UC San Diego
MOORES CANCER CENTER

A randomized Phase II trial comparing a calcineurin inhibitor-free graft-versus-host disease prophylaxis regimen with post-transplantation cyclophosphamide and abatacept to standard of care.

NCT number: NCT03680092

Protocol Number:	**180383**
Study ID:	**IM101-701**
Study Drug:	**Abatacept**
IND Number:	**140877**
IND Holder:	**Dimitrios Tzachanis, MD PhD**
Funding Source:	**Bristol Myers Squibb**
Initial version:	Version 2 Dated 15May2017
Amendment:	**8 Dated 10JAN2022**

Principal Investigator: **Dimitrios Tzachanis, MD PhD**

Associate Clinical Professor of Medicine
UCSD Blood and Marrow Transplant Program
3855 Health Sciences Drive
MC 0960
La Jolla, CA 92093-0960
Tel: (858)534-2185
Fax: (858)822-6844
dtzachanis@ucsd.edu

Co - Principal Investigator: **Divya Koura MD**, UCSD Blood and Marrow Transplant Program

Biostatisticians: **James M. Mirocha, MS**
829 Euclid Street
Santa Monica, CA 90403
Cell: 65-398-8625

Correlative Studies: **Jack Bui, MD PhD**
Director of Diagnostic Immunology, UCSD School of Medicine

Gerald Morris, MD PhD
Director, Immunogenetics and Transplantation Laboratory

Sub-Investigator(s):

Name	Department/Division
Fotios Asimakopolous MB BChir, PhD	UCSD Blood and Marrow Transplant Program
Edward Ball, MD	UCSD Blood and Marrow Transplant Program
Caitlin Costello, MD	UCSD Blood and Marrow Transplant Program
Aaron Goodman, MD	UCSD Blood and Marrow Transplant Program
Ayad Hamdan, MD	UCSD Blood and Marrow Transplant Program
Daniel Kaufman, MD PhD	UCSD Blood and Marrow Transplant Program
James Mangan, MD	UCSD Blood and Marrow Transplant Program
Carolyn Mulroney, MD	UCSD Blood and Marrow Transplant Program
Tiffany Tanaka, MD	UCSD Blood and Marrow Transplant Program
Parag Sanghvi, MD	UCSD Radiation Medicine and Applied Sciences

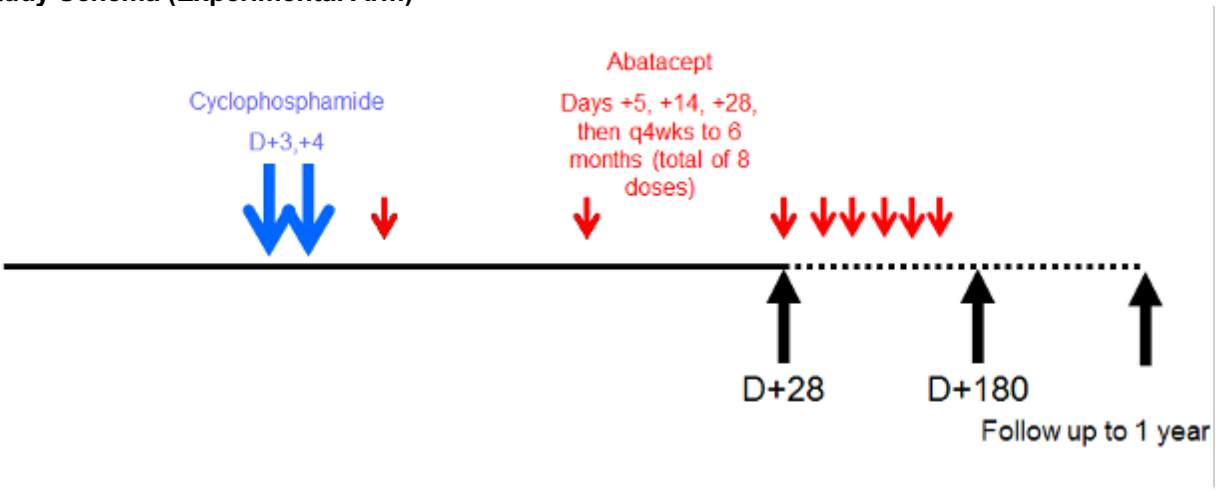
TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
aGVHD	Acute Graft Versus Host Disease
ALC	Absolute Lymphocyte Count
ALT	Alanine Aminotransferase
APC	Antigen Presenting Cells
AST	Aspartate Aminotransferase
ATG	Anti-thymocyte globulin
BM	Bone marrow
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
cGVHD	Chronic Graft versus Host Disease
CMP	Comprehensive Metabolic Panel
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitor
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr Virus
ECOG	Eastern Cooperative Oncology Group
EF	Ejection fraction
EFS	Event free survival
GVHD	Graft-Versus-Host Disease
GVT	Graft versus Tumor
H&P	History & Physical Exam
HLA	Human Leukocyte Antigen
HRPP	Human Research Protections Program
HSCT	Hematopoetic Stem Cell Transplantation
IV (or iv)	Intravenously
MA	Myeloablative
MDS	Myelodysplastic Syndrome
MMF	Mycophenolate Mofetil
MRD	HLA-identical sibling donors
MTD	Maximum Tolerated Dose
MUD	HLA matched unrelated
NCI	National Cancer Institute
ORR	Overall Response Rate
OS	Overall Survival
p.o.	per os/by mouth/orally
PBMCs	Peripheral Blood Mononuclear Cells
PBSC	Peripheral Blood Stem Cells
PD	Progressive Disease
PFS	Progression Free Survival

PR	Partial Response
PTLD	Post Transplant Lymphoproliferative Disease
RIC	Reduced Intensity Conditioning
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase
SSIs	Immunosuppressants
T Cells	Tregs
TBI	Total Body Irradiation
TCR	T cell receptor
ULN	Upper limit of Normal
VCA	Viral capsid antigen
VOD	Veno-occlusive disease
WBC	White Blood Cells

Study Schema (Experimental Arm)



STUDY SUMMARY

Title	A randomized Phase II trial comparing a calcineurin inhibitor-free graft-versus-host disease prophylaxis regimen with post-transplantation cyclophosphamide and abatacept to standard of care.
Short Title	Abatacept and Cyclophosphamide for GVHD prophylaxis
Protocol Number	180383
Phase	Phase II
Methodology	Randomized, Open-Label
Study Duration	2 years
Study Center(s)	Single-center (UCSD) with potential participation of other UC centers through the UC Hematologic Malignancy Consortium
Study Hypothesis	This is a post-transplantation study comparing two GVHD prophylaxis regimens in patients who underwent an allogeneic stem cell transplantation for the treatment of a hematologic malignancy. The experimental GVHD prophylaxis arm consists of cyclophosphamide and abatacept. Cyclophosphamide induces apoptosis of activated T cells and abatacept (CTLA4Ig) blocks activation of T cells by inhibiting the co-stimulatory signal. The standard of care arm consists of methotrexate and tacrolimus. Compared to the standard-of-care control arm, the experimental arm is much more convenient and expected to be associated with fewer toxicities. In addition there is a great theoretical potential for immunological synergy between cyclophosphamide and abatacept for inducing post-Hematopoietic Stem Cell transplant (HSCT) immunologic tolerance that clinically might translate into less GVHD without increase in relapse. If the experimental arm looks promising, then our findings will be used for the design of a larger phase III trial.

Primary Objective	To show that post HSCT cyclophosphamide + abatacept can induce tolerance. This clinically translates into a reduction in chronic GVHD by one year (compared to standard of care GVHD prophylaxis with methotrexate and tacrolimus).
Secondary and Exploratory Objectives	Secondary Objectives To compare the two treatment arms regarding GVHD- and relapse- free survival by one year Donor engraftment at day 28 Grades II-IV and III-IV acute GVHD Chronic GVHD Relapse rate Disease free survival over the course of the study Overall survival Transplant related mortality by 1 year after transplantation Infections by 1 year after transplantation Renal insufficiency and other toxicities Cognitive impairment Exploratory Objectives Post-HSCT immune reconstitution studies will include measuring T cell and NK cell phenotype, PD-1 expression,and alloreactivity to recipient and third party at predetermined time points. We will compare and contrast the findings between the treatment and control arm and correlate those with disease relapse and presence of acute or chronic GVHD.
Study population	50 subjects with hematologic malignancies

Diagnosis and Main Inclusion and Exclusion Criteria	Inclusion criteria: • High risk hematologic malignancy justifying the need for a stem cell transplantation • Patient receiving either Myeloablative Busulfan/Fludarabine or TBI/Cy or Reduced Intensity Fludarabine/ Melphalan for conditioning • ECOG performance score 0-2 • Creatinine clearance ≥ 40 (by Cockgroft-Gault) • Adequate hepatic function (total bilirubin <2.0 mg/dl, AST and ALT <3x ULN), • Normal cardiac function (EF > 50%) Exclusion criteria: • Patients with hematologic malignancies for which transplant is not the only curative option, such as AML with good or intermediate cytogenetics or molecular markers in CR1 or CML in chronic phase • Inability to identify an 8/8 or 10/10 HLA-Matched Donor (related or unrelated) • Prior allogeneic stem cell transplant • Active, uncontrolled infection, uncontrolled cardiac angina, symptomatic congestive heart failure or any other uncontrolled medical condition that in the opinion of the investigator will put the patient at increased risk by participating in this clinical trial or would interfere with data collection or interpretation • Life expectancy <3 months
Treatment plan	Patients will be randomized 1:1 to the experimental vs the standard of care arm. The two arms will be stratified by conditioning regimen (MA v RIC) and donor type (MRD vs MUD) in an effort to keep them balanced. The GVHD prophylaxis regimen on the experimental arm will consist of high dose Cyclophosphamide on Days +3 and +4 followed by abatacept for 6 months. The GVHD prophylaxis regimen on the standard of care arm will consist of methotrexate on Days +1,+3, +6 and +11 and tacrolimus.
Study Product(s), Dose, Route, Regimen	Abatacept at a dose of 10mg/kg will be administered on days +5, +14 and +28, +56, +84, +112, +140, +168
Subject duration of participation	1 year post-transplant

Statistical Methodology	The primary endpoint is the occurrence of moderate and severe chronic GVHD by one year post-HSCT. A Mantel-Haenszel test will be used to compare chronic GVHD risk between the two arms at 5% significance level. Competing risks will be treated as censoring events, including non-relapse mortality and relapse. This approach will test for a change in the cause-specific hazard ratio for GVHD. The key secondary endpoint is GVHD- and relapse-free survival (or GRFS i.e. free from acute GVHD Grade III or IV, and from moderate or severe chronic GVHD, and from relapse or non-relapse mortality and CRFS, i.e. free from chronic GVHD, relapse and non-relapse mortality) by one year post-transplant. A stratified Cox proportional hazard model will be used to compare survival curves over time and estimate a two-sided 95% confidence interval for the hazard ratio of treatment vs. control Additional secondary analyses: Cumulative incidence funcitons for GVHD, relapse, and non-relapse mortality will also be displayed, and tested for significant differences using Gray's test. Toxicities and adverse events will be summarized by arm, disease type and donor type. Their grade, relationship with the treatment, and severity will be listed.

1.0 BACKGROUND AND RATIONALE

1.1 Disease Background

Allogeneic Hematopoetic Stem Cell Transplantation (HSCT) is the only curative option for many malignant hematologic disorders mainly due to the graft versus leukemia effect. Infection and graft-versus-host disease (GVHD) is a major complication of allogeneic HSCT. Limiting GVHD and infection rates while improving Graft Versus Tumor effect (GVT) has been the elusive “Holy Grail” for transplant immunology [1].

The most commonly used GVHD prophylaxis regimen has been a calcineurin inhibitor (CNI) (cyclosporine or tacrolimus) in combination with either methotrexate, mycophenolate mofetil (MMF) or sirolimus[2]. Despite prophylactic measures, the incidence of acute GVHD (aGVHD) remains in the 40–60% range for transplants from HLA-identical sibling donors (MRD) and up to 75% for HLA matched unrelated (MUD) transplants[2]. The incidence of chronic GVHD (cGVHD) ranges between 40 and 70% and is the leading cause of mortality and morbidity in allogeneic HSCT survivors.

The currently used CNI based GVHD prophylaxis regimens cause significant toxicity (nephrotoxicity, neurotoxicity, myelosuppression, hypertension, tissue microangiopathy etc.) and close level monitoring and frequent dose adjustments, as well as aggressive hydration and electrolyte replacements are needed. Furthermore it has been proposed that CNIs block the induction of post-transplantation tolerance [3] and thymic T cell development [4] leading therefore to chronic GVHD.

1.2 Study Agent(s) Background and Associated Known Toxicities

Cyclophosphamide is an alkylating agent that has been used for decades in the treatment of cancer and autoimmune diseases. Cyclophosphamide induces apoptosis of activated T cells but is not stem cell toxic [5]. Most currently used GVHD prophylaxis agents (such as cyclosporine) inhibit T cell activation, proliferation, and interleukin-2 (IL-2) production (thereby also blocking tolerance induction). Only methotrexate and cyclophosphamide can induce apoptosis of activated T cells but methotrexate cannot be given safely in high doses because of marrow and mucosal toxicity.

