is nephrotoxicity. This toxicity appears to be dose-related is usually reversible with discontinuation of the drug. Clinical manifestations may include fluid retention, edema, and in some cases, hyperchloremic, hyperkalemic metabolic acidosis. The risk of renal toxicity increases with administration of other potentially nephrotoxic agents. Hypertension may develop, generally within the first few weeks of therapy and affects both systolic and diastolic blood pressure. Some evidence suggests that the hypertension results from the renal vasoconstrictive effects of the drug. Hypertension may respond to dosage reduction and/or antihypertensive therapy. Adverse nervous system effects occur frequently, with tremor occurring in a large percentage of patients. Tremor may be manifested as fine hand tremor,

usually is mild in severity, may improve despite continued therapy, and/or may be alleviated with dosage reduction. Seizures, headache, paresthesia, hyperesthesia, flushing and confusion have also been reported occasionally. Other adverse effects include hirsutism, gingival hyperplasia, and hepatotoxicity. Adverse GI effects occur frequently during tacrolimus therapy, including diarrhea, nausea, vomiting, anorexia and abdominal discomfort. Infectious complications have occurred frequently.

Etoposide (VePesid®, Etopophos®, VP-16)

Pharmacology: A semisynthetic derivative of podophyllotoxin that forms a complex with topoisomerase II and DNA which results in single and double strand DNA breaks. Its main effect appears to be in the S and G2 phase of the cell cycle. The initial tó is 1.5 hours and the mean terminal half-life is 4 to 11 hours. It is primarily excreted in the urine. In children, approximately 55% of the dose is excreted in the urine as etoposide in 24 hours. The mean renal clearance of etoposide is 7 to 10 mL/min/mÇ or about 35% of the total body clearance over a dose range of 80 to 600 mg/mÇ. Etoposide, therefore, is cleared by both renal and nonrenal processes, i.e., metabolism and biliary excretion. The effect of renal disease on plasma etoposide clearance is not known. Biliary excretion appears to be a minor route of etoposide elimination. Only 6% or less of an intravenous dose is recovered in the bile as etoposide. Metabolism accounts for most of the non renal clearance of etoposide.

The maximum plasma concentration and area under the concentration time curve (AUC) exhibit a high degree of patient variability. Etoposide is highly bound to plasma proteins (~94%), primarily serum albumin. Pharmacodynamic studies have shown that etoposide systemic exposure is related to toxicity. Preliminary data suggests that systemic exposure for unbound etoposide correlates better than total (bound and unbound) etoposide. There is poor diffusion into the CSF < 5%.
Etoposide phosphate is a water soluble ester of etoposide which is rapidly and completely converted to etoposide in plasma. Pharmacokinetic and pharmacodynamic data indicate that etoposide phosphate is bioequivalent to etoposide when it is administered in molar equivalent doses.

Formulation and Stability: Etoposide for Injection is available as a 20 mg/mL solution in sterile multiple dose vials (5 mL, 25 mL, or 50 mL each). The pH of the clear, nearly colorless to yellow liquid is 3 to 4. Each mL contains 20 mg etoposide, 2 mg citric acid, 30 mg benzyl alcohol, 80 mg modified polysorbate 80/tween 80, 650 mg polyethylene glycol 300, and 30.5 percent (v/v) alcohol. Vial headspace contains nitrogen. Unopened vials of etoposide are stable until expiration date on package at controlled room temperature (20°-25°C or 68°-77°F). Etoposide phosphate for injection is available for intravenous infusion as a sterile lyophilized powder in single-dose vials containing etoposide phosphate equivalent to 100 mg etoposide, 32.7 mg sodium citrate USP, and 300 mg dextran 40. Etoposide phosphate must be stored under refrigeration (2°-8°C or 36°-46°F). Unopened vials of etoposide phosphate are stable until the expiration date on the package.

Supplier: Commercially available from various manufacturers.

Toxicity: Myelosuppression is dose related and dose limiting, with granulocyte nadirs occurring 7 to 14 days after drug administration and platelet nadirs occurring 9 to 16

days after drug administration bone marrow recovery is usually complete by day 20, and no cumulative toxicity has been reported. Fever and infection have also been reported in patients with neutropenia. Death associated with myelosuppression has been reported. The occurrence of acute leukemia with or without a preleukemic phase has been reported rarely in patients treated with etoposide in association with other antineoplastic agents. Nausea and vomiting are the major gastrointestinal toxicities. The severity of such nausea and vomiting is generally mild to moderate with treatment discontinuation required in 1% of patients. Nausea and vomiting can usually be controlled with standard antiemetic therapy. Mild to severe mucositis/esophagitis may occur. Gastrointestinal toxicities are slightly more frequent after oral administration than after intravenous infusion. Anaphylactic-like reactions characterized by chills, fever, tachycardia, bronchospasm, dyspnea and/or hypotension have been reported to occur in 0.7% to 2% of patients receiving intravenous etoposide and in less than 1% of the patients treated with the oral capsules. These reactions have usually responded promptly to the cessation of the infusion and administration of pressor agents, corticosteroids, antihistimines, or volume expanders as appropriate; however, the reactions can be fatal. Hypertension and/or flushing have also been reported. Blood pressure usually normalizes within a few hours after cessation of the infusion. Anaphylactic-like reactions have occurred during the initial infusion of etoposide. Facial/tongue swelling, coughing, diaphoresis, cyanosis, tightness in throat, laryngospasm, back pain, and/or loss of consciousness have sometimes occurred in association with the above reactions. In addition, an apparent hypersensitivity-associated apnea has been reported rarely. Rash, urticaria, and/or pruritis have infrequently been reported at recommended doses. At investigational doses, a generalized pruritic erythematous maculopapular rash, consistent with perivasculitis, has been reported.

The following adverse reactions have been infrequently reported: abdominal pain, aftertaste, constipation, dysphagia, asthenia, fatigue, malaise,somnolence , transient cortical blindness, optic neuritis, interstitial pneumonitis/pulmonary fibrosis, fever, seizure (occasionally associated with allergic reactions), Stevens-Johnson syndrome, and toxic epidermal necrolysis, pigmentation, and a single report of radiation recall dermatitis.

Hepatic toxicity, generally in patients receiving higher doses of the drug than those recommended, has been reported with etoposide. Metabolic acidosis has also been reported in patients receiving higher doses.

3.9 <u>Supportive Care</u>: With the exception of investigational supportive care agents and trials, there are no specifically prohibited supportive care agents for this study. Please refer to Section 3.3f for guidance about co-enrollment on other studies.

1) **Growth factors**: It is recommended that patients not receive post-transplant growth factors before day +14 (for those receiving PBSC transplants) or day +21 (for those receiving bone marrow transplants), except in the case of serious infection where hastening neutrophil recovery by 1-3 days may be critical for survival. After day +14 (PBSC) or +21 (BMT), GCSF or GMCSF should be given for severe neutropenia (ANC <500/mcL) or as necessary to keep ANC >1000/mcL at the treating physicians discretion.

2) **Blood Products:** The transfusion of blood products (erythrocytes and platelets) will be administered according to local institutional guidelines. Granulocyte infusions may be used at the discretion of the treating physician for treating serious infection.

3) **Management of Infections**: Prophylaxis against HSV, VZV, PCP, fungal and bacterial infections shall be administered according to institution guidelines and should be in keeping with the guidelines put forth jointly by the CDC, the Infectious Disease Society of America, and the American Society of Blood and Marrow Transplantation for preventing infections among hematopoietic stem cell transplant recipients (www.CDC.gov). The use of IVIG, and the administration of post-transplant vaccines should also be conducted according to institutional guidelines. The treatment of bacterial, fungal, viral and other infections should be according to institutional practices.

   All patients are required to be monitored for CMV reactivation (regardless of the results of donor and recipient pre-transplant serology). This testing shall be initiated no later than transplant day +7 and be performed at least once a week through at least transplant day 100, and at least every 4 weeks through day 180. More frequent testing may be indicated for some patients. Either antigen (pp65) or PCR (whole blood, plasma or leukocyte) testing may be used according to institutional practices. Pre-emptive therapy for CMV should be administered according to institutional practices.

4) **EBV-Related Lymphoproliferative Disease (PTLD)**: Though the use acyclovir for preventing EBV-PTLD has not been well studied in blood and marrow transplantation, clinical studies in solid organ transplantation suggest it is effective and support its use. As such, acyclovir or valacyclovir should be administered to all patients through day +180 regardless of HSV and VZV status. Centers may dose acyclovir and valacyclovir in accordance with their institutional practices for HSV or VZV prophylaxis. Alternatively, centers may use the dosing below:

   Acyclovir 250 mg/m2 IV q 8H until able to take PO and then switch to valacyclovir 10 mg/kg (to max of 500 mg) or acyclovir 600 mg/m2 (to max of 800 mg) po bid through day +180.Prophylaxis should start no later than day -1.

All patients are required to be routinely monitored for emerging lymphoproliferative disease using EBV PCR after transplant (regardless of the results of donor and recipient pre-transplant serology). Plasma, whole blood or peripheral blood mononuclear cell assays may be used according to institutional preferences. Because PCR monitoring has a high false positive rate, that is many if not most patients with elevated levels will never develop PTLD, testing should be used primarily as a prompt for more definitive diagnostic testing (radiographic testing and biopsy).[36,37] This PCR testing shall be initiated no later than transplant day +7 Testing shall be performed every two weeks through day 100 and then every four weeks through day 180. For patients who are still requiring intensive immune suppression at day 180, consideration should be given to continuing PCR monitoring. During this time, treating teams should also monitor patients for signs of malignant PTLD, such as lymphadenopathy and fever. Extended testing may be indicated in select cases. The need for extended testing will be determined by the

treating physician. In addition, PCR testing more frequently than once every two weeks may be indicated, as determined by the treating physician and according to institutional standards, especially in the event of low level EBV reactivation.

The threshold for concern varies greatly by type of PCR assay used; for example, a threshold of >10,000,000 copies/million cells has been recommended for PBMC assays, while a threshold of >1000 copies/ml has been recommended for plasma assays. [36,37,38] Such elevations should prompt diagnostic testing.

Treatment of diagnosed PTLD will be determined by the treating team. Consideration, however, should be given to using rituximab as initial therapy for most cases, especially for early disease (polymorphic disease, plasmacytic hyperplasia, mononucleosis-like disease). More aggressive therapy, donor-lymphocyte infusions, EBV-specific cytotoxic lymphocyte infusions or chemotherapy, is often warranted for more advanced, rituximab-refractory or progressive forms of PTLD. [36,37,38]

Reduction in immune suppression is rarely effective for PTLD diagnosed in the early post-transplant period. In patients with persistently positive EBV PCR levels but no evidence of PTLD, however, reduction in immune suppression may prevent the development of PTLD. [36,37,38] Reductions in immune suppression, when safe (no GVHD) and feasible, then should be considered in this setting. [36,37,38]

3.10 Data Collection and Study Endpoints

a)Data Collection: The REDCap™ system, a secure web application for building and managing online surveys and databases, will be used for data submission. Access to the system will be provided through Children's Healthcare of Atlanta, a REDCap™ consortium partner. RedCap will be used to capture data for enrollment, baseline patient, disease and treatment characteristics and outcomes. The study CRN will enable participating centers to access the system. Clinical research associates and clinical research nurses at participating centers will then access the enrollment and case report forms on the trial website. Also on the website will be detailed guidance for accurately completing the forms and the timeframe for submission of each form. If web access is not available, the forms may be printed, filled manually and submitted by email or faxing to study personnel. The data will be compiled electronically. All questions regarding the system should be directed to the study CRA or CRN.

b) Study Endpoints and Definitions: because this is a phase II trial, short-term endpoints will be used. All endpoints, unless otherwise specified, will be defined at 1 year post-transplant.

i**) Primary Endpoint: The primary endpoint for this trial will be early (before day 100) severe (grade III-IV) acute GVHD.** This endpoint will be defined according to the Blood and Marrow Transplant Clinical Trials Network Manual of Procedures (version 2, 2005, section 1) and the NIH consensus criteria[39] as shown in Tables 4a and 4b below.

All submitted forms, which will contain data regarding staging as well as grading will be reviewed centrally by members of the protocol team. The accuracy of the grading based on the submitted staging will be assessed. Staging and grading will also be adjusted as indicated by applying an algorithm developed and validated by Weisdorf et al,[40] an algorithm designed for use in place of an expert panel.

Table 4a. Clinical Stage of Acute GVHD According to Organ System			
Stage	**Skin**	**GI**	**Liver**
+	Maculopapular rash <25% of body surface	>501-1000 ml (280-555 ml/m^2) diarrhea per day or nausea, anorexia or vomiting with biopsy (EGD) confirmation of upper GI GVHD	Bilirubin 2.1-3 mg/dl
++	Maculopapular rash 25-50% of body surface	>1001-1500 ml (5556-833 ml/m^2) diarrhea per day	Bilirubin 3.1-6 mg/dl
+++	Maculopapular rash >50% of body surface area or generalized erythroderma	>1500 ml (833 ml/m^2) diarrhea	Bilirubin 6.1-15 mg/dl
++++	Generalized erythroderma with bullous formation and desquamation	Large volume stool with severe abdominal pain with or without Ileus or stool with frank blood or melena	Bilirubin >15 mg/dl

Table 4b. Overall Clinical Grading of Severity of aGVHD			
Grade	**Skin**	**GI**	**Liver**
I	Stage 1-2	0	0
II	Stage 3 or	Stage 1 or	Stage 1
III	---	Stage 2-4	Stage 2-3
IV	Stage 4	---	Stage 4

ii)Secondary Endpoints:

1. Engraftment related (a safety endpoint).

Neutrophil recovery: This endpoint will be defined as the first of 3 consecutive days following the nadir that the absolute neutrophil count is at least 500/µl.

Platelet recovery: This endpoint will be defined as the first day that the platelet count is at least 20 thousand/µl without a transfusion in the preceding 7 days.

Non-engraftment: This endpoint will be defined as lack of neutrophil recovery (defined as ANC >0.5 $*10^9$/L for three consecutive days) by 35 days post-transplant or neutrophil recovery with lack of myeloid donor chimerism.

Secondary graft failure. This endpoint will be defined by initial engraftment but subsequent development of an ANC <0.5 $*10^9$/L for fourteen consecutive

days. Transient causes of myelosuppression (infection, drugs, etc) shall be ruled out.

Graft rejection: Defined by initial engraftment (assessed by neutrophil recovery and donor chimerism) with the subsequent loss of donor myeloid chimerism (regardless of whether persistent neutropenia develops).

2. Infection and immune related (a safety endpoint).

CMV Viremia will be defined as the occurrence of a positive blood antigen or PCR test prior to day 180.

CMV invasive disease will be defined in accordance with the Blood and Marrow Transplant Clinical Trials Network Manual of Procedures (version 2, 2005, appendix 5B)

Post-transplant lymphoproliferative disorder will be defined in accordance with the Blood and Marrow Transplant Clinical Trials Network Manual of Procedures (version 2, 2005, appendix 5B) and the 4th edition of the World Health Organization's Classification of Tumours of Haematopoietic and Lymphoid Tissues.

Other Infections will be defined in accordance with the Blood and Marrow Transplant Clinical Trials Network Manual of Procedures (version 2, 2005, appendix 5A & B).

Immune Reconstitution will be assessed by the day 100 CD4+ T cell count and by the reaccumulation of NK cells, B cells, total T cells, and CD8+ T cells as assessed by multicolor flow cytometry.

3. GVHD related

Early onset (before day 100) acute GVHD (including all grades, and stratified by grades) will be assessed according to the Blood and Marrow Transplant Clinical Trials Network Manual of Procedures (version 2, 2005, section 1) using the NIH consensus criteria.[39]

Late onset (after day 100) acute GVHD will be assessed according to the Blood and Marrow Transplant Clinical Trials Network Manual of Procedures (version 2, 2005, section 1) using the NIH consensus criteria.[41]

Chronic GVHD, including overlap syndrome, will be assessed according to the Blood and Marrow Transplant Clinical Trials Network Manual of Procedures (version 2, 2005, section 2) using the NIH consensus criteria.[42] This assessment will continue through Year 5 post-transplant.

Immune Suppression-Free Survival and Immune Suppression-Free/Disease-Free Survival will be assessed at 1 year and will be defined as survival and disease-free survival off of all immunosuppressive agents.

To compare severe (Grade III-IV) aGVHD free survival (GFS) up to Day 180 post-transplantation between the abatacelpt + standard GVHD prophylaxis and standard GVHD prophylaxis regimen. The aGVHD events in this definition of GFS are the adjudicated grade (III-IV) aGVHD events. (This is a pre-determined endpoint for the 8/8 cohort and a retrospective endpoint for the 7/8 cohort).

To compare the cumulative incidence of severe (grade III-IV) aGVHD (based on adjudicated aGVHD events) up to Day 180 post-transplant between the abatacept + standard GVHD prophylaxis and standard GVHD prophylaxis regimen. (This is a pre-determined endpoint for the 8/8 cohort and a retrospective endpoint for the 7/8 cohort).

4. <u>**Relapse and Survival**</u>

Transplant related mortality will be defined as any death occurring in a continuous complete remission.

Relapse of the underlying malignancy.

Disease-free survival will be defined as survival without relapse of underlying malignancy. This will be evaluated through Year 5 post-transplant.

Overall-survival will be defined as survival with or without relapse of underlying malignancy. This will be evaluated through Year 5 post-transplant.

3.11 Sample Size Considerations, Randomization Scheme and Statistical Analysis:

3.11.1 Sample size and statistical power:

3.11.1.1 Stratum 1 (For patients receiving 8/8 HLA matched unrelated donor transplants): Using a Bayesian test of hypothesis, the cumulative incidence of Gr III-IV severe acute GVHD in the patients randomly assigned to the investigational regimen will be compared to the cumulative incidence in patients randomly assigned to the standard regimen. This will be an intent-to-treat analysis. In these calculations, adhering to the paradigm endorsed by CTEP, [43-46] for the design of screening phase II randomized controlled trials, we employed an alpha and beta of ≤ 0.2. We estimated the cumulative incidence of Grade III-IV aGvHD by Day +100 in the standard arm to be 0.20 This estimate is consistent with the experience from the CIBMTR [3,7,47,48] and from phase III randomized controlled trials in HSCT. [11,49,58]. From the experience of the phase III randomized controlled trials of aGvHD prevention [11,49,58] we estimated the absolute reduction in the incidence of severe aGVHD that we would achieve with abatacept (the δ) to be 0.10, predicting an incidence of 0.10 for the investigational arm.

Given these assumptions, the statistical rule was set as follows: Let θ_1 be the true incidence of grade III-IV aGVHD in the standard arm and θ_2 be the corresponding incidence rate in the investigational arm. We will test the null hypothesis Ho: $\theta_1 \leq \theta_2$ against the alternative hypothesis Ha: $\theta_1 > \theta_2$. The decision rule is to reject the null hypothesis in favor of the alternative if $P(\theta_1 > \theta_2 \mid \text{data}) > 0.8$. For the patients that will be enrolled in this study, the historical incidence of grade III-IV aGVHD is approximately 20%. In order to detect a reduction

of l0% in the incidence of severe GvHD in the investigational cohort (incidence of aGvHD decreasing from 20% to 10%), a group size of 70 would be required to achieve at least 80% power. Thus, the total targeted enrollment for the randomized stratum of 8/8 matched transplant recipients is is 140 patients. The table below gives the operating characteristics of the design assuming uniform (non-informative) prior distributions for the parameters θ_1 and θ_2 under four scenarios. The last column of the table gives the probability of rejecting the null hypothesis in favor of the alternative hypothesis for each scenario and the parameter $\delta = \theta_1 - \theta_2$ is the effect size. Scenario 1 is the ideal scenario and the corresponding P(Ha) is the Bayesian power. Scenario 2 corresponds to no difference in incidence rates and P(Ha) corresponds to the "Bayesian type I error probability". We note that here, we are being less conservative in terms of this Bayesian type I error probability since this trial is following the strategy endorsed by CTEP for a screening phase II trial. Scenario 4 is a pessimistic scenario where the investigational arm does worse that the standard arm. The probability of accepting Ha in this case is 0.01. This table was derived by using the fact that the posterior distributions of θ_1 and θ_2 are beta distributions and the last column was obtained by simulating 10000 trial replicates under each scenario.