High-dose cyclophosphamide has been studied as single agent prophylaxis of GVHD after myeloablative HLA-matched related or unrelated donor HSCT [6]. In that trial 117 patients with hematologic malignancies underwent a myeloablative HSCT, 78 from a matched related and 39 from matched unrelated donor. Transplantation conditioning was oral or intravenous busulfan and cyclophosphamide followed by infusion of donor marrow. Cyclophosphamide was administered at a dose of 50mg/kg on days +3 and +4. In theory cyclophosphamide given within a short window after stem cell infusion can eliminate activated T cells of both host and donor origin while leaving memory T cells intact [5, 7]. Hematopoetic stem cells are resistant to the toxic effects of cyclophosphamide due to the high expression of aldehyde dehydrogenase [8]. High-dose post-transplantation cyclophosphamide was well tolerated. The most common toxicities were transient mild renal dysfunction or elevations of serum liver enzymes. Hepatic veno-occlusive disease developed in 10 patients (9%) and was fatal in 2. Engraftment of donor cells occurred in 98% of patients with a median time to neutrophil recovery of 23 days for recipients of related donor and 25 days for recipients of unrelated donor allografts. The cumulative incidences of grades II–IV GVHD by day 200 after transplantation were 42 and 46% among recipients of related and unrelated donor grafts, respectively. The incidence of grades III–IV GVHD for all patients was 10%. At 2 years after transplantation, the cumulative incidences of chronic GVHD for recipients of related and unrelated donor grafts were 9 and 11% respectively.

In a follow-up updated report on 139 consecutive patients (79 related and 60 unrelated) treated on the same study and with a median follow up of 26 months, the cumulative incidences of acute grades II–IV and chronic GVHD for all patients were 45 and 10%, respectively. Only three patients have died with refractory GVHD. Secondary systemic immunosuppressants (SSIs) were used in 45% of all patients. The overall and event free survival for all patients at 1 year after transplantation were 63 and 48% respectively, and at 2 years after transplantation were 55 and 39%, respectively, suggesting that with the use of high-dose cyclophosphamide for GVHD prophylaxis the GVT effect was retained in these high-risk patients[7]. In summary this regime was effective but there was still a high rate of acute GVHD and need for SSIs.

Another approach to GVHD prophylaxis is based on costimulation blockade. The two signal model of T cell activation dictates that for T cell activation to proceed two signals are required: one through the antigen specific T cell receptor (TCR) and the second though the non-antigen specific engagement of a costimulatory molecule by its counterpart on an antigen presenting cell[9]. The main costimulatory signal is delivered by the engagement of CD28 on the T cell by B7-1 and B7-2 on antigen presenting cells (APCs). Engagement of the TCR in the absence of costimulation leads to T cell anergy and peripheral tolerance [9, 10]. Anergy is thought to be the initial stage of development of regulatory T lymphocytes and there is evidence that regulatory T cells abrogate GVHD and enhance immune reconstitution without blocking the GVT effect [11, 12]. In a clinical study haploidentical donor derived regulatory T cells (Tregs) followed by CD34+ cells and mature T cells were infused to the recipient [13]. Almost all patients engrafted, acute GVHD rate was low, there was no chronic GVHD, immune recovery was rapid and the GVL effect appeared preserved [14].

A group from Harvard conducted a clinical trial where a haploidentical marrow was infused after in vitro co-culture with recipient cells in the presence of CTLA4Ig, which is an antibody that blocks the second costimulatory signal rendering the alloreactive T cells in the culture anergic[15]. 95% of the treated patients engrafted, the GVHD rate was low and the immune reconstitution was rapid resulting in very few viral infections [16]. After the in vitro treatment the frequency of helper T cells that were reactive against the recipient fell by one to four orders of magnitude, whereas third party alloreactivity remained unaffected.

There are currently two FDA approved CTLA4Ig molecules for clinical use, belatacept and abatacept. Belatacept is a fusion protein composed of the Fc fragment of a human IgG1 immunoglobulin linked to the extracellular domain of CTLA4. It differs from CTLA4Ig by two amino acid substitutions that increase the avidity for CD80 and CD86 [17]. Belatacept was rationally designed as CD28 blocker leading therefore to T cell anergy and tolerance upon TCR engagement. In addition CTLA4 by itself might play a significant role. Regulatory T cells constitutively express CTLA4 and may exert at least part of their action through the engagement of B7-1 and B7-2 on APCs as this has been shown to lead to a tolerogenic APC phenotype [18]. A possible mechanism of action is the induction of indolamine-2,3-dioxygenase (IDO) expression, which results in exhaustion of tryptophan and appearance of the tryptophan metabolite kynurenine that is toxic for T lymphocytes and an anergy inducing factor[19].

Belatacept is FDA approved for the prophylaxis of organ rejection in kidney transplant instead of cyclosporine based on strong data from phase III trials [20-23]. Rejection prophylaxis with belatacept in these trials resulted in comparable patient and graft survival and superior preservation of renal function compared with cyclosporine. In addition transplant biopsies suggest that belatacept confers a substantially different immunologic profile at the tissue level than cyclosporine with increased levels of markers of tolerance [24]. Belatacept was also safe and resulted in reduced mean blood pressures and new onset diabetes than cyclosporine at 1 year [25]. There were fewer deaths and serious infections than with cyclosporine with the exception of Post Transplant Lymphoproliferative Disease (PTLD) in EBV seronegative patients. Kidney transplantation patients receiving ATG/belatacept/sirolimus had a significantly higher percentage of T regs and showed a

more potent anti-donor suppressive activity when with a CNI-based regimen (ATG/tacrolimus/MMF)[26]. Belatacept has not been studied in HSCT [27].

Abatacept is another CTLAIg molecule that has been approved by the FDA for the treatment of rheumatoid arthritis and juvenile idiopathic arthritis [28]. It was also recently shown to slow down the progression of diabetes type 1[29]. Similar to belatacept, abatacept also has a favorable side effect profile and was associated with fewer serious adverse events and serious infections when compared to infliximab in a randomized phase III trial [30].

A “first-in-disease” feasibility trial of in vivo T cell costimulation blockade with abatacept for GVHD prophylaxis was reported[31]. In this trial abatacept was added to standard of care GVHD prophylaxis with cyclosporine and methotrexate in 10 patients undergoing matched unrelated donor HSCT for high-risk hematologic malignancies. Abatacept was administered at a dose of 10 mg/kg on days -1, +5, +14, and +28. The drug was well tolerated, and patients did not develop unexpected infections. No patient developed CMV disease or high-level EBV reactivation. All patients engrafted, no patients developed grade IV or steroid refractory GVHD, two patients developed severe chronic GVHD and seven patients were alive and disease free with a median follow up of 475 days. The pharmacokinetic parameters of abatacept in this patient population were similar to those in patients with Rheumatoid Arthritis. Another recently presented trial found abatacept to be well tolerated and effective in the treatment of steroid refractory chronic GVHD[32]. An increase in PD-1 expression and skewing toward Th2 cytokines were observed in responders.

In a recent pilot study children with aplastic anemia underwent haploidentical transplantation with post-transplant high dose cyclophosphamide, abatacept and sirolimus for GVHD prophylaxis[33]. Outcomes were compared to a control group that received post-transplant high dose cyclophosphamide based GVHD prophylaxis without abatacept. The GVHD and disease-free survival was 80% for the children who received abatacept vs 30% who didn’t. The acute GVHD incidence was respectively 10.5 vs 50%.

1.3 Other Considerations

Conditioning regimens will be administered per institutional standards. This is a GVHD prophylaxis study so we won’t be examining the effect of conditioning regimens. The following conditioning regimens are allowed on the study:
The combination of Busulfan (Bu) and Cyclophosphamide (Cy) as a conditioning regimen is associated with liver toxicity and VOD [34]. Furthermore, the antitumor activity of Cy in several hematological malignancies is questionable. (Cyclophosphamide was originally added to Total Body Irradiation (TBI) for tumor lysis prevention in relapsed acute leukemia.) On the other hand fludarabine (Flu) does not cause VOD and it has both direct and synergistic with busulfan anti-leukemic activity. In a study by the MD Anderson group 96 patients with AML or myelodysplastic syndrome (MDS; 56% with active disease at the time of transplantation) were transplanted with Bu/Flu conditioning regimen using Fludarabine (40 mg/m2/d for 4 days) and IV Busulfan (130 mg/m2/d for 4 days)[35]. This regimen was well tolerated with no deaths from VOD and low mucositis and overall toxicity rate. The one year OS and event-free survival EFS were 65% and 52% for all patients, and 81% and 75% for patients receiving transplants in CR. In another study by the same group the Bu-Flu conditioning regimen was retrospectively evaluated against BuCy using Bayesian methodology. In a cohort of mostly advanced patients (half of the patients were > CR1), the OS and EFS were significantly better in the Bu-Flu group compared with BuCy (70 vs 59%, P = .03, and 62 vs 37%, P = .04, respectively). Similar results were observed in the subgroup of patients transplanted in CR[36]. The safety and efficacy of Bu-Flu were also shown in a cohort of patients older than 55 years[37]. In Acute Lymphoblastic Leukemia (ALL) there is some evidence in favor of a TBI-based regimen [38]. For patients who will receive a RIC conditioning regimen, we propose the use of Fludarabine and Melphalan (140 mg/m2) which is the most widely used RIC regimen.

Most transplant centers prefer to use mobilized peripheral blood stem cells (PBSCs) over bone marrow (BM) as a stem cell source because of the ease of collection, reduced risk for the donor who doesn't need to undergo general anesthesia and faster engraftment[39]. It was shown though in a randomized trial that PBSCs are associated with a significant increase in the risk of chronic GVHD compared to BM[40]. On the other hand there is evidence suggesting a reduced risk of graft failure and of relapse with the use of PBSCs[40, 41].

1.4 Rationale

It was shown in the study by the Hopkins group that post-transplant high dose cyclophosphamide can be safely used for GVHD prophylaxis and the patients can be spared from the toxicity associated with CNIs and methotrexate[6]. This approach was also associated with a lower than expected cGVHD rate. However there was a relatively high rate of VOD (9%), acute GVHD (42% for MRD and 46% for MUD grade II-IV aGVHD) and an EFS at 2 years of 39%. 45% of the patients required secondary immunosuppression. Therefore there is clearly room for improvement.

In the abatacept study [31] it was demonstrated that the use of abatacept is safe and feasible for patients who undergo stem cell transplantation with promising results in a limited number of patients studied. However, the patients still had to take a CNI and methotrexate. In addition it was recently shown that abatacept as a single agent is safe and effective for the treatment of chronic GVHD[32].

One of our goals is to avoid the CNI associated toxicity and the need for frequent drug level monitoring and dose adjustments. In addition we might be able to improve both disease and transplant related mortality by combining two novel safe GVHD prophylaxis regimens.

The combination of cyclophosphamide and abatacept should promote tolerance given their immunological mechanism of action. We will examine if we can indeed promote tolerance induction. This will clinically translate into a low rate of chronic GVHD.

Tolerance induction might also paradoxically prevent relapses [11, 12] either by reducing the need for nonspecific immunosuppression or through another unexplained mechanism. For instance costimulation blockade was shown to lead to earlier natural killer (NK) Cell recovery [31] and NK cell alloreactivity protects from relapse[42].

Furthermore CD86 (one of CTLA4Ig's ligands) is an activation receptor for NK cells and it has been shown in vitro that CTLA4Ig enhances tumor cell killing by NK cells[43, 44]. CTLA4Ig treated Donor Lymphocyte Infusions (DLI) were used recently in a clinical study with the aim to prevent relapses[45]. In this study priming of the DLI with CTLA4Ig resulted in greater proliferation of NK cells with mature phenotype and improved relapse free survival especially in patients with AML.

We plan to enroll 50 patients with hematologic malignancies undergoing a myeloablative stem cell transplantation over the course of two years. The conditioning regimen will be myeloablative or RIC followed by the infusion of donor PBSCs. The patients will be randomized to either standard of care GVHD prophylaxis (MTX and tacrolimus) or the experimental arm with cyclophosphamide followed by abatacept. (Please refer to section 10.6 for more details on randomization.) Cyclophosphamide will be administered at a daily dose of 50mg/kg IV on days +3 and +4. Abatacept will be administered at a 10mg/kg IV dose on days +5, +14, +28, +56, +84, +112, +140, and+168 [23].

On December 15, 2021, the Food and Drug Administration approved abatacept (Orencia, Bristol-Myers Squibb Company) for the prophylaxis of acute graft versus host disease (aGVHD), in combination with a calcineurin inhibitor (CNI) and methotrexate (MTX), in adults and pediatric patients 2 years of age and older undergoing hematopoietic stem cell transplantation (HSCT) from a matched or 1 allele-mismatched unrelated donor.
Efficacy was evaluated in two studies in patients six years and older undergoing HSCT from a matched or 1 allele-mismatched unrelated donor.

GVHD-1 (NCT 01743131) was a randomized (1:1), double-blind, placebo-controlled clinical trial of patients who underwent an 8 of 8 Human Leukocyte Antigen (HLA)-matched HSCT and received abatacept or placebo in combination with a CNI and MTX. While severe (grade III-IV) aGVHD-free-survival assessed at Day 180 after transplantation was not significantly improved in patients who received Orencia compared to patients who received a placebo (HR 0.55; 95% CI 0.26, 1.18), the OS rate at Day 180 after HSCT was 97% (95% CI: 89%, 99%) for patients who received abatacept compared to 84% (95% CI: 73%, 91%) for patients who received a placebo (HR 0.33; 95% CI: 0.12, 0.93). The moderate-severe (grade II-IV) aGVHD-free survival rate at Day 180 after HSCT was 50% (95% CI: 38%, 61%) for patients who received abatacept compared to 32% (95% CI: 21%, 43%) for patients who received a placebo (HR 0.54; 95% CI: 0.35, 0.83).

Additional evidence of effectiveness was provided by GVHD-2, a clinical study using data from the Center for International Blood and Marrow Transplant Research (CIBMTR) in patients who underwent a 7 of 8 HLA-matched HSCT between 2011 and 2018. This registry-based study analyzed outcomes of 54 patients treated with abatacept for the prophylaxis of aGVHD, in combination with a CNI and MTX, versus 162 patients randomly selected from the CIBMTR registry treated with a CNI and MTX alone. The OS rate at Day 180 after HSCT was 98% (95% CI: 78%, 100%) for patients who received abatacept in combination with CNI and MTX compared to 75% (95% CI: 67%, 82%) for patients who received CNI and MTX alone.

With the approval of abatacept for GVHD prophylaxis (which validates some of our hypotheses), the SOC for GVHD prophylaxis changed and we've decided to stop enrolling in the SOC arm but complete enrollment in the experimental arm.

1.5 Hypothesis

There is a great theoretical potential for immunological synergy between cyclophosphamide and abatacept. Cyclophosphamide induces apoptosis of activated T cells and abatacept blocks activation of T cells by inhibiting the co-stimulatory signal. Therefore the combination has the potential of inducing immunologic tolerance that clinically might translate into less GVHD without increase in relapse. Furthermore abatacept activates NK cells and might enhance the graft versus tumor effect. Abatacept's immunologic mechanism of action will be examined further with our correlative studies.

Compared to the standard-of-care control arm, the experimental arm is a calcineurin inhibitor-free graft-versus-host disease prophylaxis regimen that is much more convenient for patient care. The patients on standard of care oral CNIs have to come in two to three times a week to have drug levels checked and the dose of the CNI needs to be adjusted accordingly. In addition, there are always concerns about absorption and interactions with other medications and foods with CNIs; the pills need to be taken precisely at the exact same time twice a day. Besides convenience, the experimental arm is expected to have fewer toxicities such as renal insufficiency, electrolyte abnormalities such as hypokalemia and hypomagnesemia requiring oral and frequently even IV repletion and CNS toxicity.

If the experimental arm looks promising, then our findings will be used for the design of a larger phase III trial.

1.6 Correlative Studies

1.6.1 GVHD Specific Immune Cell Activity Samples

This study provides a unique setting that will enable to further characterize the mechanism of action of abatacept.

In order to assess the effect of abatacept on immune responses, we will collect peripheral blood samples for immunologic studies. We hypothesize that the effect of abatacept would be to prevent the priming or activation of allogeneic donor cells against host antigens, thereby blocking or ameliorating acute and/or chronic GVHD. Previous studies found that the effector phase of acute GVHD involves a significant increase in CD8+ T cells expressing co-stimulatory molecules CD134 (OX40) and CD154 (CD40L)[46]. Other studies found that abatacept decreased the percentage of CD4+ T cells secreting IL-17 (Th17) and IFN-gamma (TH1) in the peripheral blood of patients with rheumatoid arthritis [47]. Notably, abatacept treated rheumatoid arthritis patients also demonstrated a decrease in repertoire restriction [48].

Based on these previous studies, we propose the following studies to determine how abatacept might influence immune cell activity, specific for GVHD, in our patient cohort. We will collect the following samples:

- 2 baseline blood samples at different timepoints from donor preferably prior to mobilization but ok during mobilization and at time of apheresis.
- 2 baseline blood samples at different timepoints from recipient prior to conditioning
- Post-transplant blood samples from recipient at days +28, +100, +180 and 1 year (representing engrafted donor immune cells)
- Post-transplant blood samples from recipient at study exit in the case of disease relapse

All samples are EDTA 10 mL x 5 tubes.

1 tube will be used immediately for flow cytometry studies using the following panel:

- Tube 1 – lymphocytes: CD4 / HLA-DR / 7-AAD / CD8 / CD56 / CD3
- Tube 2 – Treg tube: CD4 / CD127 / 7AAD / CD8 / CD25 / CD3
- Tube 3 – Helper T: CXCR3 / CCR4 / 7-AAD / CCR6 / CD4 / CD3
- Tube 4 – Cytotoxic T: CD45RA / CD44 / 7-AAD / CD8 / CD45RO / CD62L
- Tube 5 – NK cells: CD16 / CD69 / 7-AAD / CD33 / CD56 / CD3
- Tube 6 – T markers 1: CD4 / PD1 / 7-AAD / LAG-3 / CD8 / CD3
- Tube 7 – T markers 2: CD4 / CTLA4 / 7-AAD / CD28 / CD8 / CD3
- Tube 8 – T markers 3: CD4 / CD40L / 7-AAD / OX40 / CD8 / CD3
- Tube 9 – T markers iso: CD4 / IgG / 7-AAD / IgG / CD8 / CD3

4 tubes will be processed and stored in the MCC biorepository as plasma, serum, and PBMCs. Once the trial is close to completion, these samples will be analyzed in batched studies.. We hypothesize that abatacept will prevent the oligoclonal expansion of donor T cells mediating GVHD. Thus, we will compare recipient blood samples post-transplant with donor samples prior to transplant. As a functional readout, we will perform mixed lymphocyte reactions (MLRs) using donor samples at baseline and recipient samples after transplant as Responder Cells. Stimulator Cells will be recipient PBMCs collected at baseline prior to conditioning. In these studies, recipient plasma during abatacept treatment can be spiked in to determine whether abatacept in the plasma is sufficient to block MLR responses in vitro. In addition, third-party MLR studies can be performed

to test if abatacept has induced suppressive cells such as T-regulatory cells. We will also directly examine the effect of abatacept on T-regulatory cell proliferation using intracellular Ki-67 and FOXP3 staining of frozen PBMCs.

We envision that the studies proposed will profile the immune response to an extent that correlations between various immune cell parameters and clinical course can be drawn. Given that we will have a control cohort, we can also assign a causal effect on immune cells due to abatacept.

1.6.2 Immunogenicity Studies

Blood samples for determination of antibodies to abatacept will be collected at timepoints specified below. Samples will be assayed for presence of abatacept-sepecifc antidbodies by ICON Clinical Laboratories. A validated, sensitive, electrochemiluminescence assay (ECL) method will be used to analyze anti-abatacept antibodies in serum [61].

Time Points:
Baseline (screening)
Days +28, +56, +84, +112, +140, +168(all timepoints +/- 7 days)

Sample Collection:
Approximately 7 mL Whole blood samples are collected to provide approximately 3 mL of serum for drug concentration or ADA measurement at time points specified in the protocol. Blood samples are collected into appropriately labeled additive-free glass tubes (note that silicone-coated plastic tube are acceptable if non-additive glass red top tube is unavailable). Serum separator tubes (SST) should not be used.

Processing Guidelines:
Blood samples are allowed to clot at room temperature for at least 20 minutes for a complete clot to form. Serum is separated from the whole blood within approximately 40 minutes of collection. Specimens should be centrifuged at 1,500 x g for approximately 10 minutes in a room temperature centrifuge (a refrigerated centrifuge is acceptable if available) to harvest the serum. After centrifugation, the upper serum layer is carefully transferred with a disposable pipette and equally aliquoted into 2 labeled screw capped plastic storage tubes (each with approximately 1.5 mL of serum, one for PK analysis and one for a back-up sample for PK analysis, respectively). Similar samples are collected for ADA sample and backup sample as noted in the protocol. After centrifugation, the upper serum layer is carefully transferred with a disposable pipette and into one labeled screw capped plastic storage tube. If red blood cells are inadvertently drawn into the serum, the sample should be recentrifuged immediately and processed appropriately. Serum samples will be frozen in an upright position within 120 minutes of sample collection at approximately -70°C (-20°C is acceptable if -70°C not available at the collection site).

Shipping Guidelines:
Samples are to be shipped to ICON per the included requisition forms for processing and analysis.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

To examine if the novel combination of post transplant high dose cyclophosphamide and abatacept can induce tolerance.

2.2 Secondary Objectives

- To estimate the rate of other important transplant outcomes with the two GVHD prophylaxis strategies:
    - donor engraftment by day 28
    - Grades II-IV and III-IV acute GVHD
    - Chronic GVHD of all grades
    - Relapse rate
    - Disease free survival over the course of the study
    - Overall survival
    - Transplant related mortality by 1 year after transplantation
- To estimate the infectious, renal, cognitive and other toxicity caused by the two GVHD prophylaxis strategies.

2.3 Exploratory Objectives

Post-transplantation immune reconstitution studies will include measuring T cell and NK cell phenotype, PD-1 expression, and alloreactivity to recipient and third party at predetermined time points. We will compare and contrast the findings between the treatment and control arm and correlate those with disease relapse and presence of acute or chronic GVHD.

2.4 Endpoints

2.4.1 Primary

Occurrence of (moderate and severe) chronic GVHD by one year post–transplant . Chronic GVHD will be diagnosed and staged according to the previously published and widely accepted National Institutes of Health consensus criteria [49]. Only moderate and severe chronic GVHD will be included.

2.4.2 Secondary

1. GVHD- and relapse-free survival by one year post transplant. GRFS: GVHD- and relapse-free survival will be defined as the absence of acute GVHD Grade III or IV or moderate or severe chronic GVHD or relapse or non-relapse mortality by one year post transplant. CRFS: Chronic GVHD and relapse free survival will be defined as the absence of moderate or severe chronic GVHD or relapse or non-relapse mortality by one year post transplant. Acute GVHD will be diagnosed and graded according to Glucksberg criteria[50].

2. **The rate of donor engraftment by day 28**
   The day of neutrophil engraftment is defined as the first day of three consecutive increased lab values on different days, after the conditioning regimen induced nadir of blood counts that the absolute neutrophil count is ≥ 500/microL. The day of platelet engraftment is defined as the first day of three consecutive increased lab values on different days, after the conditioning regimen induced nadir of blood counts that the platelet count is ≥ 20,000/microL without platelet transfusion support in the seven days prior. Chimerism will also be measured on Days +28, +100 and +180. Donor engraftment is defined as presence of both donor and platelet engraftment.

3. **Grades II-IV and III-IV acute GVHD**

Acute GVHD will be diagnosed and graded according to Glucksberg criteria[50].

4. **Chronic GVHD**

5. **Disease free survival over the course of the study**
   Recurrent malignancy will be defined by standard hematologic criteria.

6. **Overall survival**
   Proportion of patients who are alive post transplantation.

7. **Transplant related mortality at 1 year after transplantation**
   Defined as death in the absence of recurrent malignancy.

8. **Infections by 1 year after transplantation**
   Defined as Grade 3 or higher infections

9. **Renal insuffiency and other toxicities**
   Defined as Grade 3 or higher renal insufficiency or other grade 3 or higher.

10. **Cognitive impairment**
    Defined as change in Mini-Mental State Examination between baseline, Days +100, +180 and one year after transplant

3.0 PATIENT ELIGIBILITY

Eligibility waivers are not permitted. Subjects must meet all of the inclusion and exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered (see Section 3.3 for registration requirements).

3.1 INCLUSION CRITERIA

- High risk hematologic malignancy justifying the need for an allogeneic hematopoetic stem cell transplantation: AML, ALL, CML in accelerated or blast phase, MDS/MPN, NHL, Hodgkin lymphoma, and multiple myeloma
- Patient receiving either Myeloablative Busulfan/Fludarabine or TBI/Cy or Reduced Intensity Fludarabine/ Melphalan for conditioning
- Patient age $\geq$ 18 years
- ECOG performance score 0-2
- Creatinine clearance $\geq$ 40 (by Cockgroft-Gault)
- Adequate hepatic function (total bilirubin <2.0 mg/dl, AST and ALT <3x ULN)
- Normal cardiac function (EF > 50%)
- Acceptable pulmonary function, FEV1 of >/= 50% of predicted and DLCO of >/= 40% predicted.
- Psychological assessment, social arrangement and family support indicate reasonable expectation that patient will adhere to the medication regimen required after stem cell transplantation.
- No clinical evidence of metastatic CNS disease
- Laboratory testing for infectious disease performed on a sample collected within 30 days prior to or concurrently with procurement of the stem cell product. No clinical or laboratory evidence for active infections such as hepatitis B or C, HIV.
- Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 90 days following completion of therapy. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. A female of child-bearing potential is any woman (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:
    - Has not undergone a hysterectomy or bilateral oophorectomy; or
    - Has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).
- Ability to understand and willingness to sign a written informed consent.