Table 5. Design Operating Characteristics

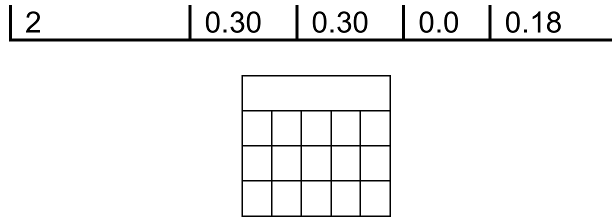
Scenario	θ_1	θ_2	δ	P(Ha)
1	0.2	0.1	0.1	0.80
2	0.2	0.2	0.0	0.19
3	0.2	0.14	0.06	0.55
4	0.2	0.3	-0.1	0.01

Table 5 was derived by using the fact that the posterior distributions of θ_1 and θ_2 are beta distributions and the last column was obtained by simulating 10000 trial replicates under each scenario.

3.11.1.2 Stratum 2 (For patients receiving 7/8 HLA matched unrelated donor transplants): Sample size and statistical power: The cumulative incidence of grade III – IV acute GVHD by day 100 in patients enrolled in the 7/8 matched stratum will be compared to the cumulative incidence of grade III – IV acute GVHD by day 100 in control subjects from the CIBMTR database. A maximum of 3 matched controls will be randomly selected for each case using the following criteria:
Based on data from the CIBMTR (personal communication, Marcelo Pasquini), we are estimating that the cumulative incidence of grade III – IV acute GVHD by day 100 for the 7/8 matched transplant control group (calcineurin inhibitor and methotrexate alone for GVHD prophylaxis) will be 30%. Let θ_1 be the true incidence of grade III-IV aGVHD in the control arm and θ_2 be the corresponding incidence rate in the (case) investigational arm. We will test the null hypothesis Ho: $\theta_1 \leq \theta_2$ against the alternative hypothesis Ha: $\theta_1 > \theta_2$. The decision rule is to reject the null hypothesis in favor of the alternative if $P(\theta_1 > \theta_2 \mid data) > 0.8$. Table 6 shows the Bayesian power (row 1) to detect a 20% reduction in grade III – IV acute GVHD by day 100 with abatacept and Bayesian type I error probability (row 2) using a 1:2 case control matching ratio, 40 patients in the 7/8 matched group *and* 80 *for* the control group.

Table 6. Design Operating Characteristics

Scenario	θ_1	θ_2	δ	P(Ha)
1	0.30	0.10	0.2	0.96



2	0.30	0.30	0.0	0.18

3.11.2 Randomization: For stratum 1, non-adaptive randomization will be used with a block size of 8 and an allocation ratio of 1:1 to assign each patient to one of the two arms: (1) Arm A-standard GVHD prophylaxis with a calcineurin inhibitor, methotrexate and placebo; and (2) Arm B-investigational prophylaxis with abatacept, a calcineurin inhibitor and methotrexate. Allocation will be performed across one factor: patient age: patients ≤21 years of age *versus* patients > 21 years. Given that each site involved in the project will be closely correlated with age (pediatric versus adult centers), we will not be stratifying on center. Randomization will be performed using the open source randomization software RANDI2 [52,53] via a secure web-browser, hosted and maintained by the Cedars-Sinai Statistical Analysis Core. The study biostatistician, the study pharmacist, the primary pharmacist and the study monitors will be unmasked to group specification; all others will be blind to subject randomization.

3.11.3 Statistical Analyses

Primary endpoint:

The primary analysis will consist of comparing the cumulative incidence of severe aGVHD at day +100 post-transplant in patients receiving investigational GVHD prophylaxis to patients receiving standard GVHD prophylaxis. This comparison will be made as follows for the two Study Strata:

For Stratum 1 (8/8 HLA-matched), patients randomly assigned to investigational prophylaxis will be compared to those randomly assigned to standard prophylaxis.

For Stratum 2 (7/8 matched), patients enrolled on-study, and receiving investigational prophylaxis will be compared to a matched control group drawn from the CIBMTR registry. Each patient receiving investigational prophylaxis will be matched to at least two registry control patients (up to four as feasible). To be eligible for inclusion in the control group, registry patients must meet the following criteria: 1) Received a 7/8 matched unrelated marrow or peripheral blood transplant between 2008 and 2014. 2) Be at least 6 years old. 3) Have received cyclosporine or tacrolimus and methotrexate alone (no lymphocyte depleting antibodies) for GVHD prophylaxis. Trial patients and registry control patients will be matched on three variables: age (within ten years), performance status, and disease stage (early, intermediate and advanced using CIBMTR criteria).

For the few patients (<5) with 7/8 HLA-matched donors who were enrolled on the study prior to the creation of Stratum 2, those randomly assigned to receive placebo will be added to the CIBMTR control group. Those randomly assigned to receive abatacept will be assigned to the abatacept-treatment group.

All registered patients will be considered for this analysis. The primary null hypothesis of the study is that there will be no difference in the incidence of severe aGVHD in patients receiving investigational and standard GVHD prophylaxis. .The primary outcome will be assessed in a

final analysis to be performed after the last enrolled patient has been followed for 100 days post-transplant. Death prior to day 100 without grade 3-4 acute GVHD will be considered a competing risk. Relapse will also be considered a competing risk to negate the effect of measures, such as withdrawal of immune suppression and donor-lymphocyte infusion, often used in response to relapse The cumulative incidence and confidence interval will be calculated. The cumulative incidence will be compared between treatment arms using Gray's test.

Secondary endpoints: A cumulative incidence curve will be computed along with a 95% confidence interval for infection, serious infection, CMV viremia, CMV invasive disease, EBV viremia, PTLD, BK viremia and disease, adenovirus viremia and disease, engraftment of: neutrophils, platelets, lymphocyte count (>1000 cells/μl), total T cell counts (>500 cells/μl), CD4+ T cell counts (>200 cells/μl), and CD8+ T cell counts (>200 cells/μl), secondary graft failure, graft rejection, all grades of early and late aGvHD, overlap syndrome, chronic GvHD, RRT, TRM, DFS, immunosuppression-free survival, immunosuppressive-free/relapse-free survival, relapse, and OS. A log-rank test will be used to compare the two treatment arms. Death will be considered as a competing risk for neutrophil engraftment, platelet engraftment, lymphocyte-, total T cell-, CD4+ T cell-, CD8+ T cell- engraftment, relapse acute GVHD and chronic GVHD. Relapse will be considered a competing risk for TRM. Relapse will also be considered a competing risk for aGVHD, overlap syndrome and cGvHD to negate the effect of measures, such as withdrawal of immune suppression and donor-lymphocyte infusion, often used in response to relapse. Gray's test will be used for statistical analyses where competing risks exist and log-rank testing where they do not.

The proportion of patients who fail to engraft by day 35 will be compared between the two arms using the chi square test or nonparametric tests as appropriate. Descriptive statistics will be computed for all the immune reconstitution assays. Treatment arms will be compared using chi-square tests, t-tests or non-parametric tests as appropriate for the assay. In addition to the planned clinical and biologic assessments through day +365 post-transplant, patients will be evaluated clinically on a yearly basis through Year 5 post-transplant to document overall survival and chronic GvHD.

4. Protection of Human Subjects

4.1 IRB review and Patient Informed Consent: This protocol will be first reviewed and approved by the **IRB at the study's Data Coordinating Center (Lead IRB for the study). Each participating site will then be required to submit the Lead IRB approved protocol to their local IRB for approval at their site**. Consent forms will be modified as needed to comply with local institutional guidelines. The Clinical Trials Coordinator for the study will review and approve modified versions of consent forms before participating centers submit to their IRBs. The trial will not open for enrollment at a given center until the protocol has been approved by its IRB and a copy of the approval letter has been provided to the Clinical Trials Coordinator. The protocol will also be reviewed by the National Marrow Donor Program (NMDP).

4.2 IRB review as of 7/1/2018. Dr. Leslie Kean, the Overall PI and Sponsor, moved to the Division of Hematology/Oncology at Boston Children's Hosptial and Dana-Farber Cancer Institute. The Dana-Farber Cancer Institute IRB will become the Lead IRB (previously Fred Hutch) for this study. The DFCI IRB will do review of all amendments, and once approved, will be provided to performance sites to be reviewed by their institutional IRB. The Clinical Trials Coordinator, Brandi Bratrude, will contintue to coordinate the review of any events, protocol

deviations or other issues with the Lead IRB and can be reached at: brandi.bratrude@childrens.harvard.edu .

Prospective participants and their families will be informed of the nature of the study and will be given pertinent information as to the intended purpose, possible benefits, and possible adverse events. The procedures and possible hazards to which the patient will be exposed will be explained, as well as alternatives to participation in this trial, will be presented in detail to the patient and donor and to the patient's and donor's responsible family members. An approved informed consent statement will be read and signed by the patient (or responsible family member if under the age of 18), a witness and an investigator. Through the NMDP, an approved informed consent statement will also be read and signed by the donor and a witness..

For patients participating at Children's Healthcare of Atlanta or Emory University: Verbal assent will be obtained from children ages 6 to 10 and documented in the patients' medical record, as well as being noted in the case report forms. Written assent will be obtained from children ages 11 to 16, using an approved assent statement, along with use of the informed consent by their parent or guardian. Children 17 years or older will read/sign the consent form along with their parent or guardian.

For patients participating at other centers: assent will be obtained according to local institutional guidelines.

4.3 Investigational New Drug application. The use of abatacept in this trial is covered by IND# 111738.

4.4 Interim Analyses and stopping guidelines:

Interim analyses for safety: Combining Strata 1 and 2, we will conduct an interim analysis for safety after the 10th, 20th, 40th, 70th and 120th patients enrolled have reached day +100 (100 days post-transplant) or died. Enrollment will not be suspended while the analyses are being performed. Enrollment will be suspended, if and when the analyses yield early evidence of harm according to the statistically defined guidelines.