3.2 EXCLUSION CRITERIA

a. Patients with hematologic malignancies for which transplant is not the only curative option, such as AML with good or intermediate cytogenetics or molecular markers in CR1 or CML in chronic phase

b. Inability to identify an 8/8 or 10/10 HLA-Matched (related or unrelated) Donor

c. Prior allogeneic stem cell transplant

d. Active malignant disease relapse

e. Active, uncontrolled infection, uncontrolled cardiac angina, symptomatic congestive heart failure or any other uncontrolled medical condition that in the opinion of the investigator

will put the patient at increased risk by participating in this clinical trial or would interfere with data collection or interpretation

f. Life expectancy <3 months

g. Pregnancy or lactation

h. Inability to comply with treatment regimen

i. History of allergic reactions attributed to compounds of similar chemical or biologic composition to abatacept,

j. Patients may not be receiving any other investigational agents in the last 28 days

k. Patients with chronic myeloid leukemia in first chronic phase.

3.3 REGISTRATION PROCEDURES

3.3.1 Registration Process

All patients must obtain a study ID number from the UCSD BMT Clinical Trials Office before enrollment. An eligibility check list signed by the PI or Sub-I must be faxed or emailed to the BMT Clinical Trials Office at 858-822-1473. Confirmation of eligibility by UCSD is not required prior to starting treatment.

Patients will be given a unique sequential patient ID number based on the study number, and patient enrollment number.

Oversight by the principal investigator is required throughout the entire registration process.

4.0 TREATMENT PLAN

4.1 Treatment Dosage and Administration

4.1.1 Randomization

Patients will be randomized to the either the standard of care or the experimental arm prior after enrollment and prior to initiating the conditioning regimen.

4.1.2 Pre-transplant Work-up and Intake

Pre-transplant donor and recipient work up and intake will proceed per the institutional standard operating procedure (SOP).

Patients age, sex, disease and disease status, donor source (sibling, unrelated), HLA matching and donor and recipient CMV status and blood groups will be recorded for research purposes.

4.1.3 Conditioning Regimen

Conditioning regimens will be administered per institutional standard. The following conditioning regimens are allowed on the study: TBI/Cy, myeloablative BuFlu and RIC Flu/Mel.

Drug administration dates and dose received will be recorded on the study Case Report Forms (CRFs).

Dosing will be based on institutional standard of care.
Dosing guidelines are provided as suggestions in Appendix V.

4.1.4 Transplant (Day 0)

Patients will receive donor peripheral stem cells per institutional standards. Stem cell dose will be recorded for research purposes, but will be at minimum 2x10^6 CD34+ cells/kg.

4.1.5 GVHD prophylaxis

Patients were randomized to the experimental or the standard of care arm. The two arms will be stratified by conditioning regimen (MA vs RIC) and donor type (MRD vs MUD).
Drug administration dates and dose received will be recorded on the study Case Report Forms (CRFs).

On December 15 2021 the FDA approved abatacept for GVHD prophylaxis (in combination with Methotrexate and a CNI). Given that the SOC for GVHD prophylaxis changed, we've decided to stop enrolling in the SOC arm but complete enrollment in the experimental arm.

Starting with Amendment 8 all patient will be enrolled in the experimental arm.

4.1.5.1 Standard of Care arm

Patients randomized to the standard of care arm will receive the following GVHD prophylaxis regimen:

- Tacrolimus 0.022 mg/kg/day (using ideal body weight) starting on day -2; adjust for trough of 5-15
- Methotrexate
    - For Flu/Bu and Flu/Mel: 5 mg/m2 on Days 1, 3, 6 and 11 (The methotrexate dose might be adjusted or held at the investigator's discretion)
    - For Cy/TBI and TBI/Cy: 15 mg/m2 on day 1, then 10 mg/m2 on days 3, 6, and 11 (the methotrexate might be adjusted or held at the investigator's discretion)

Use of ATG in the standard of care arm should be avoided but will ultimately be at the investigator's discretion for patient safety. It is prohibited in the experimental arm.

4.1.5.2 Experimental arm

Patients randomized to the experimental arm will receive the following GVHD prophylaxis regimen:

- A 10mg/kg dose of abatacept (maximum dose, 1000 mg) will be given intravenously on days, +5, +14 , +28, +56, +84, +112, +140, +168[31].
- Dosing will be based on actual body weight on the day of treatment (-1 day allowed).
- The doses on days +5 and +14 will be administered on that exact day.
- The other doses may be administered within a 3 day window from the planned day to allow for clinic closures (weekends, holidays etc).
- Cyclophosphamide will be administered at a dose of 50 mg/kg/day will be given intravenously on days +3 and +4 after transplantation [6]. Mesna will be administered on both days of cyclophosphamide administration as per institutional guidelines.

Cyclophosphamide will be dosed according to the recipient's ideal body weight (IBW), unless the patient weighs more than 125% of IBW, in which case the drug will be dosed according to the adjusted IBW (AIBW= IBW + [(0.25) x (ABW-IBW)]).

4.1.6 Post-Transplant Treatment and Maintenance

Growth factors are allowed 24-48 hr after the completion of the chemotherapy, ie on Day +13 for patients on the SOC arm and **Day +6 on the experimental arm**.

Post-transplant supportive care and infection prophylaxis will be otherwise provided according to institutional standards.

Post transplant maintenance therapies to prevent relapse will generally be allowed unless there is a known GVHD prophylactic effect.

4.2 Toxicities and Dosing Delays/Dose Modifications

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed for the development of toxicity according to the Time and Events table. Toxicity will be assessed according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE), version 5.0.

As busulfan, fludarabine (or TBI based regimens), abatacept and cyclophosphamide are part of the transplantation regimen, they will only be held if in the opinion of the investigator, the patient's safety is at risk. The reason for all dosing delays must be described in the subject's medical record.

Infusion related reactions to cyclophosphamide and abatacept have been observed and are usually mild and self-limited.

If an anaphylactic or other serious allergic reaction to abatacept occurs, administration of abatacept should be stopped immediately with appropriate therapy instituted, and the use of abatacept should be permanently discontinued. Please refer to the Orencia (abatacept) Package Insert for further information.

If there are grade 3 or higher nonhematologic toxicities due to abatacept, hold abatacept until grade 2 or less, then resume dosing. If with resumed dosing grade 3 or higher nonhematologic toxicity recurs, then discontinue abatacept permanently.

If there is a delay of abatacept dosing by 4 weeks or more due to toxicity, abatacept will be discontinued.

Common toxicities with abatacept

Infections.

- The most commonly reported side effects (occurring in one out of 100 or more of patients treated with drug) were headache, upper respiratory tract infection (cough sneezing, runny nose, nasal congestion, sore throat), nasopharyngitis (commonly known as cold), and nausea
- Serious infections: pneumonia, cellulitis, urinary tract infection, bronchitis, diverticulitis, and acute pyelonephritis

Malignancies

- The potential role of ORENCIA in the development of malignancies in humans is unknown. One major safety concern with belatacept is the numerical increase of early Post-transplant Lymphoproliferative Disease (PTLD) in the BENEFIT trial. However, whether or not belatacept really increases the risk of developing PTLD is unclear. While the numbers in the belatacept groups were higher than in controls in the first trial, they were still very low. Given the spontaneous frequency of occurrence (1.6%), 14 cases in about 1000 patients is not unexpectedly high. Furthermore a recent meta-analysis of five Randomized Controlled Trials did not show a difference in PTLD.

Infusion-Related Reactions and Hypersensitivity Reactions

- The most frequently reported events (1%-2%) were dizziness, headache, and hypertension. Acute infusion-related events that were reported in >0.1% and ≤1% of patients treated with ORENCIA included cardiopulmonary symptoms, such as hypotension, increased blood pressure, and dyspnea; other symptoms included nausea, flushing, urticaria, cough, hypersensitivity, pruritus, rash, and wheezing. Most of these reactions were mild (68%) to moderate (28%).

4.3 Concomitant Medications/Treatments

Concomitant use of any other immunosuppressive medications (such as cyclosporine, MMF or corticosteroids) for GVHD prophylaxis is prohibited. Concurrent use of other investigational drugs is prohibited. Immunosuppressive medications such as steroids may be used for the treatment of acute or chronic GVHD or other indications per the investigator's discretion. If a patient develops acute GVHD, abatacept will be continued as scheduled up to day+168.

Supportive care, such as hydration and pre-medications should be provide according to institutional standard of care and is at the discretion of the treating physician.

4.4 Duration of Therapy

Treatment on the experimental arm will continue until the last dose of abatacept on Day +168 after transplantation or until:

- Disease progression
- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study, OR
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

4.5 Duration of Follow Up

Patients will be followed for 1 year after transplant or until death whichever occurs first. Patients removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event or 1 year after transplant, whichever is later.

4.6 Removal of Patients from Protocol Therapy

Patients will be removed from therapy when any of the criteria listed in Section 5.4 apply. Notify the Principal Investigator, and document the reason for study removal and the date the patient was removed in the Case Report Form. The patient should be followed-up per protocol.

4.7 Handling of dropouts

Patients in the treatment arm who drop out of the study prior to the first dose of abatacept will be replaced. Similarly, patients in the control arm who drop out prior to day +5 will be replaced. All patients, including those who are replaced, will be included in the intent-to-treat analysis, but replaced patients will be excluded from the as-treated analysis.

5.0 STUDY PROCEDURES

5.1 Screening/Baseline Procedures

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

All screening procedures must be performed within 90 days prior to registration unless otherwise stated. The screening procedures include:

1. **Informed Consent –** The investigator must obtain documented consent from each potential subject prior to performing research procedures.

2. **Medical history -** Complete medical and surgical history, history of infections

3. **Demographics -** Age, gender and race

4. **Review subject eligibility criteria -** All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial

5. **Review previous and concomitant medications –** The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject with 30 days before starting the trial. All concomitant medications will be recorded during the trial.

6. **Physical exam including vital signs, height and weight.** Vital signs include heart rate, blood pressure, respiratory rate, and temperature.

7. **Performance status (ECOG**) - Performance status evaluated prior to study entry according to Appendix III.

8. **Adverse event assessment-** See Section 6 for Adverse Event monitoring and reporting. Adverse events will be graded and recorded throughout the study according to NCI-CTCAE Version 5.0. Toxicities will be characterized in terms

including seriousness, causality, toxicity grading, and action taken with regard to study therapy. All safety evaluations may be performed at any time based on the clinical judgement of the Investigator.

9. **Hematology -** CBC with auto differential, platelets, coagulation

10. **Serum chemistries**
    Comprehensive metabolic panel (CMP) to include: albumin, alkaline phosphatase, ALT/SGPT, AST/SGOT, BUN, creatinine, electrolytes (sodium, potassium, calcium, chloride, bicarbonate), glucose, and total bilirubin.

11. **Quantiferon**

12. **Pregnancy test (for females of child bearing potential)**
    See section 3.1.6.1 for definition.

13. **Tumor assessment**
    Disease type specific (eg. bone marrow biopsy for leukemias, PET or CT scan for lymphomas, serum/urine protein electrophoresis and free light chains for multiple myeloma)

14. **Chest X ray**

15. **EKG**

16. **Echocardiogram**

17. **Mini-mental status exam**

5.2 Study visits (days +5, +14, +28, +56, +84, +112, +140, +168)

History and Physical exam

Vital signs- vital signs to be performed as clinically indicated during abatacept administration.

Hematology

Serum chemistries

Toxicity assessment

Mini mental status exam

Disease assessment will be at the investigator's discretion

Recording of GVHD (date of diagnosis, symptoms/signs, scoring and treatment)

Monitoring for CMV, EBV, and adenovirus PCR post-transplantation.

5.3 Blood for correlative studies will be collected:

- 2 baseline blood samples at different timepoints from donor preferably prior to mobilization but ok during mobilization and at collection
- 2 baseline blood samples at different timepoints from recipient prior to conditioning
- Post-transplant blood samples from recipient at days +28, +100, +180, and +365
- Blood samples for the immunogenicity studies will be collected at screening, days +28, +56, +84, +112, +140, +168 (all timepoints +/- 7 days)

5.4 Removal of Subjects from Study

Patients can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation will be documented and may include:

- Patient voluntarily withdraws from treatment (follow-up permitted)
- Patient withdraws consent (termination of treatment and follow-up)
- Patient is unable to comply with protocol requirements
- Patient demonstrates disease progression (unless continued treatment with study drug is deemed appropriate at the discretion of the investigator)
- Patient experiences toxicity that makes continuation in the protocol unsafe
- Treating physician judges continuation on the study would not be in the patient's best interest
- Patient becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event)
- Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study
- If a research subject cannot be located to document survival after a period of 1 year, the subject may be considered "lost to follow-up."