Safety will be monitored by tracking two outcomes: (a) post-transplant lymphoproliferative disease (PTLD), and (b) day 100 transplant-related mortality in both arms.

(a) The PTLD stopping rule: The trial will stop if the probability that the development of malignant PTLD rate (θ) exceeds 20% is equal or higher than 0.95, i.e., stop if $Pr\{\theta>0.20|data\}\geq 0.95$. A noninformative prior distribution for θ will be used. Table 7 gives the stopping rule for each interim analysis, with column 2 indicating the maximum number of cases of malignant PTLD that are permitted. For example, when the experience for the first 10 patients is evaluated (interim analysis 1) if 3 or more cases of PTLD are observed, the trial will be stopped. The third column gives the probability of stopping the trial when in fact, the true $\theta = 0.2$. This is the equivalent of the Bayesian type I error probability. The target type I error probability was set at 0.05.

Table 7. Stopping Rules for PTLD			
Number of patients	**Max # permitted**	**Probability to Stop**	**Cumulative Probability to stop**
10	**2**	**0.0115**	**0.01150**
20	**3**	**0.01067**	**0.02217**
40	**5**	**0.00811**	**0.03029**
70	**7**	**0.01408**	**0.04436**
120	**11**	**0.00811**	**0.05247**

(b) The day 100 transplant-related mortality stopping rule: The trial will stop if the probability that the day 100 transplant-related mortality rate (θ) exceeds 5% is equal or higher than 0.95, i.e., stop if $Pr\{\theta>0.05|data\}\geq0.95$. A noninformative prior distribution for θ will be used. Table 8 gives the stopping rules for the design for each interim analysis, with column 2 providing the maximum number of transplant related deaths permitted at each point. For example, if 5 or more transplant related deaths are observed by day 100 when the experience on the first 10 patients is assessed (interim analysis 1), the trial will be stopped. The third column gives the probability of stopping the trial when in fact, the true θ = 0.20. This is the equivalent of the Bayesian type I error probability. The target type I error probability was set at 0.05.

Table 8. Stopping Rules for Day +100 Transplant Related Mortality			
Number of patients	**Max # permitted**	**Probability to Stop**	**Cumulative Probability to stop**
10	**5**	**0.00637**	**0.00637**
20	**8**	**0.00765**	**0.01402**
40	**13**	**0.01449**	**0.02851**
70	**21**	**0.00872**	**0.03723**
120	**33**	**0.00997**	**0.04720**

Interim Analysis for Efficacy: This will involve the randomized stratum (stratum 1) only at a single time point after the 100th patient enrolled in that stratum has reached day 100 (50 patients per group). The rule will be intended to halt enrollment better than expected efficacy. The Bayesian power for detecting a 10% decrease in the incidence of grade III-IV aGVHD rate and the corresponding Bayesian type I error probability is calculated such that at the interim analysis, we will stop the trial for efficacy if $P(\theta_1 > \theta_2 \mid data) > 0.8$. The minimum detectable difference between the incidence of grade III-IV aGVHD rates in this interim analysis is given by δ using the following calculation: Power = 0.7, Type I error probability = 0.195, delta = 0.134.

4.4 Data and Safety Monitoring and Suspension of Enrollment: This study will be centrally reviewed by the Data Safety and Monitoring Committee (DSMC) of the Pediatric Blood and Marrow Transplant Consortium (PBMTC). This DSMC is chartered to monitor trials enrolling both children and adult patients. The DSMC is a standing committee, composed of a chair, patient advocate, biostatistician, nurse representative and 2 bone marrow transplant physicians with procedures and processes as defined in the PBMTC DSMC Charter. The DSMC will review the study protocol prior to study activation and IRB review, and will continue to review the study on a regular basis according to the committee rules.

The DSMC will meet at regular intervals to review all adverse events and deaths and determine whether any patient safety problems necessitate protocol modifications or discontinuation of the trial. The DSMC will also meet on an *ad hoc* basis to review the results of interim analyses or if

unexpected safety events occur that may necessitate study suspension or closure. The DSMC will discontinue the review of outcomes when this protocol is closed to accrual. The DSMC has the authority to unblind study personnel prior to study closure if, in their opinion, doing so will be critical to patient safety.

Before each regularly scheduled DSMC meeting, the study coordinator will submit a report including tabular summaries of all reported SAEs, AEs and deaths on study to date. The report will also include a brief summary of each previously unreported SAE and death, including an assessment of whether the event was unexpected or related to the study.

If the DSMC recommends protocol or informed consent changes during the study, the recommendations will be reviewed by the Protocol Chair and incorporated into the protocol as deemed appropriate. The protocol with incorporated changes will be distributed to the participating PIs after approval by the Fred Hutchinson IRB. As of July 2018 this will change to the DFCI IRB. It It is the responsibility of each Transplant Center PI to forward the distributed communications from the DSMC to their local IRB.

4.5 Adverse events, unanticipated problems and other immediately reportable events.

a) **Definitions**:

a. An **adverse event (AE)** is any untoward medical occurrence regardless of causality assessment. An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease associated with or occurring during the use of an investigational product whether or not considered related to the investigational product.

b. An adverse event is defined as a **serious adverse event (SAE)** when the AE 1) results in death, 2) is considered life-threatening, 3) results in hospitalization or cause the prolongation of hospitalization, 4) results in permanent disability or irreversible impairment, 5) leads to a congenital anomaly, or 6) represents a significant medical condition which, without urgent medical intervention, would lead to one of the above outcomes. Life-threatening means that the AE represented an immediate threat of death without medical intervention. **For the purposes of this study, post-transplant lymphoproliferative disorder (PTLD), non-engraftment, graft rejection and secondary graft failure (see section 3.10. ii for definitions) shall always be considered SAEs regardless of their impact on the patient's condition.**

c. **Unexpected adverse event**s are those events whose nature, severity, or frequency is inconsistent with what is known about the patient's prior medical history, the possible adverse effects of HSCT and the safety profile of abatacept. On the other hand there are many **expected adverse events** in HSCT for patients with hematological malignancies. Expected adverse events usually considered to be related to intensive pre-transplant conditioning, allogeneic grafts and graft versus host disease prophylaxis with a calcineurin inhibitor and methotrexate include serious bacterial and fungal infections, veno-occlusive disease, complications arising from GVHD and its treatment, idiopathic pneumonia syndrome and complications arising from oral or gastrointestinal mucositis. Such events may be deemed *expected*. Adverse events associated with the previous treatment of or the post-transplant relapse of a patient's malignancy may, likewise, be deemed expected.

d. **Unanticipated problems (UP) include unexpected adverse events** and also unexpected problems, events, or new information which are not adverse events but which indicate that research participants or others are at greater risk of harm than previously believed prior to recognition of the unanticipated problem.

e. **Other immediately reportable events (IRE):** Hypersensitivity and other infusional reactions to the study drug that do not meet criteria for an SAE will be considered an immediately reportable event and shall be reported according to the guideline below (4.5 c)

b) **Characterizing an adverse event**: Adverse events will be described using event terms and severity grading from the NCI Common Toxicity Criteria for Adverse Events (CTCAE) version 4. The expectedness of the event (see above) and the relation of the event to the study drug shall also be characterized.

The relation **or attribution** of the event to the investigational product may be characterized as follows:

- **definitely** related, clearly associated with study drug
- **probably** related, likely associated with study drug
- **possibly** related, may be associated with study drug
- **unlikely to be** related, or
- **definitely not** related to the study drug

Because this is a blinded, placebo controlled trial, the ability of investigators to assess the attribution for most events will be limited. In most cases, then, assigning an attribution of definitely or probably related will be inappropriate.

c) **Reporting of serious adverse events, unanticipated problems and immediately reportable events**: Guidelines for reporting SAEs, IREs and UPs are outline in Table 8A.

Table 8A: Guidelines for Reporting SAEs, IREs, and UPs			
Type of Event	**Reporting to Study**	**Time Frame for Reporting to Study**	**Reporting to Local IRB**
Serious adverse event before day 100	Yes, regardless of whether expected or unexpected	Within 24 hours if fatal; within 5 business days if not fatal.	According to local IRB guidelines.
Serious adverse event on or after day 100	Only if PTLD, fatal or unexpected	Within 24 hours if fatal; within 5 business days if not fatal.	According to local IRB guidelines.
Unanticipated problem	Yes	Within 5 business days	According to local IRB guidelines.
Immediately reportable event	Yes	Within 5 business days	According to local IRB guidelines.

All SAEs occurring through day 100, whether expected or unexpected, will be reported in an expedited manner. SAEs occurring after day 100 will be reported in an expedited manner only if they are PTLD, fatal or unexpected. If reportable SAEs are fatal, they are required to be reported within 24 hours of knowledge of the event. If non-fatal, they are required to be reported within five business days. The **SAE CRF shall be used for reporting SAEs.**

IREs and UPs are required to be reported within 5 business days. **The IRE CRF and the UP CRF shall be used for reporting IREs and UPs, respectively.**

The appropriate eCRF shall be completed in REDCap per the deadlines detailed above. Once the eCRF has been completed, the trial coordinator and research nurse are required to be notified via e-mail. Please see contact information below.
Brandi Bratrude, CRA
brandi.bratrude@childrens.harvard.edu

Local IRBs are required to be notified of reported SAEs, UPs and IREs by institutional PIs in accordance with local IRB guidelines.

d) **Monitoring of SAEs, unexpected problems and IREs**: The trial coordinator will notify Dr. Leslie Kean, the protocol chair and sponsor of the trial, Dr. John Horan, the clinical vice-chair, and the chair of the PBMTC DSMC within three business days of receiving a report.

e) **Reporting of non-serious adverse events:** For non-serious adverse events only those that are unexpected are required to be reported. They shall be reported using the AE eCRF to Brandi Bratrude who will notify Dr. Kean, the protocol chair and sponsor and Dr. Horan the clinical vice-chair. All such events will be tabulated for routine meetings of the DSMC.
Contact information for Brandi Bratrude:
Brandi Bratrude
e-mail: brandi.bratrude@childrens.harvard.edu

Safety reports: Written IND safety reports will be submitted to the FDA by Dr. Kean, the IND sponsor, for serious,unexpected suspected adverse reactions within 15 calendar days of the sponsor determining that the information requires reporting. If the event is fatal or is deemed to be life threatening, the report will be made within 7 calendar days. Dr. Kean will also distribute safety reports to the Dana-Farber Cancer Institute IRB and participating transplant centers. Local PIs will be responsible for submitting these safety reports to their IRBs. Finally, Dr. Kean will make an assessment of whether the event constitutes an unanticipated problem posing risks to subjects or others (UP). She will provide her assessment to the Dana-Farber Cancer Institute IRB, which, in turn will make a final determination. If the Dana-Farber Cancer Institute IRB determines an event is a UP it will notify the appropriate regulatory agencies and institutional officials.

4.6 Unblinding of study assignment: in situations where the treating physician believes that knowledge of the treatment assignment will clearly enhance patient care, the treating physician should make all efforts to contact the study chair or the vice-chair of clinical oversight, who will direct the local study pharmacist to unblind the treating physician. However, if the urgency of the clinical situation demands it, the treating physician can direct the pharmacist to unblind him or her without prior consultation with the study chair or vice-chair. All members of the study committee will remain blinded to assignment.

5. APPENDIX 1: Research Evaluations: To be performed at the Central Reference Laboratory

5.1 Handling of samples at the Central Laboratory

1.Donor Samples:
Donor samples will arrive unprocessed. They will be fully processed at the donor lab.

2.Recipient Samples:

A.Cytochex tubes: These tubes are processed within 24 hours of arrival at the Central Reference Laboratory for flow cytometry using standard extracellular and intracellular flow cytometric techniques. .No part of the cytochex tube is cryopreserved.

B. PAXgene tubes:
1.Store the PAXgene® Blood RNA Tube upright at room temperature for a minimum of 2 hours and a maximum of 72 hours before aliquoting and freezing.

2. Enter into the LIMS system.

3. Freeze the first at -20°C for 24 hours, then transfer them to -80°C.

C. CPT tubes:

Purification and Cryopreservation of PBMCs for Functional T cell Assays and Plasma Preservation using Cell Processing Tubes (CPT tubes).

Equipment, Supplies and Reagents Needed:

1.BD Vacutainer® CPT™ Tube with Sodium Citrate at room temperature.
2.5, 10 & 50 ml sterile serological Pipettes (not Pasteur pipettes)
3. Characterized Fetal Bovine Serum (FBS) - Fisher/Hyclone #SH30071.03 This reagent must be heat inactivated by thawing at 37°C with occasional mixing. Once thawed bottle is placed at 56°C for 60 mins with occasional mixing. Aliquots are stored at -70°C until specified expiration date.
4.DMSO – Sigma #154938
5.FBS and DMSO are used to make up "Freezing Media 1" and "Freezing Media 2" to be used in procedures that follow.

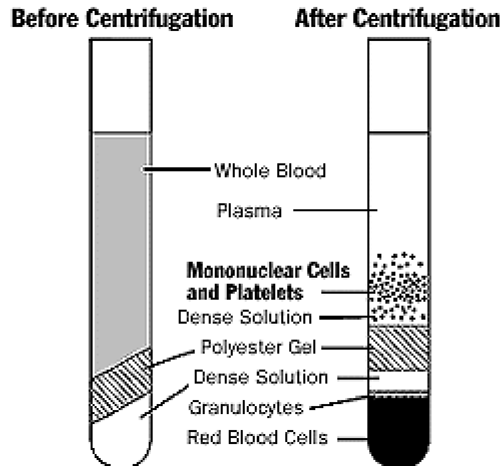
i. Freezing Media 1 – 100% filtered heat inactivated FBS
ii. Freezing Media 2 – 80% filtered heat inactivated FBS + 20% DMSO (32ml FBS + 8ml DMSO)

6.PBS + 2% FBS– Combine 980mls PBS (Cellgro #21-031-CM) with 20ml "Freezing Media 1" to make PBS with 2% FBS to be used in procedures that follow.
7.High-Yield Lyse – Invitrogen #HYL250
8.0.4% Trypan Blue dye – Cellgro #25-900-CL
9.15 ml conical tubes
10.50 ml conical tubes
11.Nalgene 1.8 ml cryovials
12.Nalgene cryofreezing container with 2-propanol
13.-80º freezer
14.Liquid nitrogen cell freezer

Special Precautions and notes:

1.Make sure tubes are balanced & secured in centrifuge
2.Do not place pipette in the polyester gel (Middle Layer) as it will clog the pipette
3.This procedure must be performed in a sterile environment.
4.All reagents must be at room temperature.
5.Thaw the freezing media, but keep it on ice or at 0-4ºC.

PBMC Isolation:



1.For shipped CPT tubes, upon arrival at the Central Laboratory, gently invert the previously centrifuged tubes 8-10 times to remix the PBMCs with the plasma. No plasma is stored from CPT tubes that have been previously shipped.
2. If the blood has been drawn at CHOA, Emory University or SCCA, for the 8mL CPT tubes, immediately after centrifugation, proceed with the full plasma and PBMC cryopreservation protocol stipulated below. **NOTE: For the 4mL CPT tube, PBMCs are isolated and analyzed fresh at the Central Reference Laboratory. No cryopreservation is performed on PBMCs isolated from 4cc CPT. Only isolate plasma and cryopreserve PBMCs from blood that is drawn into 8cc CPT tubes and processed at the Central Site (CHOA, Emory and the** SCCA**). Plasma cryopreservation cannot be performed on shipped CPT tubes.**
3.After centrifugation, for the large (8cc) CPT tubes, remove plasma layer being careful not to disrupt cell layer (cloudy layer between CPT gel and plasma). Plasma will be saved.
4.Plasma specific protocol:

a)Aliquot 0.5ml of plasma into the five labeled and barcoded 1mL Nunc tubes be careful not to disrupt cell layer (cloudy layer between CPT gel and plasma)

b)Keep CPT tubes for PBMC isolation

c)Place plasma in -80 Freezer

5.Pour remaining plasma and cells from CPT tubes into a 50mL Falcon tube
NOTE: No more than 6 CPTs per 50mL Falcon tube, if you have more than 6 divide them evenly into 2 or more Falcon tubes (they can be combined at the lyse step).
6.Rinse CPTs using ≈7mL of PBS + 2% FCS (room temperature). If you have multiple CPTs, they will be rinsed sequentially using the same 7mls of PBS + 2% FCS. Rinsing should remove most; if not all the blood from the gel matrix (the wash will be red in color). **Be careful not to touch the outside of the CPT tubes with the pipette**
7.Dispense anything remaining in the pipette into a 50ml Falcon tube.

8.Pour the rinse mixture from the CPTs into the 50ml Falcon tube, without touching the inside of the Falcon tube with the CPTs**.** Discard CPT tubes.

Washing PBMCs:

1. Bring Falcon tube volume up to 50mL using PBS with 2% FBS (room temperature)
2. Centrifuge at 1200 RPM for 10 minutes at room temperature, 9-9 acceleration/deceleration (handle with care after centrifuging)

3. Aspirate the supernatant and discard, then mechanically disrupt pellet by raking

4. Resuspend the raked cell pellet in 10mL High-Yield Lyse. Then, lyse for 10 minutes at room temperature

NOTE: At this point you can use the Lyse to combine the pellets (1:10 ratio, pellet to lyse volume)

5. Bring volume up to 50mL using PBS with 2% FBS(room temperature), mix gently

6. Place labeled and barcoded 1mL Nunc tubes on ice in préparation for eventually receiving the PBMCs.

7. Centrifuge at 1200 RPM for 10 minutes at room temperature, 9-9 acceleration/deceleration (handle with care after centrifuging)

8. Aspirate the supernatant and discard. Then, mechanically disrupt pellet by raking

9. Add **exactly** 10mL of PBS with 2% FBS(room temperature), gently mix

10. To count the cells, add 20µL of the cell suspension to 10mL of isotonic solution, gently mix (Make sure all bubbles have dissolved before using Coulter Counter).

Freezing PBMCs:

1. While counting cells, centrifuge cells at 1200 RPM for 10 minutes at room temperature, 9-9 acceleration/deceleration

2. Alternative Method for Manually Counting the cells using a haemocytometer.
   - **a.** Add 10ul of cells from the 10ml cell suspension to 90ul of 0.4% trypan blue dye for a dilution of 1:10
   - **b.** Count the cells in 3 squares and take an average and perform the following calculations:
     - i. Number of cells counted X 10 (the dilution factor)X 10^4= cells/ml
     - ii. ________cells/ml x 10ml= ________x10^6 Total cells
       (Example: average of 120 cells per square. Cell concentration will calculate out to be 12 x10^6 cells/ml. Then, multiply by 10 (because your cell suspension is 10ml) to 120 x10^6 total cells)

   - **c.** Determine the number of 1mL aliquots you want to freeze based on the number of cells that you have purified. **NOTE: If possible, a minimum of 10x10^6 and no more than 30x10^6 cells per aliquot**

   - **d.** The number of tubes multiplied by 1mL is the total volume you will be freezing.

i. Example: Total cells = $120x10^6$ / $30x\ 10^6$cells per tube, total 1mL Nunc tubes = 4; Total volume equals 4x1mL=4mL

e. Calculate the volume of Freezing Media 1 and Freezing Media 2 you will need based of the total volume **(Keep freeze media on ice at all times)**
   i. Total volume/2= Freezing Media 1 volume=Freezing Media 2 volume
   ii. Example: 4ml/2=2ml, Freezing Media 1 =2mL and Freezing Media 2 =2mL

3. Centrifuge at 1200 RPM for 10 minutes at room temperature, 9-9 acceleration/deceleration (handle with care after centrifuging)

4. Aspirate the supernatant, mechanically disrupt pellet by raking

5. Resuspend cells in Freezing Media 1 (volume calculated in **Section 2.d)**

6. Add Freezing Media 2(volume calculated in **Section 2.d)** one drop at a time while continuously mixing gently

7. Aliquot 1mL of cells into previously determined quantity of labeled and barcoded 1mL Nunc tubes (**Section 2.c)**

8. Transfer cells to a cell freezer (Mr. Frosty) and place in a -80°C freezer for 24-72hrs. Then transfer to a liquid nitrogen freezer.