6.0 Study Calendar: Experimental Arm

Period/Procedure	**Screening**	**Treatment**													
	-90 to -1	Post Transplant													End of treatment
Day		+3	+4	+5	+14	+28	+56	+84	+100	+112	+140	+168	+180	+365	
Informed Consent	X														
Randomization	X														
Treatment															
Conditioning Regimen															
Stem cell Infusion															
Cyclophosphamide		X	X												
Abatacept[6]				X	X	X	X	X		X	X	X			
Clinical Procedures															
Review of Eligibility Criteria	X														
Demographics	X														
Medical History	X														
Physical Exam[7]	X			x	x	x	x	x	X	x	x	x	X	X	X
Performance Status (ECOG)[7]	X								X				X	X	X
Vital Signs, Weight[7, 8]	X			x	x	x	x	x	X	x	x	x	X	X	X
Height	X														
ECHO	X														
EKG	X														
Chest X-ray	X														
GVHD Assessment									X				X	X	X
Tumor Assessments (as indicated for disease)															
Bone Marrow Biopsy (Leukemia)[7]	X								X[10]						
	-90 to -1														End of treatment

		Post Transplant													
Day	_	+3	+4	+5	+14	+28	+56	+84	+100	+112	+140	+168	+180	+365	
PET/CT or CT (Lymphoma)[7]	X								X[10]						
Serum/Urine Protein Electrophoresis (MM)[7]	X								X[10]					X	X
Free Light Chains (MM)[7]	X								X[10]					X	X
Laboratory Procedures															
CBC[7]	X			x	x	x	x	x	x	X	x	x	x	X	X
PT[7]	X														
PTT[7]	X														
CMP[7]	X			x	x	x	x	x	x	X	x	x	x	X	X
Quantiferon	X														
Pregnancy Test	X														
CMV, EBV, and adenovirus PCR post-transplantation[7]						X			X						
Immunogenicity samples[5]	X					X	X	X		X	X	X			
Research labs	X					X			X				X	X	X
Chimerism[11]						X			X				X		
Study Assessments															
Adverse Events				x	x	x	X	x	x	X	x	x	x	X	X
Concomitant Medications	X			x	x	x	X	x	x	X	x	x	x	X	X
Mini-Mental Status Exam[7]	X								X				X	X	X

Blood samples

1. 2 baseline blood samples at different timepoints from donor preferably prior to mobilization but ok during mobilization and at collection
2. 2 baseline blood samples at different timepoints from recipient prior to conditioning
3. Post-transplant blood samples from recipient at days +28, +100, +180 (+/- 7 days) and 1 year (+/- 14 days)
4. Blood samples to be checked if disease relapse
5. Sent to central lab, collected at screening, days +28, +56, +84, +112, +140, +168 (all timepoints +/- 7 days)
6. Beginning on Day +28, Abatacept may be administered within +/- 3 day window
7. Beginning on Day +28, procedures may be performed within +/- 7 day window
8. Dosing weight for the first two doses of Abatacept (Days +5 & +14) may be taken within -1 day window.

9. Vital signs to be collected as clinically indicated during Abatacept administration. Vital signs include heart rate, blood pressure, respiratory rate, and temperature.
10. The window for the first post-transplant assessment is between Day +60 and Day +120
11. The window for chimerism is +/- 10 days
12. The window for 1 year and End of treatment visits is +/- 14 days

7.0 Measurement of Effect

Acute GVHD will be diagnosed and graded according to the Glucksberg Criteria [50]. (Appendix II)

Chronic GVHD will be diagnosed and staged according to the previously published and widely accepted National Institutes of Health consensus criteria [49] (Appendix I)

The day of neutrophil engraftment is defined as the first day of three consecutive lab values on different days, after the conditioning regimen induced nadir of blood counts that the absolute neutrophil count is ≥ 500/microL. The day of platelet engraftment is defined as the first day of three consecutive lab values on different days, after the conditioning regimen induced nadir of blood counts that the platelet count is ≥ 20,000/microL without platelet transfusion support in the seven days prior. Chimerism will also be measured on Days +28, +100 and +180. Donor engraftment is defined as presence of both donor and platelet engraftment.

Recurrent malignancy will be defined by standard disease specific criteria and it is recommended to be proven by biopsy (bone marrow for leukemia/ myeloma, lymph node for lymphoma).
Event free survival is defined as survival in the absence of recurrent malignancy.

Transplant related mortality is defined as death in the absence of recurrent malignancy.

7.1 Safety/tolerability

Analyses will be performed for all patients after the initiation of the conditioning regimen. The study will use the CTCAE version 5.0 for reporting of adverse events http://ctep.cancer.gov/reporting/ctc.html) (Appendix IV).

8.0 ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

8.1 Definitions

Definition of Adverse Event (AE): Any untoward medical occurrence or worsening of a preexisting medical condition associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

Related: There is a reasonable causal relationship between study drug administration and the AE.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

NONSERIOUS ADVERSE EVENT

- Non-serious Adverse Events (AE) are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [eg, IND US trial] as part of an annual reporting requirement.
- Non-serious AE information should also be collected from the start of a placebo lead-in period or other observational period intended to establish a baseline status for the subjects.

A non-serious adverse event is an AE not classified as serious.

Non-serious Adverse Event Collection and Reporting

The collection of non-serious AE information should begin at initiation of study drug. All non-serious 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.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious 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 to BMS 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 participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (eg, anemia versus low hemoglobin value).

Potential Drug Induced Liver Injury (DILI)

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:

1. AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)

   AND

2. Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)

   AND

3. No other immediately apparent possible causes of AT 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.

8.1.1 Unanticipated Problems: An unanticipated problem involving risk to subjects or others (UP) is defined as any unexpected incident, event, or problem that is related or possibly related to the research and poses greater risk of harm than was previously known to an individual or group of individuals (including research subjects, research staff, or others not directly involved in the research).

Examples of Unancipated Problems may include:
- Adverse Events
- Subject complaints
- Medication or device errors
- Other errors in the conduct of the research
- Protocol deviations or violations
- Protocol exceptions (changes made to the research without prior approval in order to eliminate apparent immediate harm to subjects)
- Breach of confidentiality
- Billing problems that pose unanticipated financial risk to subjects

8.1.2 Serious Adverse Event (SAE): Any adverse event that results in ANY of the following outcomes:

1) Death
2) A life-threatening adverse drug experience
3) Inpatient hospitalization or prolongation of existing hospitalization (for >24 hours). (see NOTE below)
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition
7) Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.

Although pregnancy, overdose, potential drug-induced liver injury (DILI), and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).

The following hospitalizations are not considered SAEs:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).
- Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)

8.1.3 Severity vs. Seriousness: Severity is not synonymous with seriousness. SAE is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. SAEs need to fulfill additional reporting process (reported to corporate global drug safety group or pharmacovigilence group, regulatory authorities, IRBs). On the other hand, Severity of an AE is a point on a scale of intensity of the adverse event in question. See Section 8.3 below for the severity grading system.

Pregnancy:

If, following initiation of abatacept, it is subsequently discovered that a study participant is pregnant or may have been pregnant at the time of the abatacept exposure, including during at least 5 half-lives after abatacept administration, abatacept will be permanently discontinued.

The investigator must immediately notify Worldwide.Safety@bms.com of this event via either the CIOMS, MedWatch, or appropriate Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Protocol-required procedures for study discontinuation and follow-up must be performed on the participant.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the CIOMS, MedWatch or appropriate Pregnancy Surveillance Form. A BMS Pregnancy Surveillance Form may be provided upon request.

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. In

order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must give written permission for disclosure of this information.

8.1.4 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.

8.1.5 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 non-serious or serious AE, as appropriate, and reported accordingly.

8.2 Data Collection procedures for adverse events

The principal investigator is responsible for evaluating all adverse events, obtaining supporting documents, and determining that documentation of the event is adequate. He/she is responsible for determining the seriousness, severity, and relationship of the adverse event to the investigational drug. The principal investigator may delegate these duties to sub-investigators and must assure that these sub-investigators are qualified to perform these duties under the supervision of the principal investigator. All adverse events will be documented in the subject's source and recorded on Case Report Form(s).

The term of the adverse event should reflect the diagnosis rather than its symptoms, when available. In the event of death, the cause of death should be recorded as the adverse event. The detailed description of the event will include appropriately graded severity of the adverse event and its relationship to the study drug.

"Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfill the definition of an AE or SAE.

Events that **do not** meet the definition of an AE include:

- Any clinically significant abnormal laboratory finding or other abnormal safety assessments that is associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.
- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition
- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital)
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen

8.3 Grading and Attribution

Grading (severity grading): Terminology Criteria for Adverse Events will be evaluated using the following criteria (The NCI Common Toxicity Criteria, Version 4.0 shall be used):

- **Grade 1 - Mild**: Awareness of symptom, but easily tolerated; usually transient requiring no special treatment; does not interfere with usual status or activities
- **Grade 2 – Moderate:** May be ameliorated by simple therapeutic measures; may interfere with usual activities
- **Grade 3 – Severe:** Incapacitating, inability to perform usual activities
- **Grade 4 – Life-threatening/Disabling:** Subject was at risk of death or significant disability at the time of the event
- **Grade 5 – Death** related to AE

Attribution is an assessment of the relationship between the AE/SAE and the medical intervention**.** Although all of the drugs used in this study have been used in man before, this combination of drugs has not, therefore the phase 1 study is considered a "first in human" study and therefore **all adverse events should be considered relevant to determining dose-limiting toxicities and to reporting unless the event can clearly be determined to be unrelated to the study drug.** Relationship of the adverse event to the investigational drug will be determined by the principal investigator, or qualified designee, and will be categorized as:

Relationship	Attribution	Description
Unrelated to investigational agent/intervention[1]	Unrelated	The AE is clearly ***NOT* related** to the intervention
	Unlikely	The AE is ***doubtfully related*** to the intervention
Related to investigational agent/intervention[1]	Possible	The AE ***may be related*** to the intervention
	Probable	The AE is ***likely related*** to the intervention
	Definite	The AE is ***clearly related*** to the intervention

[1]**NOTE**: AEs listed as possibly, probably, or definitely related to the investigational agent/intervention are considered to have a suspected reasonable causal relationship to the investigational agent/intervention. For routine, adverse event reporting purposes, "Attribution" defines the relationship between the adverse event and the investigational agent(s)/intervention. Additional Instructions and Guidelines that can be found at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/cdus_ig_3r4.pdf.

8.4 Steps to Determine If an Adverse Event Requires Expedited Reporting

Step 1: Identify the type of adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE v5.0).

Step 2: Grade the adverse event using the NCI CTCAE v5.0.

Step 3: Determine whether the adverse event is related to the protocol therapy Attribution categories are as follows:

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

Note: This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

Step 4: Determine the prior experience of the adverse event.
Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

- the current known adverse events listed in the Agent Information Section of this protocol;
- the drug package insert;
- the current Investigator's Brochure

8.5 Reporting Requirements for Adverse Events

8.5.1 Routine Reporting

All serious adverse events (SAE) regardless of causality must be documented according to the table outlined in Section 8.3

Contact for Expedited reporting –

Dimitrios Tzachanis, MD PhD
Tel: 858-822-6600
Pager: 619-290-8792
Email: dtzachanis@ucsd.edu

Divya Koura, MD
Tel: 858-822-6600
Pager: 619-290-8079
Email: dkoura@ucsd.edu

Serious adverse events, occurring after the informed consent is signed but prior to the initial dose of abatacept will be collected as part of the subject's medical history/baseline symptoms but will only be reportable if they are considered by the Investigator to be causally related to required research procedures. Non-serious adverse events (AEs) and serious adverse events (SAEs) will be collected throughout the treatment period maintenance phase and for 30 days after discontinuing abatacept or if otherwise classified as possibly related to study intervention. Events occurring during this period must be followed until resolution or death unless in the Investigator's opinion, the condition is unlikely to resolve due to the patient's underlying disease. SAEs must be reported to oversight agencies as described below

8.5.2 Reporting to the FDA

8.5.3 Reporting to the IRB

The Principal Investigator must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug. Serious Adverse Event (SAE) reporting will be in accordance with the UCSD IRB Regulations and Code of Federal Regulation Title 21 Volume 5 Part 312.32. MedWatch forms will be utilized for reporting purposes. For a copy of the form, go to http://www.fda.gov/medwatch/getforms.htm.

All SAEs from outside institutions must be reported to:

UCSD BMT Clinical Trials Office
3855 Health Science Drive MC0698
La Jolla, CA, 92093
Phone: 858-822-6390/6396/6397
Fax 858-822-6398

within 24 hours of learning of its occurrence, even if it is not felt to be treatment related. Follow-up information about a previously reported SAE must be sent to the UCSD BMT Clinical Trials Office as soon as complete details of the SAE are known.

If the SAE is death, and is determined to be possibly, probably or definitely related to the investigational drug or any research related procedure, the event must be reported to the UCSD DSMB Chair by the UCSD Clinical Trials Office within 24 business hours. The reporting procedure is by personal communication via phone or with written documentation of the one to one communication via e-mail, with a copy of the e-mail to DSMB Administrator and DSMB Coordinator.

Follow-up data should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or discontinued study participation.

The UCSD Human Research Protections Program (HRPP) must be notified within 10 business days of “any unanticipated problems involving risk to subjects or others” (UPR).

The following events meet the definition of UPR:
1. Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures.
2. Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
3. Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
4. Any new information (e.g., publication, safety monitoring report, updated sponsor safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
5. Any breach in confidentiality that may involve risk to the subject or others.
6. Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.

Routine Reporting

The UCSD HRPP will be notified of any adverse events that are not unanticipated problems involving risk to subjects or others (non-UPRs) at the time of the annual Continuing Review.

8.5.4 Reporting by and to the Drug Manufacturer

- 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, whether related or not related to study drug. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (eg, a follow-up skin biopsy).
- 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 aforementioned time periods, which is believed to be related to study drug or protocol-specified procedure.
- An SAE report should be completed for any event where doubt exists regarding its seriousness;
- 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 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.
- An appropriate SAE form (USA = Medwatch form) should be used to report SAEs to BMS . The BMS protocol ID number must be included on whatever form is submitted by the Sponsor/Investigator.
    - The MedWatch form is available at: https://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf
- For studies with long-term follow-up periods in which safety data are being reported, include the timing of SAE collection.
- The Sponsor will reconcile the clinical database SAE cases (case level only) transmitted to BMS Global Pharmacovigilance (Worldwide.Safety@bms).
    - Frequency of reconciliation should be every 3 months and prior to the database lock or final data summary.
    - BMS GPV&E will email, upon request from the Investigator, the GPV&E reconciliation report.
    - Requests for reconciliation should be sent to aepbusinessprocess@bms.com. The data elements listed on the GPV&E reconciliation report will be used for case identification purposes.

If the Investigator determines a case was not transmitted to BMS GPV&E, the case should be sent immediately to BMS.

- In 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). 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 a SUSAR Report.
    - Other important findings which may be reported by BMS as an Expedited Safety Report (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 to the Sponsor Investigator's responsibility to report events to their local HA, 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 eMedWatch form & pregnancies must be reported on a Pregnancy Surveillance Form or can be submitted on the aforementioned SAE form to BMS.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: +1 609-818-3804

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 BMS (or designee) using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

SAEs should be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/. The website will instruct you where to send the SAE forms.

9.0 DRUG INFORMATION

9.1 Abatacept

9.1.1 Abatacept Description

ORENCIA (abatacept) is a soluble fusion protein that consists of the extracellular domain of human cytotoxic T-lymphocyte-associated antigen 4 (CTLA-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.
ORENCIA is supplied as a sterile, white, preservative-free, lyophilized powder for parenteral administration. Following reconstitution with 10 mL of Sterile Water for Injection, USP, the solution of ORENCIA is clear, colorless to pale yellow, with a pH range of 7.2 to 7.8. Each single-use vial of ORENCIA provides 250 mg abatacept, 500 mg maltose, 17.2 mg monobasic sodium phosphate, and 14.6 mg sodium chloride for administration.

9.1.2 CLINICAL PHARMACOLOGY

Mechanism of Action

Abatacept, a selective costimulation modulator, inhibits T cell (T lymphocyte) activation by binding to CD80 and CD86, thereby blocking interaction with CD28. This interaction provides a costimulatory signal necessary for full activation of T lymphocytes. Activated T lymphocytes are implicated in the pathogenesis of RA and are found in the synovium of patients with RA. In vitro, abatacept decreases T cell proliferation and inhibits the production of the cytokines TNF alpha (TNFα), interferon-γ, and interleukin-2. In a rat collagen-induced arthritis model, abatacept suppresses inflammation, decreases anti-collagen antibody production, and reduces antigen specific production of interferon-γ. The relationship of these biological response markers to the mechanisms by which ORENCIA exerts its effects in RA is unknown.

Pharmacodynamics

In clinical trials with ORENCIA at doses approximating 10 mg/kg, decreases were observed in serum levels of soluble interleukin-2 receptor (sIL-2R), interleukin-6 (IL-6), rheumatoid factor (RF), C-reactive protein (CRP), matrix metalloproteinase-3 (MMP3), and TNFα. The relationship of these biological response markers to the mechanisms by which ORENCIA exerts its effects in RA is unknown.

Pharmacokinetics and Drug Metabolism

Healthy Adults and Adult RA The pharmacokinetics of abatacept were studied in healthy adult subjects after a single 10 mg/kg intravenous infusion and in RA patients after multiple 10 mg/kg intravenous infusions (see Table 3).

Table 3: Pharmacokinetic Parameters (Mean, Range) in Healthy Subjects and RA Patients After 10 mg/kg Intravenous Infusion(s)

PK Parameter	Healthy Subjects (After 10 mg/kg Single Dose) n=13	RA Patients (After 10 mg/kg Multiple Doses[a]) n=14
Peak Concentration (C_{max}) [mcg/mL]	292 (175-427)	295 (171-398)
Terminal half-life ($t_{1/2}$) [days]	16.7 (12-23)	13.1 (8-25)
Systemic clearance (CL) [mL/h/kg]	0.23 (0.16-0.30)	0.22 (0.13-0.47)
Volume of distribution (Vss) [L/kg]	0.09 (0.06-0.13)	0.07 (0.02-0.13)

[a] Multiple intravenous infusions were administered at days 1, 15, 30, and monthly thereafter.

The pharmacokinetics of abatacept in RA patients and healthy subjects appeared to be comparable. In RA patients, after multiple intravenous infusions, the pharmacokinetics of abatacept showed proportional increases of Cmax and AUC over the dose range of 2 mg/kg to 10 mg/kg. At 10 mg/kg, serum concentration appeared to reach a steady-state by day 60 with a mean (range) trough concentration of 24 (1 to 66) mcg/mL. No systemic accumulation of abatacept occurred upon continued repeated treatment with 10 mg/kg at monthly intervals in RA patients.
Population pharmacokinetic analyses in RA patients revealed that there was a trend toward higher clearance of abatacept with increasing body weight. Age and gender (when corrected for body weight) did not affect clearance. Concomitant MTX, NSAIDs, corticosteroids, and TNF blocking agents did not influence abatacept clearance.
No formal studies were conducted to examine the effects of either renal or hepatic impairment on the pharmacokinetics of abatacept.

Juvenile Idiopathic Arthritis
In patients 6 to 17 years of age, the mean (range) steady-state serum peak and trough concentrations of abatacept were 217 (57 to 700) and 11.9 (0.15 to 44.6) mcg/mL. Population pharmacokinetic analyses of the serum concentration data showed that clearance of abatacept increased with baseline body weight. The estimated mean (range) clearance of abatacept in the juvenile idiopathic arthritis patients was 0.4 (0.20 to 1.12) mL/h/kg. After accounting for the effect of body weight, the clearance of abatacept was not related to age and gender. Concomitant methotrexate, corticosteroids, and NSAIDs were also shown not to influence abatacept clearance.

9.1.3 Dosage form

Commercial marketed formulation will be provided

9.1.4 Preparation and Administration Instructions

For detailed preparation and administration instructions please refer to the most current Investigator's Brochure version.

10.0 STATISTICAL CONSIDERATIONS

This is a prospective, randomized phase 2 trial of the safety and efficacy of high dose cyclophosphamide and abatacept vs. standard of care GVHD prophylaxis. The study will enroll 50 patients with hematologic malignancy who are eligible for allogeneic peripheral blood stem cell transplant (PBSCT) following conditioning with Busulfan and Fludarabine (or a TBI based regimen for ALL) from a matched related donor (MRD) or matched unrelated donor (MUD).

On December 15 2021 the FDA approved abatacept for GVHD prophylaxis (in combination with Methotrexate and a CNI). Given that the SOC for GVHD prophylaxis changed, we've decided to stop enrolling in the SOC arm but complete enrollment in the experimental arm.

Starting with Amendment 8 all patient will be enrolled in the experimental arm.

10.1 STATISTICAL AND ANALYTICAL PLANS

Compared to the standard-of-care control arm, the experimental arm is a Calcineurin inhibitor-free graft-versus-host disease prophylaxis regimen that is much more convenient for patient care. Besides convenience, the experimental arm is expected to have lower chronic GVHD incidence, fewer toxicities such as renal insufficiency, electrolyte abnormalities and mainly hypokalemia and hypomagnesemia requiring oral and frequent IV repletion and CNS toxicity. It is expected that disease relapse will be unaffected by this new regimen, compared to standard of care.

10.2 STATISTICAL HYPOTHESES

The primary hypothesis is that post transplantation cyclophosphamide + abatacept will reduce the risk of moderate and severe chronic GVHD by one year compared to standard of care GVHD prophylaxis with methotrexate and CNI.

In addition, there is a key secondary hypothesis:

High dose cyclophosphamide and abatacept will not significantly lower the GVHD- and-relapse free survival rate at one year post transplant. Due to sample size limitations, this will be assessed as a secondary endpoint of major interest, using descriptive statistics.

Additional secondary hypotheses are that the overall safety profile of cyclophosphamide + abatacept will be favorable compared to standard of care GVHD prophylaxis with methotrexate and CNI.

10.3 10.3 ANALYSIS DATASETS

For intent-to-treat analysis, all patients who are randomized will be used in comparisons. Please refer to section 10.6 for details on randomization. For as-treated analysis, analysis data sets will be limited only to the experimental arm patients who have had at least one dose of abatacept and the control arm patients who have stayed on treatment for at least 5 days post transplant.

10.4 DESCRIPTION OF STATISTICAL METHODS

10.4.1 GENERAL APPROACH

The proportion of subjects experiencing adverse events, serious adverse events, and treatment delays/modifications will be summarized by arm, disease type and donor type.
Event rates and survival rates at one year will be estimated by arm and 95% confidence intervals will be presented per arm. Competeing risks will be treated as censoring events when estimating cause-specific hazard rates. To compare two arms, a Mantel-Haenszel test or Cox regression will be used for a proportion, treating competeing risks as censoring events; in case of rare cell frequencies, a stratified Fisher's exact test may be used instead. A log rank test will be used to compare a survival outcome between the two arms. Test for homogeneity among strata will be performed using a logrank test; if there is a significant difference among the strata, a stratified logrank test will be used to compare treatment vs. control; otherwise a non-stratified logrank test will be performed to compare the two arms. Logistic regression models, multiple linear models or Cox models will be used if there is need to adjust for covariates. Cumulative incidence curves for of relapse, non-relapse mortality, and GVHD will also be estimated and tested for significant differences using Gray's test.
Unless otherwise indicated, all tests will be two-sided at the 5% significance level.

10.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT

The primary endpoint is the occurrence of moderate and severe chronic GVHD by one year post-transplant. A Mantel-Haenszel test will be used to compare chronic GVHD risk between the two arms while taking into account the two stratification factors: conditioning regimen (MA vs RIC) and donor type (MRD vs MUD), using a two-sided test at the 5% significance level. In this analysis, competing risks will be treated as censoring events, including non-relapse mortality and relapse. This approach, which is equivalent to testing for a change in the hazard ratio in a stratified Cox regression model, will test for an change in the cause-specific hazard ratio for GVHD, as suggested by Pintle (2002) and in Kalbfliesch and Prentice (2002; Ch 8 p 257 and example 8.2)

10.4.3 ANALYSIS OF SECONDARY EFFICACY ENDPOINTS

The key secondary endpoint is GVHD- and relapse-free survival (GRFS i.e. free from acute GVHD Grade III or IV, and from moderate or severe chronic GVHD, and from relapse or non-

relapse mortality and CRFS, i.e. free from chronic GVHD, relapse and non-relapse mortality) by one year post-transplant. A stratified Cox proportional hazard model will be used to compare survival curves over time and estimate a two-sided 95% confidence interval for the hazard ratio of treatment vs. control [52]. Stratified Cox models will first be built to stratify on conditioning regimen and donor types. A test for homogeneity among strata will be performed using a logrank test. If there is not a significant difference among the strata, a non-stratified Cox model will be used to increase power [53] The proportional hazard assumption will be assessed based on weighted residuals [54].

We will also test for any change in the cause-specific hazard ratio for relapse or non-relapse mortality, using the same approach as above. Cumulative incidence funcitons for GVHD, relapse, and non-relapse mortality will also be displayed, and tested for significant differences using Gray's test.

Please refer to section 10.4.1 for more details about the analysis for other secondary endpoints.

10.4.4 SAFETY ANALYSES

Toxicities and adverse events will be summarized by arm, disease type and donor type. Their grade, relationship with the treatment, and severity will be listed. Treatment delays/modifications will also be reported.

10.4.5 ADHERENCE AND RETENTION ANALYSES

Patients who drop out of the study early will be reported; their follow-up time will be calculated and early dropouts reasons will be listed.

10.4.6 BASELINE DESCRIPTIVE STATISTICS

Baseline characteristics will be compared between the two arms to see if the randomization is successful.

10.4.7 SAFETY STOPPING RULES

There will be a continuously monitored safety stopping rule applied to deaths related to the experimental treatment (cyclophosphamide and abatacept). . The rule uses an exact sequential Pockock boundary [60]. It will be applied sequentially as subjects enroll in the experimental arm of the study. The stopping rule will be assessed after each subject has passed 100 days post-transplant. We assume UCSD experience is a ~20% transplant related mortality at 1 year, occurring with uniform hazard rate over the year; this translates to approximately 7% morality by 100 days. The stopping rule is designed to stop early with probability 60% if 100-day experimental treatment related mortality is 10% or higher.