D Cryopreserved Serum from BD Red Top Serum (Clot) Tubes:

1.Serum will arrive frozen.
2. Enter into the LIMS system.
3.Store serum at -80°C.

E. Cryopreserved Plasma PPT Tubes:

1. Plasma will arrive frozen.
2.Enter into the LIMS system.
3.Store plasma in -80 Freezer.

F.Urine Samples:

1.Urine samples will arrive frozen.
2.Enter into LIMS
3. Store at -80°C.

G.Marrow or PBSC Graft Sample:

1.These samples arrive from the recipient center, shipped on Day 0 at ambient temperature to the Central Reference Laboratory.
2.2cc are used for flow cytometric analysis.
3. PBMCs are prepared from the remaining sample using a hystopaque gradient and the standard Emory Transplant Center Hystopaque SOP.
4. Cryopreserve the PBMCs using the SOP described above for the CPT tubes.

5.2. Pharmacokinetic and pharmacodynamic analysis of abatacept:

5.2.1 Serum peak and trough abatacept levels will be measured by ELISA. Samples for this testing will be obtained at the following time points: day -1 (pre-infusion and post-infusion); day +5, day +14, day +21, day +28 (pre-infusion and post-infusion), day +35, day +42, day +63, day +100, day +180, day +270, day +365.

5.2.2 Peripheral blood CD20+ B cells and CD11c+ antigen presenting cells will be monitored for expression of CD80 and CD86 (the targets of abatacept) to determine the pharmacodynamic profile of the abatacept dosing regimen, as previously described.[54] Samples for this testing will be obtained at the following time points: pre-conditioning (within 30 days of the start of conditioning), day +14, day +28, day +63, +100, +180, + 270, +365.

5.3 Immune Monitoring:

Immune reconstitution studies: Multicolor flow cytometry (9-10 colors) will be performed to determine the character and the pace of immune recovery in all transplanted patients in comparison to both the recipient's baseline values and the baseline values of the donor. The relative percentages, as well as absolute numbers (based on TruCount flow cytometry and CBC) of the following leukocyte populations will be determined. (1) T cells: CD3+ CD20- CD14-. (2) CD4+ or CD8+ T cells: CD4+ CD8- or CD4- CD8+ CD3+ CD20- CD14-. (3) B cells: CD20+ CD3- cells. (4) NK cell subsets: CD3- CD20- CD16+ CD56- cells, CD3- CD20- CD16+ CD56+ cells, CD3- CD20- CD16- CD56+ cells. (5) Naïve T cells: CD3+ CD20- CD4+ CD8- (or CD4- CD8+) CCR7+ CD45RA+. (6) Central memory T cells: CD3+ CD20- CD4+ CD8- (or CD4- CD8+) CCR7+ CD45RA-. (7) Effector memory T cells: CD3+ CD20- CD4+ CD8- (or CD4- CD8+) CCR7- CD45RA-. (8) Effector memory RA T cells: CD3+ CD20- CD4+ CD8- (or CD4- CD8+) CCR7- CD45RA+, (9) Myeloid Dendritic Cells: CD3-CD20-CD8-HLA-DR+CD11c+ CD123-. (10) Plasmacytoid DC: CD3-CD20-CD8-HLA-DR+CD11c-CD123+.
Samples for this testing will be obtained at the following time points from the recipient and donor. Recipient: pre-conditioning (within 30 days of the start of conditioning), days, -1, +5, +14, +21, +28, +63, +100, +180, +270, +365, and at the time of new-onset GvHD or viral infection. Donor: One peripheral blood sample from the donor will also be obtained for this analysis at the time of bone marrow donation.

Monitoring of T cell activation and regulation: Multicolor flow cytometry will be performed to quantify the emergence of T cells displaying both activated and regulatory phenotypes. Activated T cells will be defined as follows: (1) CD38 and HLADR expression: CD3+ CD20- CD4+ CD8- (or CD4- CD8+) HLADR+ CD38+. (2) Ki67 upregulation and BCl-2 downregulation: CD3+ CD20- CD4+ CD8- (or CD4- CD8+) Bcl2+ Ki67+ (3) Perforin and Granzyme upregulation: CD3+ CD20- CD4+ CD8- (or CD4- CD8+) Perforin+ GranzymeB +. CD31 expression will be queried in each of these subpopulations to determine whether the cells are new thymic emigrants.
Samples for this testing will be obtained at the following time points from the recipient and donor. Recipient: pre-conditioning (within 30 days of the start of conditioning), days, +14, +28, +63, +100, +180, +270, +365, and at the time of new-onset GvHD or viral infection. Donor: One peripheral blood sample from the donor will also be obtained for this analysis at the time of bone marrow donation.

Regulatory T cells will be defined as follows: CD3+ CD20- CD4+ CD8- $CD25^{high}$ $CD127^{low}$. Where appropriate, the regulatory phenotype will also be confirmed by measuring expression of FoxP3+.This data will be correlated with clinical data on GVHD and other immune mediated

processes such as engraftment and infection. Results in patients receiving abatacept-containing GVHD prophylaxis will be compared to those in the patients receiving the standard, two-drug regimen.
Samples for this testing will be obtained at the following time points from the recipient and donor. Recipient: pre-conditioning (within 30 days of the start of conditioning), days +14, +28, +63, +100, +180, +270, +365, and at the time of new-onset GvHD or viral infection. Donor: One peripheral blood sample from the donor will also be obtained for this analysis at the time of bone marrow donation.

Gene array analysis of post-transplant expression profiles: We will perform transcriptional analysis on both whole blood and PBMCs after transplant. Whole blood transcriptional analysis will be performed on a subset of patients (those weighing >50Kg, from RNA stabilized in PAXgene tubes), with samples obtained at the following time points from the recipient and donor: Recipient: pre-conditioning (within 30 days of the start of conditioning), days -1, +5 +14, +21, +28, +63, +100, +180, +365, and at the time of new-onset GvHD or viral infection. Donor: One peripheral blood sample from the donor will also be obtained for this analysis at the time of bone marrow donation.

Transcriptional analysis will also be performed from PBMCs, which will be obtained at the following time points from the recipient and the donor: Recipient: Samples will be obtained at the following time points: pre-conditioning (within 30 days of the start of conditioning), days +21, +28, +63, +100, +180, +270 and +365, and at the time of new-onset GvHD or viral infection. Donor: one peripheral blood sample from the donor will also be obtained at the time of bone marrow or peripheral blood stem cell donation.

For patients over 18 years of age and treated in Atlanta (Emory University or Children's Healthcare of Atlanta) or Seattle (SCCA) who weigh >60Kg, an additional 60cc will be drawn at Day +100 and Day +365 to perform gene array and TREC analysis on sorted T cell populations.

Assays for viral-specific T cells:

(1) **Quantitative analysis CMV-specific, EBV-specific and BK-specific CD8+ T cells.** Tetramer-based reagents will be used to measure immune responses against CMV, EBV and BK virus (Adenovirus-specific tetramers are not available). These tetramers will be placed into standard flow-cytometric assays to enumerate the percentage and absolute number of CMV- or EBV-specific CD8+ T cells, as well as the naïve or memory phenotypes (as described above) of the viral-specific T cells. Samples for tetramer analysis will be obtained from the recipient and the donor as follows: Recipient: Samples will be obtained at the following time points: pre-conditioning (within 30 days of the start of conditioning), days +28, +63, +100, +180, +270 and +365, and at the time of new-onset GvHD or viral infection. Donor: one peripheral blood sample from the donor will also be obtained at the time of bone marrow or peripheral blood stem cell donation.

(2) Functional analysis of anti-CMV, anti-EBV and anti-BK protective immunity: The functional integrity of T cells towards CMV,EBV and BK viral antigens will also be determined. To perform this analysis, PBMCs will be isolated and both CD4+ and CD8+ T cells will be assessed for their ability to produce functional cytokines (IL-2, IFNγ and TNF) in response to both viral lysates (purchased from Advanced Biotechnologies, Columbia, MD) or viral peptide pools (CMV peptides from BD, Franklin Lakes, NJ, EBV peptides from Anaspec, Fremont, CA, BK peptides will be synthesized at Emory University). Results in patients receiving the augmented, abatacept containing GVHD prophylaxis regimen will be compared to those in the patients receiving the standard, two-drug regimen. For this analysis, samples will be obtained as follows: Recipient: Samples will be obtained at the following time points: pre-conditioning (within

30 days of the start of conditioning), days +28, +63, +100, +180, +270 and +365, and at the time of new-onset GvHD or viral infection. Donor: one peripheral blood sample from the donor will also be obtained at the time of bone marrow or peripheral blood stem cell donation.

(3) Serum cytokine and chemokine analysis: We will utilize cytometric bead array analysis to evaluate cytokine and chemokine secretion into serum in transplant recipients. The goal of these two techniques is to yield an aGvHD-specific biomarker signature and determine its perturbations with effective aGvHD prevention. The cytokines that will be assessed are based on ours and others previous studies [34,55,56] and include the following: IFNγ, TNF, TNFR1, HGF, TGF-b, IL-2, IL-2 receptor α, IL4, IL6, IL7, IL8, IL10, IL17, and CD8. Results in patients receiving the augmented, abatacept containing GVHD prophylaxis regimen will be compared to those in the patients receiving the standard, two-drug regimen. Samples for this testing will be obtained as follows: Recipient: Samples will be obtained at the following time points: pre-conditioning (within 30 days of the start of conditioning), days +28, +63, +100, +180, +270 and +365, and at the time of new-onset GvHD or viral infection. Donor: one peripheral blood sample from the donor will also be obtained at the time of bone marrow or peripheral blood stem cell donation.

(4) Viral Infection: PCR testing will be performed to monitor for infection from four viruses commonly encountered in allogeneic HSCT recipients, CMV, EBV, Adenovirus and BK virus. Testing will be performed on blood (all viruses) and urine (only BK virus will be measured in urine) samples. These results will be correlated with clinical evidence of viral diseases (e.g. pneumonitis, enterocolitis, hemorrhagic cystitis, hepatitis, PTLD and marrow failure), and data on the rate of reconstitution as well as the function of viral-specific T cells. Results in patients receiving the augmented, abatacept-containing GVHD prophylaxis regimen will be compared to those in the patients receiving the standard, two-drug regimen. Samples for this testing will be obtained as follows: Recipient: Samples will be obtained at the following time points: pre-conditioning (within 30 days of the start of conditioning), days +28, +63, +100, +180, +270 and +365, and at the time of new-onset GvHD or viral infection. Donor: one peripheral blood sample from the donor will also be obtained at the time of bone marrow or peripheral blood stem cell donation.

Donor immunologic Evaluation: A peripheral blood sample will be obtained from the donor on the day of marrow harvest or peripheral blood collection and will be analyzed with each of the modalities discussed above. In addition, EBV and HSV serology will be tested on the donor from these research samples.

Marrow or PBSC Graft Evaluation: A sample (5cc) of the marrow or PBSC graft and/or the empty bag or syringe that held the stem cell product will be evaluated for immune phenotype and virus-specific T cells as described above.
Calculation of absolute cell counts for manipulated products: Some products are manipulated between the time of arrival at the transplant center and infusion. In some cases, plasma or red cells are depleted, while in others, a subset of the product is cryopreserved. For such products, the doses of cell subsets transplanted can be estimated by adjusting the cells subset count at the Central Reference Laboratory multiplied by the proportion of total cells infused divided by the total cell received by the transplant center. Such a method assumes that travel and manipulation of the product did not affect any cell subset in a selective manner.

6. APPENDIX 2. Statistical Analysis: Adopting a Bayesian paradigm and assuming a flat uninformative prior distribution, the posterior probability Pr{θ>t|k,n} that the frequency of a toxicity event *θ* exceeds a given threshold *t* after *n* accrued patients and *k* observed toxicity events is given by

$$\Pr\{\theta>t|k,n\} = \frac{\int_t^1 p^k(1-p)^{n-k}\,dp}{\int_0^1 p^k(1-p)^{n-k}\,dp} = 1 - \frac{B_t(k+1,n-k+1)\Gamma(n+2)}{\Gamma(k+1)\Gamma(n-k+1)}, \text{ where}$$

$B_z(a,b)=\int_0^z t^{a-1}(1-t)^{b-1}dt$ is the incomplete beta function, and $\Gamma(z)=\int_0^\infty t^{z-1}e^{-t}dt$ is the gamma function.

Table 9 gives the design operating characteristics under selected values of the true probability of PTLD θ. It gives the probability of stopping the trial under the alternative hypothesis, the expected sample size, and the average sample size given that the trial stopped. For example, if the true value of *θ* is 0.15, then there is an 86% chance that the trial is stopped early and the average sample size is about 47. On the other hand, there is a small chance of stopping the trial if *θ* is small; 0.14% chance of stopping the trial when in fact *θ* = 0.02. Based on these results, we conclude that the design has good operating characteristics for stopping the trial early due to excessive PTLD rate.

Table 9. Design operating characteritics under different scenarios for the true probability of PTLD *θ*.

True Value of θ	Probability to Stop	Expected N	Expected N Given That We Stopped
0.02	0.0014	104	17
0.05	0.0444	101	37
0.1	0.4497	77	44
0.15	0.8616	47	38
0.2	0.9888	29	28
0.25	0.9988	21	21

Table 10. Design operating characteritics under different scenarios for the true probability of day 100 transplant-related mortality rate *θ*.

True Value of θ	Probability to Stop	Expected N	Expected N Given That We Stopped
0.15	0.0117	102.95	14.09
0.2	0.0578	99.47	25.62
0.25	0.2199	89.08	36.14
0.3	0.5268	69.81	39.11
0.35	0.8165	48.68	36.25
0.4	0.9576	33.19	30.06

Table 10 gives the design operating characteristics under selected values of the true probability of day 100 transplant-related mortality rate θ. It gives the probability of stopping the trial under the alternative hypothesis, the expected sample size, and the average sample size given that the trial stopped. For example, if the true value of θ is 0.35, then there is an 81% chance that the trial is stopped early and the average sample size is about 49. On the other hand, there is a small chance of stopping the trial if θ is small; 1.2% chance of stopping the trial when in fact θ = 0.15. Based on these results, we conclude that the design has good operating characteristics for stopping the trial early due to excessive day 100 transplant-related mortality rate.

Since these stopping rules follow the Bayesian paradigm, no corrections are needed for multiple looks.

7. Appendix 3: **Blood Samples from Healthy Volunteers**-Healthy volunteers, who are at least 18 years, will be recruited at Emory University and the **Seattle sites including** University of Washington**/Seattle Children's Hospital/Seattle Cancer Care Alliance/Seattle Children's Research Institute** for blood samples. This blood will be used for quality control for the flow cytometry analysis. Approximately one volunteer will be recruited each week at each of the two locations. Up to 50cc of blood will be drawn via peripheral venipuncture. A volunteer may not donate more than 50cc of blood per month.. Only the volunteers' age, sex and race will be recorded. Volunteers will receive compensation, a $10 gift card, each time a sample is obtained. Written informed consent will be obtained.

8. Appendix 4: Adverse Event Reporting to Bristol-Myers Squibb

- All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through 30 days of discontinuation of dosing must be reported to BMS Worldwide Safety.
- If the BMS safety address is not included in the protocol document (eg, multicenter studies where events are reported centrally), the procedure for safety reporting must be reviewed/approved by the BMS Protocol Manager. Procedures for such reporting must be reviewed and approved by BMS prior to study activation.
- The BMS SAE form should be used to report SAEs. If the BMS form cannot be used, another acceptable form (ie, CIOMS or Medwatch) must be reviewed and approved by BMS. The BMS protocol ID number must be included on whatever form is submitted by the Sponsor/Investigator.
- Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. The investigator should report any SAE occurring after these time periods that is believed to be related to study drug or protocol-specified procedure. worldwide.safety@bmsaebusinessprocess@bms.comIn accordance with local regulations, BMS will notify investigators of all reported SAEs that are suspected (related to the investigational product) and unexpected (ie, not previously described in the IB). In the European Union (EU), an event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Investigator notification of these events will be in the form of an expedited safety report (ESR).
    - Other important findings which may be reported by the as an ESR include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (eg, animal) study, important safety recommendations from a study data monitoring committee, or sponsor decision to end or temporarily halt a clinical study for safety reasons.
    - Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the IB. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision.

The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.

- In addition, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours. SAEs must be recorded on BMS or an approved form; pregnancies must be reported on a Pregnancy Surveillance Form.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: 609-818-3804

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to the BMS (or designee) using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

For studies conducted under an Investigator IND in the US include the following:

For studies conducted under an Investigator IND in the US, any event that is both serious and unexpected must be reported to the Food and Drug Administration (FDA) as soon as possible and no later than 7 days (for a death or life-threatening event) or 15 days (for all other SAEs) after the investigator's or institution's initial receipt of the information. BMS will be provided with a simultaneous copy of all adverse events filed with the FDA.

SAEs should be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/.

MedWatch SAE forms should be sent to the FDA at:
MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

- An SAE report should be completed for any event where doubt exists regarding its seriousness.
- For studies with long-term follow-up periods in which safety data are being reported, include the timing of SAE collection in the protocol.
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.
- If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

- If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to BMS using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization. All SAEs should be followed to resolution or stabilization.

Nonserious Adverse Event Collection and Reporting

The collection of nonserious AE information should begin at initiation of study drug. All nonserious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 30 days following the last dose of study treatment.

Nonserious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for nonserious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

Laboratory Test Abnormalities

All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the subject to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

<u>Potential Drug Induced Liver Injury (DILI) - confirm language with medical team for each product</u>

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs. Potential drug induced liver injury is defined as:

- ALT or AST elevation > 3 times upper limit of normal (ULN)
  AND
- Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)
  AND
- No other immediately apparent possible causes of AST/ALT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 5 half lives after product administration, the investigational product will

be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for subject safety).

The investigator must immediately notify Worldwide Safety @BMS of this event via the Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form [provided upon request from BMS]

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a nonserious or serious AE, as appropriate, and reported accordingly.

9. Appendix 5: Dana-Farber Cancer Institute Multi-Center Data Safety Monitoring Plan

Definitions:

- **DF/HCC Multi-Center Protocol**: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor.
- **Lead Institution:** One of the Dana-Farber/Harvard Cancer Center consortium members in this case, Boston Children's Hospital (BCH), responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (Food and Drug Administration (FDA), etc.). The Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.
- **DF/HCC Sponsor:** The person sponsoring the submitted Multi-Center protocol. Within DF/HCC, this person is the Overall Principal Investigator who takes responsibility for initiation, management and conduct of the protocol at all research locations. In applicable protocols, the DF/HCC Sponsor will serve as the single liaison with any regulatory agencies (i.e. the FDA). The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions.
- **Participating Institution:** An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor (Dr. Leslie Kean) is a DF/HCC Investigator. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.
- **Coordinating Center:** The entity (Boston Children's Hospital) that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the

responsibilities outlined in the protocol document, and as specified in applicable regulatory guidelines. BCH is the Coordinating Center for this DF/HCC Multi-Center Protocol.

GENERAL ROLES AND RESPONSIBILITIES
For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

DF/HCC Sponsor
The DF/HCC Sponsor, Leslie Kean, MD, PhD, will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Ensure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team member receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all applicable site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC, and FDA reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with the FDA.
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.
- Monitor accrual and address Participating Institutions that are not meeting their accrual requirements.

Coordinating Center (Boston Children's Hospital)
The general responsibilities of the Coordinating Center may include but are not limited to:

- Maintain FDA correspondence.