The trial will be stopped if the number of treatment related deaths is equal to or exceeds b_n out of n patients with completed follow-up.

Number of Patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3
Number of Patients, n	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	3	3	3	4	4															

After each patient on the experimental arm reaches 100 days on post-transplant, data will be updated in the study database and a formal report will be issued documenting the status of the stopping rule. Enrollment will be suspended during the time period that the stopping rule is being updated (between day 100 and issuance of the report). If the number of experimental treatment related deaths b_n out of the first n enrolled patients exceeds the number in the table above, the study will be halted due to safety concerns.

The operating characteristics of the stopping rule are given below, where

Θ = the mortality rate
Y = the number of deaths
N = the number of patients enrolled prior to stopping
φ* = the actual probability of early stopping (hitting the boundary)
E[ ] denotes the expected value (mean)
SD[ ] denotes the standard deviation

θ	φ^*	$E[Y]$	$SD[Y]$	$E[N]$	$SD[N]$
0.10	0.5987	1.39	0.77	13.94	10.29
0.20	0.9233	1.41	0.68	7.04	7.49
0.30	0.9919	1.20	0.49	4.02	4.45
0.40	0.9995	1.09	0.31	2.71	2.65

In addition to this formal rule focused on early mortality events, the Moores Cancer Center DSMC will review this study twice per year. In addition to the usual summary of adverse events, the DSMC report will include comparative cumulative incidence curves of treatment related mortality and of aGVHC (grade III and IV), compared between treatment and control arms. Non-treatment related mortality and death from any cause will be considered competing events, respectively, in these analyses. The DSMC will consider these curves in their decision whether to continue the study. [35, 36, 55, 56].

10.4.8 SAFETY REVIEW

Safety data will be summarized and sent to the team for review every other month. The study may be stopped early due to excessive or unexpected number of serious adverse events.

10.4.9 ADDITIONAL SUB-GROUP ANALYSES

Additional subgroup analyses may be considered such as patients with good adherence; however these will be considered exploratory.

10.4.10 MULTIPLE COMPARISON/MULTIPLICITY

There is only one primary endpoint for this study. All the results from secondary endpoints will be considered exploratory. Thus, no adjustment will be done for multiple testing.

10.4.11 TABULATION OF INDIVIDUAL RESPONSE DATA

10.4.12 EXPLORATORY ANALYSES

Advanced statistical modeling techniques including logistic regression models, multiple linear models or Cox models may be applied in exploratory analyses where the stratification factors will be adjusted.

10.5 SAMPLE SIZE AND POWER

Considering the comparison of the primary endpoint between the two arms (occurrence of moderate and severe chronic GVHD by one year post transplant), with 25 patients per arm, at a type I error rate of 0.05, using a two-sided test on the treatment effect from Cox regression, and assuming that all strata have similar odds ratios, we will have 82% power to detect a significant difference if the incidence is 10% in the experimental arm and 50% in the control arm, and the incidence of competing risk from non-relapse mortality or relapse is 20% in each arm. Based on data observed from published studies [57,58, 59] without or with the use of Abatacept, we believe these incidence estimates are feasible for this study. This computation was done using the approach in Pintilie (2002).

Considering confidence intervals for the hazard rate of the key secondary endpoint, note that we expect to see on the order of 10 events in the control arm, with hazard rates in the range of 0.6 to 0.8. In this range of values, the width of an two sided 95% confidence interval ranges from about 0.7 to 1.0. While these are wide confidence intervals, they should provide a reasonable preliminary estimate of the comparability of the two approaches. In summary, while we acknowledge the limitations of the small proposed sample size, we feel that useful information will be gained.

These power calculations were done in PASS 14 (version 14.0.3)s[57].

On December 15 2021 the FDA approved abatacept for GVHD prophylaxis (in combination with Methotrexate and a CNI). Given that the SOC for GVHD prophylaxis changed, we've decided to stop enrolling in the SOC arm but complete enrollment in the experimental arm.

Starting with Amendment 8 all patient will be enrolled in the experimental arm.

New Power Analysis

Power analysis will utilize the Fisher exact test with a 2-sided 0.05 significance level.
Primary outcome: occurrence of moderate and severe chronic GVHD by 1 year post transplant.
Note: Because this is a Phase II trial, the 2-sided significance level could be 0.10 or 0.20.

Original Power for comparing event rates of 10% in ABA versus 50% in SOC with 25 in each arm, using the Fisher exact test: Power = 82.9% (0.829). I will use the Fisher approach below.

New Power for the same event rate comparison (10% v 50%)
Allowing the sample size in ABA to increase, starting at N = 25
(holding constant N = 15 in SOC)

N in ABA	N in SOC	Power
25	15	0.684
30	15	0.723
35	15	0.751

40	15	0.773
45	15	0.790
50	15	0.803

We'll plan on enrolling a total of 25 patient on the experimental arm. We have already enrolled 15 patients on the SOC arm.

10.6 MEASURES TO MINIMIZE BIAS

10.6.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

We expected to accrue all the 50 patients within a year at UCSD. All the patients will be followed for one year after transplant. The total duration of this study will be about two years.

Stratified randomization using permuted blocks of size 4 will be performed to randomize participants to the experimental or control arm. There are two stratification factors: conditioning regimen (MA vs RIC) and donor type (MRD vs MUD). Randomization will be performed prior to the use of any study related drug which includes the conditioning therapy to be used for the control arm.

11.0 STUDY MANAGEMENT

11.1 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must disclose the conflict to the IRB and in the subject's informed consent form. All investigators will follow the Institutional conflict of interest policy.

11.2 Institutional Review Board (IRB) Approval and Consent

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

11.2 Data Management and Monitoring/Auditing

11.3 Data Safety Monitoring

Safety data will be summarized and sent to the team for review bi-monthly. The study may be stopped early due to excessive or unexpected number of serious adverse events.

In addition to adverse event monitoring and clinical oversight by the Study Chair, site principal investigator and co-investigators, quality assurance of the study will be performed by the UCSD Moores Cancer Center Clinical Trials Office internal monitor. Monitoring intervals will be every 6 months.

This study will also use the UCSD Moores Cancer Center Data Safety and Monitoring Board (DSMB) to provide oversight in the event that this treatment approach leads to unforeseen toxicities. Data from this study will be reported annually and will include:

1) the protocol title, IRB protocol number, and the activation date of the study.
2) the number of patients enrolled to date
3) the date of first and most recent patient enrollment
4) a summary of all adverse events regardless of grade and attribution
5) a response evaluation for evaluable patients when available
6) a summary of any recent literature that may affect the ethics of the study.

11.4 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

11.4.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, a IRB modification form must be completed within five (5) business days of making the change.

11.4.2 Protocol Exceptions

Planned exceptions to the protocol that are more than logistical (e.g., minor changes to the study schedule for an individual subject)and/or have the potential to affect the subject's safety or study integrity may not be implemented without prior approval from the IRB. In this case, the PI should submit a Protocol Exception request.

11.4.3 Other Protocol Deviations/Violations

Logistical deviations from the protocol (e.g., minor changes to the study schedule for an individual subject) do not require prior IRB approval unless the deviation has the potential to affect the subject's safety. Such planned deviations that do not affect the subject's safety should be noted in the subject's research record.

Unintentional deviations from the protocol that might affect subject safety or study integrity should be reported to the IRB within 10 days from when the investigator becomes aware that such a deviation has occurred. In this case, a Protocol Deviation report must be submitted to the IRB.

All submissions should include a description of the plan to avoid similar deviations in the future.

11.5 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB for approval prior to implementation.

11.6 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

11.7 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

12.0 REFERENCES

1. Appleman, L.J., et al., *Induction of immunologic tolerance for allogeneic hematopoietic cell transplantation.* Leuk Lymphoma, 2002. **43**(6): p. 1159-67.
2. Ram, R., et al., *Prophylaxis regimens for GVHD: systematic review and meta-analysis.* Bone Marrow Transplant, 2009. **43**(8): p. 643-53.
3. Li, Y., et al., *Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance.* Nat Med, 1999. **5**(11): p. 1298-302.
4. Jenkins, M.K., R.H. Schwartz, and D.M. Pardoll, *Effects of cyclosporine A on T cell development and clonal deletion.* Science, 1988. **241**(4873): p. 1655-8.
5. Luznik, L., R.J. Jones, and E.J. Fuchs, *High-dose cyclophosphamide for graft-versus-host disease prevention.* Curr Opin Hematol, 2010. **17**(6): p. 493-9.
6. Luznik, L., et al., *High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease.* Blood, 2010. **115**(16): p. 3224-30.
7. Durakovic, N., et al., *Secondary Systemic Immunosuppressants (SSI) After Myeloablative HLA Matched Related And Unrelated Bone Marrow Transplantation (BMT) Using High Dose Cyclophosphamide (HiCy) As Sole GVHD Prophylaxis.* Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation, 2010. **16**(2): p. S161-S162.
8. Jones, R.J., et al., *Assessment of aldehyde dehydrogenase in viable cells.* Blood, 1995. **85**(10): p. 2742-6.
9. Tzachanis, D., et al., *Intrinsic and extrinsic regulation of T lymphocyte quiescence.* Leuk Lymphoma, 2004. **45**(10): p. 1959-67.
10. Kuklina, E.M., *Molecular mechanisms of T-cell anergy.* Biochemistry (Mosc), 2013. **78**(2): p. 144-56.
11. Nguyen, V.H., et al., *The impact of regulatory T cells on T-cell immunity following hematopoietic cell transplantation.* Blood, 2008. **111**(2): p. 945-53.
12. Jones, S.C., G.F. Murphy, and R. Korngold, *Post-hematopoietic cell transplantation control of graft-versus-host disease by donor CD425 T cells to allow an effective graft-versus-leukemia response.* Biol Blood Marrow Transplant, 2003. **9**(4): p. 243-56.
13. Di Ianni, M., et al., *Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation.* Blood, 2011. **117**(14): p. 3921-8.
14. Di Ianni, M., et al., *Immunoselection and clinical use of T regulatory cells in HLA-haploidentical stem cell transplantation.* Best Pract Res Clin Haematol, 2011. **24**(3): p. 459-66.
15. Guinan, E.C., et al., *Transplantation of anergic histoincompatible bone marrow allografts.* N Engl J Med, 1999. **340**(22): p. 1704-14.
16. Davies, J.K., et al., *Outcome of alloanergized haploidentical bone marrow transplantation after ex vivo costimulatory blockade: results of 2 phase 1 studies.* Blood, 2008. **112**(6): p. 2232-41.
17. Larsen, C.P., et al., *Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties.* Am J Transplant, 2005. **5**(3): p. 443-53.

18. Tzachanis, D., et al., *Blockade of B7/CD28 in mixed lymphocyte reaction cultures results in the generation of alternatively activated macrophages, which suppress T-cell responses.* Blood, 2002. **99**(4): p. 1465-73.
19. Mellor, A.L. and D.H. Munn, *IDO expression by dendritic cells: tolerance and tryptophan catabolism.* Nat Rev Immunol, 2004. **4**(10): p. 762-74.
20. Vincenti, F., et al., *Three-year outcomes from BENEFIT, a randomized, active-controlled, parallel-group study in adult kidney transplant recipients.* Am J Transplant, 2012. **12**(1): p. 210-7.
21. Larsen, C.P., et al., *Belatacept-based regimens versus a cyclosporine A-based regimen in kidney transplant recipients: 2-year results from the BENEFIT and BENEFIT-EXT studies.* Transplantation, 2010. **90**(12): p. 1528-35.
22. Vincenti, F., et al., *A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study).* Am J Transplant, 2010. **10**(3): p. 535-46.
23. Durrbach, A., et al., *A phase III study of belatacept versus cyclosporine in kidney transplants from extended criteria donors (BENEFIT-EXT study).* Am J Transplant, 2010. **10**(3): p. 547-57.
24. Furuzawa-Carballeda, J., et al., *Infiltrating cellular pattern in kidney graft biopsies translates into forkhead box protein 3 up-regulation and p16INK4alpha senescence protein down-regulation in patients treated with belatacept compared to cyclosporin A.* Clin Exp Immunol, 2012. **167**(2): p. 330-7.
25. Charpentier, B., *Belatacept: a novel immunosuppressive agent for kidney transplant recipients.* Expert Rev Clin Immunol, 2012. **8**(8): p. 719-28.
26. Bestard, O., et al., *Costimulatory blockade with mTor inhibition abrogates effector T-cell responses allowing regulatory T-cell survival in renal transplantation.* Transpl Int, 2011. **24**(5): p. 451-60.
27. Fuchs, E.J., *Haploidentical transplantation for hematologic malignancies: where do we stand?* Hematology Am Soc Hematol Educ Program, 2012. **2012**: p. 230-6.
28. Furst, D.E., et al., *Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2012.* Ann Rheum Dis, 2013. **72 Suppl 2**: p. ii2-34.
29. Orban, T., et al., *Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial.* Lancet, 2011. **378**(9789): p. 412-9.
30. Schiff, M., et al., *Efficacy and safety of abatacept or infliximab vs placebo in ATTEST: a phase III, multi-centre, randomised, double-blind, placebo-controlled study in patients with rheumatoid arthritis and an inadequate response to methotrexate.* Ann Rheum Dis, 2008. **67**(8): p. 1096-103.
31. Koura, D.T., et al., *In Vivo T Cell Costimulation Blockade with Abatacept for Acute Graft-versus-Host Disease Prevention: A First-in-Disease Trial.* Biol Blood Marrow Transplant, 2013. **19**(11): p. 1638-49.
32. Nahas, M.R., et al., *Phase I clinical trial evaluating abatacept in patients with steroid-refractory chronic graft-versus-host disease.* Blood, 2018.
33. Jaiswal, S.R., et al., *T cell costimulation blockade promotes transplantation tolerance in combination with sirolimus and post-transplantation cyclophosphamide for haploidentical transplantation in children with severe aplastic anemia.* Transpl Immunol, 2017. **43-44**: p. 54-59.

34. Blaise, D. and L. Castagna, *Do different conditioning regimens really make a difference?* Hematology Am Soc Hematol Educ Program, 2012. **2012**: p. 237-45.
35. de Lima, M., et al., *Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS.* Blood, 2004. **104**(3): p. 857-64.
36. Andersson, B.S., et al., *Once daily i.v. busulfan and fludarabine (i.v. Bu-Flu) compares favorably with i.v. busulfan and cyclophosphamide (i.v. BuCy2) as pretransplant conditioning therapy in AML/MDS.* Biol Blood Marrow Transplant, 2008. **14**(6): p. 672-84.
37. Alatrash, G., et al., *Myeloablative reduced-toxicity i.v. busulfan-fludarabine and allogeneic hematopoietic stem cell transplant for patients with acute myeloid leukemia or myelodysplastic syndrome in the sixth through eighth decades of life.* Biol Blood Marrow Transplant, 2011. **17**(10): p. 1490-6.
38. Khaled, S.K., S.H. Thomas, and S.J. Forman, *Allogeneic hematopoietic cell transplantation for acute lymphoblastic leukemia in adults.* Curr Opin Oncol, 2012. **24**(2): p. 182-90.
39. Appelbaum, F.R., *Pursuing the goal of a donor for everyone in need.* N Engl J Med, 2012. **367**(16): p. 1555-6.
40. Anasetti, C., et al., *Peripheral-blood stem cells versus bone marrow from unrelated donors.* N Engl J Med, 2012. **367**(16): p. 1487-96.
41. Mielcarek, M., et al., *Long-term outcomes after transplantation of HLA-identical related G-CSF-mobilized peripheral blood mononuclear cells versus bone marrow.* Blood, 2012. **119**(11): p. 2675-8.
42. Velardi, A., *Natural killer cell alloreactivity 10 years later.* Curr Opin Hematol, 2012. **19**(6): p. 421-6.
43. Peng, Y., et al., *CD86 is an activation receptor for NK cell cytotoxicity against tumor cells.* PLoS One, 2013. **8**(12): p. e83913.
44. Martin-Fontecha, A., et al., *Triggering of murine NK cells by CD40 and CD86 (B7-2).* J Immunol, 1999. **162**(10): p. 5910-6.
45. Jaiswal, S., et al., *A Novel Approach Towards Natural Killer Cell Immunotherapy Following Haploidentical Transplantation: CTLA4Ig Primed Donor Lymphocyte Infusions (DLI).* Biology of Blood and Marrow Transplantation. **24**(3): p. S76-S77.
46. Paz Morante, M., et al., *Activation-associated phenotype of CD3 T cells in acute graft-versus-host disease.* Clin Exp Immunol, 2006. **145**(1): p. 36-43.
47. Pieper, J., et al., *CTLA4-Ig (abatacept) therapy modulates T cell effector functions in autoantibody-positive rheumatoid arthritis patients.* BMC Immunol, 2013. **14**: p. 34.
48. Imberti, L., et al., *Reduced T-cell repertoire restrictions in abatacept-treated rheumatoid arthritis patients.* J Transl Med, 2015. **13**: p. 12.
49. Filipovich, A.H., et al., *National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report.* Biol Blood Marrow Transplant, 2005. **11**(12): p. 945-56.
50. Glucksberg, H., et al., *Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors.* Transplantation, 1974. **18**(4): p. 295-304.

51. Snyder, D.S., et al., *Fractionated total body irradiation and high-dose etoposide as a preparatory regimen for bone marrow transplantation for 99 patients with acute leukemia in first complete remission.* Blood, 1993. **82**(9): p. 2920-8.
52. D'Agostino, R.B., Sr., J.M. Massaro, and L.M. Sullivan, *Non-inferiority trials: design concepts and issues - the encounters of academic consultants in statistics.* Stat Med, 2003. **22**(2): p. 169-86.
53. Feng, C., H. Wang, and X.M. Tu, *Power Loss of Stratified Log-Rank Test in Homogeneous Samples.* International Journal of Quality, Statistics, and Reliability, 2010. **2010**: p. 4.
54. Grambsch, P.M. and T.M. Therneau, *Proportional hazards tests and diagnostics based on weighted residuals.* Biometrika, 1994. **81**(3): p. 515-526.
55. Lee, J.H., et al., *Randomized trial of myeloablative conditioning regimens: busulfan plus cyclophosphamide versus busulfan plus fludarabine.* J Clin Oncol, 2013. **31**(6): p. 701-9.
56. Rambaldi, A., et al., *Busulfan plus cyclophosphamide versus busulfan plus fludarabine as a preparative regimen for allogeneic haemopoietic stem-cell transplantation in patients with acute myeloid leukaemia: an open-label, multicentre, randomised, phase 3 trial.* Lancet Oncol, 2015. **16**(15): p. 1525-1536.
57. PASS 14 Power Analysis and Sample Size Software. NCSS, L.K., Utah, USA, ncss.com/software/pass. 2015.

13.0 APPENDICES

Appendix I Diagnosis and Scoring of chronic GVHD according to the NIH consensus criteria[49]

The diagnosis of chronic GVHD requires the following:
1. Distinction from acute GVHD.
2. Presence of at least 1 diagnostic clinical sign of chronic GVHD or presence of at least 1 distinctive manifestation confirmed by pertinent biopsy or other relevant tests.
3. Exclusion of other possible diagnoses.

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capabl of self-care, >50% of waking hours ou of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN† **SCORE % BSA** ☐ *GVHD features to be scored by BSA:* **Check all that apply:** ☐ Maculopapular rash/erythema ☐ Lichen planus-like features ☐ Sclerotic features ☐ Papulosquamous lesions or ichthyosis ☐ Keratosis pilaris-like GVHD	☐ No BSA involved	☐ 1-18% BSA	☐ 19-50% BSA	☐ >50% BSA
SKIN FEATURES SCORE:	☐ No sclerotic features		☐ Superficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** ☐ Deep sclerotic features ☐ "Hidebound" (unable to pinch) ☐ Impaired mobility ☐ Ulceration
Other skin GVHD features (NOT scored by BSA) **Check all that apply:** ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Poikiloderma ☐ Severe or generalized pruritus ☐ Hair involvement ☐ Nail involvement ☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
MOUTH *Lichen planus-like features present:* ☐ **Yes** ☐ **No**	☐ No symptoms	☐ Mild symptoms **with** disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* ☐ **Yes** ☐ **No** ☐ **Not examined**	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	☐ Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
GI Tract ***Check all that apply:*** ☐ Esophageal web/ proximal stricture or ring ☐ Dysphagia ☐ Anorexia ☐ Nausea ☐ Vomiting ☐ Diarrhea ☐ Weight loss ≥5%* ☐ Failure to thrive	☐ No symptoms	☐ Symptoms without significant weight loss* (<5%)	☐ Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	☐ Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
LIVER	☐ Normal total bilirubin and ALT or AP < 3 x ULN	☐ Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	☐ Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	☐ Elevated total bilirubin > 3 mg/dL
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
LUNGS**				
Symptom score:	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
Lung score: **% FEV1** ☐	☐ FEV1≥80%	☐ FEV1 60-79%	☐ FEV1 40-59%	☐ FEV1 ≤39%
Pulmonary function tests ☐ Not performed				
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

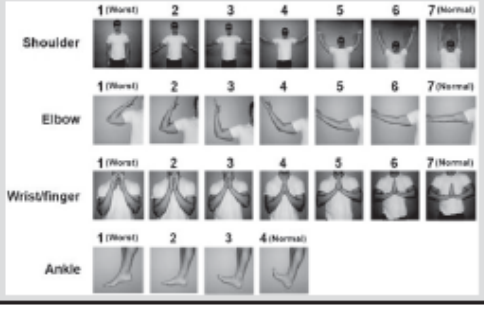
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GENITAL TRACT (*See Supplemental figure*‡) ☐ Not examined *Currently sexually active* ☐ Yes ☐ No	☐ No signs	☐ Mild signs‡ and females with or without discomfort on exam	☐ Moderate signs‡ and may have symptoms with discomfort on exam	☐ Severe signs‡ with or without symptoms
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to severity (0-3) based on functional impact where applicable none – 0,mild -1, moderate -2, severe – 3)

☐ Ascites (serositis)___	☐ Myasthenia Gravis___	
☐ Pericardial Effusion___	☐ Peripheral Neuropathy___	☐ Eosinophilia > 500/µl___
☐ Pleural Effusion(s)___	☐ Polymyositis___	☐ Platelets <100,000/µl ___
☐ Nephrotic syndrome___	☐ Weight loss>5%* without GI symptoms___	☐ Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)* ☐ **No GVHD** ☐ **Mild** ☐ **Moderate** ☐ **Severe**

Photographic Range of Motion (P-ROM)



Appendix II Acute GVHD grading [50]

Grading of acute graft-versus-host disease

Organ	Stage	Description
Skin	1	Maculopapular rash over <25 percent of body area
	2	Maculopapular rash over 25 to 50 percent of body area
	3	Generalized erythroderma
	4	Generalized erythroderma with bullous formation and often with desquamation
Liver	1	Bilirubin 2.0 to 3.0 mg/dL; SGOT 150 to 750 international units
	2	Bilirubin 3.1 to 6.0 mg/dL
	3	Bilirubin 6.1 to 15.0 mg/dL
	4	Bilirubin >15.0 mg/dL
Gut	1	Diarrhea >30 mL/kg or >500 mL/day
	2	Diarrhea >60 mL/kg or >1000 mL/day
	3	Diarrhea >90 mL/kg or >1500 mL/day
	4	Diarrhea >90 mL/kg or >2000 mL/day; or severe abdominal pain with or without ileus

Glucksberg grade

I – Stage 1 or 2 skin involvement; no liver or gut involvement; ECOG PS 0
II – Stage 1 to 3 skin involvement; Grade 1 liver or gut involvement; ECOG PS 1
III – Stage 2 or 3 skin, liver, or gut involvement; ECOG PS 2
IV – Stage 1 to 4 skin involvement; Stage 2 to 4 liver or gut involvement; ECOG PS 3

Appendix III ECOG Performance Status Scale

SCORE	DESCRIPTION
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Appendix IV: NCI CTC Version 5.0

Toxicity will be scored using NCI CTC Version 5.0 for toxicity and adverse event reporting. A copy of the NCI CTC Version 5.0 can be downloaded from the CTEP homepage: (http://ctep.cancer.gov). All appropriate treatment areas have access to a copy of the CTC Version

Appendix V Suggestions for dosing of conditioning regimens

Dosing will be based on patients' actual weight up to 120% of ideal body weight, above which it will be based on adjusted ideal body weight (ideal weight plus 50% of the difference between ideal and actual weight).

Myeloablative regimen:

- Fludarabine 40 mg/m2 IV over 30 minutes daily for 4 days (day -6, -5, -4, -3) followed by:
- Busulfan 130 mg/m2 IV over 3 hours daily (day -6, -5, -4, -3)
- Both fludarabine and busulfan are infused via a controlled-rate infusion pump through a central venous catheter. [35].

Cyclophosphamide/TBI

- Cyclophosphamide 60 mg/kg IV over 2 hours daily x 2 (Day -5, -4)
  - Consider a cyclophosphamide dose of 50mg/kg for patients who will receive PTCy
- Mesna 50 mg/kg continuous infusion IV over 24 hours q24h x 2 doses each day on (Day -5, -4).
  First dose is 30 min prior to cyclophosphamide.
- Total body irradiation 2.0 Gy bid on (Day -3, -2, -1) for a total of 12.0 Gy.

TBI/Cyclophosphamide

- Total body irradiation 2.0 Gy BID on (Day -6, -5, -4) for a total of 12.0 Gy.
- Cyclophosphamide 60 mg/kg IV over 2 hours daily x 2. (Day -3, -2)
- Mesna 50 mg/kg continuous infusion IV over 24 hours q24h x 2 doses each day on (Day -3, -2).
  First dose is 30 min prior to cyclophosphamide.

Reduced Intensity Conditioning regimen:

- Fludarabine 30 mg/m2 will be given IV over 30 minutes daily for 4 days (Day -6,-5,-4,-3)
- Melphalan 140 mg/m2 via central line over 15 minutes x 1 on Day -2