- Distribute protocol and informed consent document updates to Participating Institutions as needed.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violation submitted by Participating Institutions and provide to the Sponsor (Dr. Leslie Kean) for timely review and submission to the DFCI IRB, as necessary.
- Distribute serious adverse events reported to the Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all Participating Institutions.
- Provide Participating Institutions with information regarding DF/HCC requirements with which they will be expected to comply.
- Carry out plan to monitor Participating Institutions either by on-site or remote monitoring.
- Maintain Regulatory documents of all Participating Institutions which includes but is not limited to the following: local IRB approvals/notifications from all Participating Institutions; confirmation of Federal wide Assurances (FWAs) for all sites; all SAE submissions; IRB approved consents for all sites.
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc) and maintain documentation of all relevant communications.

Participating Institution
Each Participating Institution is expected to comply with all applicable federal regulations and DF/HCC requirements, the protocol and HIPAA requirements.
The general responsibilities for each Participating Institution may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Submit protocol and/or amendments to their local IRB.
- Maintain regulatory files as per sponsor requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Update Coordinating Center with research staff changes on a timely basis.
- Submit Serious Adverse Event (SAE) reports to local IRB per local requirements and to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to local IRB per local requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Order, store and dispense investigational agent per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.

- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.

- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.

DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS
The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

Protocol Distribution
The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

Protocol Revisions and Closures
The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately.

10. Appendix 6: Scope of Work and Transfer of Sponsor Obligations (TORO)

Leslie S. Kean/Boston Children's Hospital (BCH) wishes to transfer some of the obligations as IND Sponsor under 21 CFR 312. The Sponsor-Investigator retains responsibility for oversight of the tasks transferred. Where noted in the Sponsor Obligations list (following pages), the specific obligations have been transferred to the following entities:

1. Center for International Blood and Marrow Transplant Research (CIBMTR)
2. Bristol-Myers Squibb (BMS)
3. Pediatric Blood & Marrow Transplant Consortium (PBMTC)
4. Emory University (Emory)
5. Children's Healthcare of Atlanta (CHOA)
6. Seattle Children's Hospital (SCH)
7. Boston Children's Hospital (BCH)
8. Dana-Farber Cancer Institute (DFCI)

Effective Date: 7/1/2018.

<table>
<tr><th colspan="2">Clinical Trial Design</th></tr>
<tr><td>Author a protocol, including all protocol amendments, that complies with DF/HCC policies
Develop the DF/HCC consent which will serve as the model consent for all external sites
Review the investigator brochure (IB) for protocol or consent impact</td><td>Leslie S. Kean/BCH</td></tr>
<tr><th colspan="2">Site Agreements and Negotiation, Contracts, and Payments</th></tr>
<tr><td>Coordinate inter-institutional agreements/contracts
Negotiate site payments and execute individual site task orders
Negotiate and execute any ancillary agreements
Process and Pay Site Payments as per site agreements
Determination and Management of Conflict of Interest</td><td>Leslie S. Kean/BCH</td></tr>
<tr><th colspan="2">Project Management</th></tr>
<tr><td>Site Communication
Conduct ongoing communication with each site. Communication via email, phone, and/or site visits, as needed.
Conduct routine study team meetings with external sites. These meeting could include, but are not limited to review of the following:
Overall study status
Potential subjects
Safety updates
Data collection/query issues
Collection of correlative samples</td><td>Leslie S. Kean/BCH</td></tr>
</table>

<table>
<tr><th colspan="2">Study Start-up and Maintenance</th></tr>
<tr><td>Trial Master File (TMF)
The BCH Sponsor-Investigator is responsible for the TMF.

The supporting organization may collect and track essential documents but there must be a plan in place for the supporting organization to send the documents to BCH so they can be saved to the TMF.</td><td>Leslie S. Kean/BCH</td></tr>
<tr><td>Investigator and Site Selection
Select only investigators qualified by training and experience to investigate the drug (21 CFR 312.23a)
Select external sites
Identify and screen potential sites
Conduct site feasibility assessments using the DF/HCC Site Feasibility Questionnaire
Confirm that each site has agreed to the DF/HCC site requirements and DF/HCC indemnification policy in the SFQ</td><td>Leslie S. Kean/Emory/SCH</td></tr>
<tr><td>IRB Approval of Protocol
Provide DFCI IRB approved documents to participating sites after there is a signed Confidentiality Agreement (CDA) is in place.</td><td>Leslie S. Kean/BCH</td></tr>
<tr><td>Site Initiation Visits
Prepare site initiation visit materials
Site Initiation materials must be approved by BCH Sponsor-Investigator
Conduct site initiation visits
Participate as needed to provide Study background/rationale and answer protocol specific questions.</td><td>Leslie S. Kean/Emory/SCH</td></tr>
<tr><td>Site Regulatory Document Maintenance
Collect and track site-specific required documents including but not limited to 1572, financial disclosure, CV/license, and lab documents. These essential personnel/study documents must be collect at the time of start-up and remain up-to-date throughout the course of the study.
Collect and track documentation of DF/HCC policy and protocol (initial and amendments) training for all investigators and study staff during start-up and throughout the course of the trial.
Collect and track delegation of authority logs for all investigators and study staff during start-up and throughout the course of the trial.
Collect and track external site IRB submission and approval documents. The initial external site IRB approval must be provided to BCH for site activation the DFCI IRB system.
Collect and track documentation of annual IRB continuing review approvals</td><td>Leslie S. Kean/BCH</td></tr>
</table>

Study Start-up and Maintenance (cont.)	
Study Support Documents • Study Procedure Manual • Study Visit Checklist	**Leslie S. Kean/Emory/SCH**
Amendments, Safety Information, and Other Updated Study-Related Materials • Distribute DFCI IRB approved protocol amendments to external sites • Review of site specific consent form to ensure compliance with DF/HCC consent language requirements • Distribution of IND Safety Reports that occurred on study and/or safety reports received from any source to participating sites • Distribute updated Investigator Brochures to external sites	**Leslie S. Kean/BCH**
Deviation/Violation Review • DF/HCC Sponsor-Investigator must receive and review major deviations/violations forms and minor deviation/violation logs from participating sites for completeness.	**Leslie S. Kean/BCH**
SAE Reporting • DF/HCC Sponsor-Investigator must receive and review SAE forms prior to submission to drug supplier, FDA, lead sites IRB and/or any other required regulatory agency	**Leslie S. Kean/BCH**
Deviation/Violation and SAE Reporting Communication • Collect initial submission of all deviation/violations and SAE Reports and send to DF/HCC Sponsor-Investigator for review and outcome/attribution • Facilitate further follow-up communication and approval to participating sites	**Leslie S. Kean/BCH**
Randomization/Registration Registration and randomization information will be provided to participating sites Regardless of registration/randomization system used for the trial all participants must be registered in OnCore.	**Leslie S. Kean/Emory/SCH**
Clinical Monitoring • Provide on-site or remote monitoring services for all participating sites per the Data Safety Monitoring Plan	**CIBMTR**
ClinicalTrials.gov • Maintain clinicaltrials.gov record	**Leslie S. Kean/BCH**

<table>
<tr><th colspan="2">Data Management</th></tr>
<tr><td>Clinical Trial Management (Data and Monitoring)
• Provide electronic data capture (EDC) and/or biospecimen management
• Provide any paper logs necessary outside of the EDC system
• Provide site support including:
    o Training
    o Study tools and aids to facilitate Study implementation

If a non-DF/HCC EDC system will be used, a plan for the supporting organization to provide the data to BCH for review by the Data Safety Monitoring Board/Committee and for the IND Annual Report needs to be in place.</td><td>Leslie Kean/BCH, CHOA</td></tr>
<tr><td>eCRF Data Review and Validation</td><td>Leslie Kean/BCH, CHOA</td></tr>
<tr><td>Data Query Resolution to Database Closure
• Resolve all queries generated either automatically via the EDC system or by the data validators and monitors within the EDC system.</td><td>Leslie Kean/BCH, CHOA</td></tr>
<tr><td>SAE Data Management
• SAE database reconciliation
• Provide reports of safety data, as needed</td><td>Leslie Kean/BCH, CIBMTR, CHOA, BMS</td></tr>
<tr><td>Data Safety Monitoring
• Provide administrative support for Data Safety Monitoring Board/Committee
• If DF/HCC is not performing the Data Safety Monitoring, a plan for how an external safety board would communicate that information to the DF/HCC Sponsor-Investigator needs to be in place.</td><td>PBMTC</td></tr>
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<table>
<tr><th colspan="2">IND Management</th></tr>
<tr><td>IND Content
• Submit an Investigational New Drug (IND) application (21 CFR 312.23a)
• Submit protocol amendments, including new protocols, changes in a protocol, the addition of new participating sites, and change in DF/HCC PI to FDA (21 CFR 312.30)
• Submit in an information amendment essential information on the IND that is not within the scope of a protocol amendment, IND safety report, or annual report (21 CFR 312.31)
• Submit a report of the progress of the investigation (21 CFR 312.33)
IND Safety Reports
• Review all information relevant to the safety of the drug obtained or otherwise received from any source including information derived from any clinical or epidemiological investigations, animal investigations, commercial marketing experience, reports in scientific literature, as well as reports from foreign regulatory authorities not previously reported (21 CFR 312.32b)
• Notify the FDA in a written safety report of any adverse experience associated with the use of the drug that is both serious and unexpected or any finding from tests in laboratory animals that suggests a significant risk for human subjects including mutagenicity, teratogenicity, or carcinogenicity (21 CFR 312.32c)</td><td>Leslie S. Kean/BCH</td></tr>
<tr><th colspan="2">Investigational Drug Management</th></tr>
<tr><td>• Maintaining adequate records showing receipt, shipment, or other disposition of the investigational drug (21 CFR 312.57(a), 21 CFR 312.58(b))
    ○ The supporting organization may collect and track the documents but there must be a plan in place for the supporting organization to send the documents to BCH so they can be saved to the TMF.
• Assuring return of unused investigational drug from each investigator whose participation in the clinical study is discontinued or terminated (21 CFR 312.59)
• Authorizing alternative disposition of unused supplies of the investigational (21 CFR 312.59)</td><td>n/a
(all investigational drug management activities were completed by Emory University prior to the effective date of this document)</td></tr>
</table>

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