A PILOT STUDY OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PEDIATRIC AND ADOLESCENT-YOUNG ADULTS PATIENTS WITH HIGH RISK SOLID TUMORS

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Synopsis: A PILOT STUDY OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PEDIATRIC AND ADOLESCENT-YOUNG ADULTS PATIENTS WITH HIGH RISK SOLID TUMORS

Primary Objectives: The primary objectives are to assess tolerability of allogeneic HCT for patients with chemo-responsive recurrent/refractory solid tumors as defined by transplant-related mortality (TRM) at day 30 and the rate of grade III or higher organ toxicity (Bearman Regimen-Related Toxicities Scale) attributable to conditioning occurring within 30 days.

Secondary Objectives: Assess: (i)Median time to platelet and neutrophil engraftment,(ii)incidence of acute graft-versus-host disease (aGVHD) by day 100, (iii)incidence of chronic GVHD(cGVHD) at day 100 and one year (iv)rate of grade II organ toxicity through day 100,(v)rate of graft failure (primary and secondary) through day 100,(vi)rate of infectious complications through day 100,(vii)progression free survival (PFS) at day 100,180 and 365, (viii) cumulative incidence of relapse,(ix) overall survival(OS) at day 100 and 365.

Eligibility Criteria: Patients aged 0-25 years, confirmed diagnosis of a malignant recurrent/refractory solid tumors with chemo- responsive disease, an available suitable HCT donor and patients have adequate organ function to undergo the conditioning regimen as outlined in section 7.0. Up to 200 patients will be enrolled.

Method: This is a non-randomized, non-blinded ,phase II clinical trial. Patients will be assigned to one of two arms based on the HCT donor and the stem cell source that is available as detailed in section 7.3.A non radiation based conditioning regimen will be used as detailed in section 9.1.Patients will receive standard supportive care as per best clinical practice in addition to the required observations detailed in Appendix B.

1.0 Purpose

This is a pilot study of allogeneic hematopoietic stem cell transplantation (HCT) among patients with chemo-responsive recurrent/refractory solid tumors.

2.0 Objectives

2.1 Primary Objectives

To assess tolerability of allogeneic HCT for patients with chemo-responsive recurrent/refractory solid tumors as defined by transplant-related mortality (TRM) at day 30 and the rate of grade III or higher organ toxicity (Bearman Regimen-Related Toxicities Scale)[1] attributable to conditioning occurring within 30 days.

2.2 Secondary Objectives:

Assess

I. Median time to platelet and neutrophil engraftment.

II. Incidence of acute graft-versus-host disease (aGVHD) by day 100

III. Incidence of chronic GVHD(cGVHD) at day 100 and one year

IV. Rate of grade II organ toxicity through day 100

V. Rate of graft failure (primary and secondary) through day 100.

VI. Rate of infectious complications through day 100.

VII. Progression Free Survival (PFS) at day 100,180 and 365

VIII. Cumulative incidence of relapse, overall survival (OS) at 100 days and 1 year.

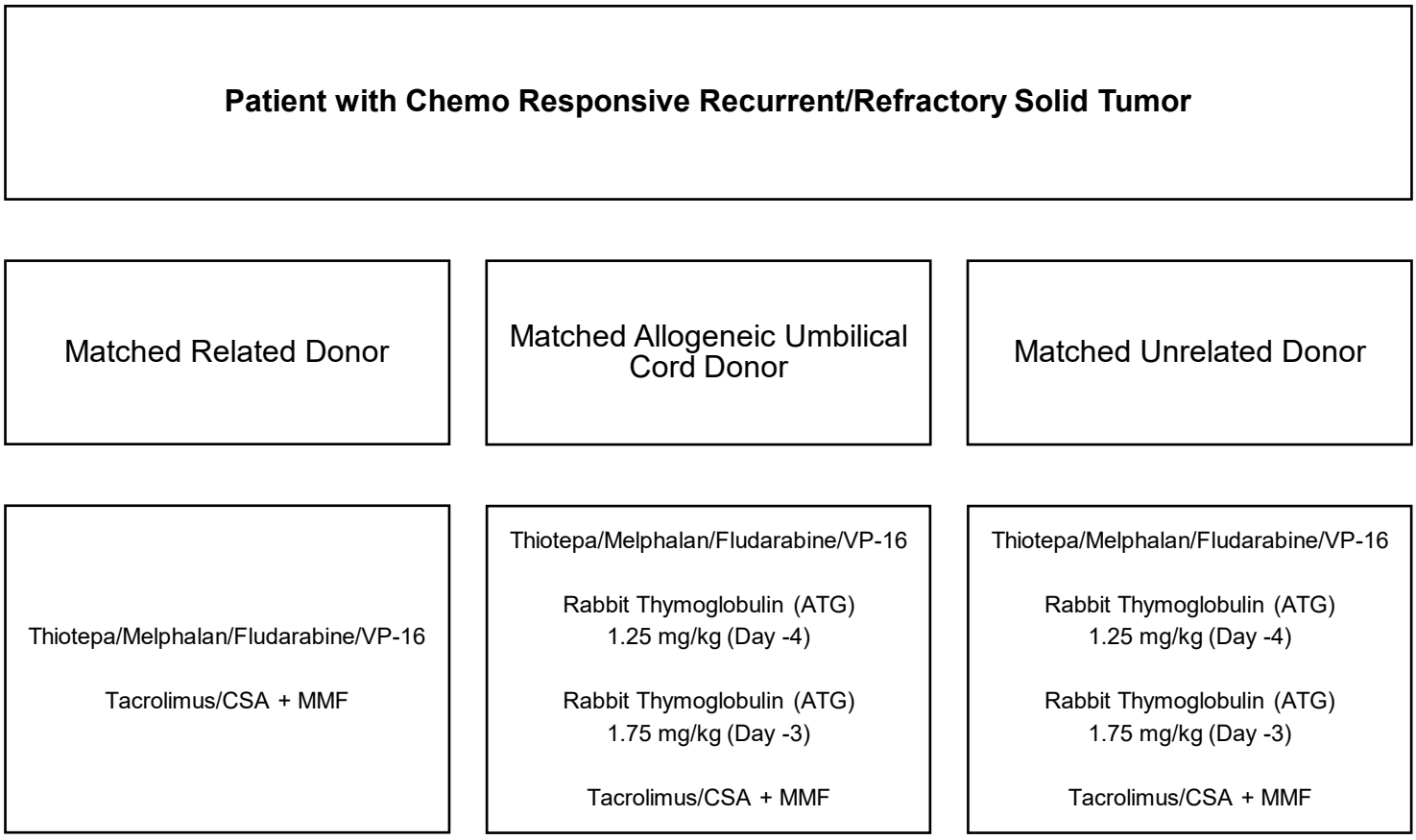
3.0 Hypotheses

Allogeneic HCT may be a consideration for the treatment of patients with recurrent/refractory chemo- responsive solid tumors. High dose chemotherapy may reduce tumor burden and associated inhibitory immune escape signals. Allogeneic HCT confers a Graft- Versus-Tumor effect (GvT) to eliminate residual disease after conditioning chemotherapy which may decrease disease recurrence and improve overall survival [2].

4.0 Trial Design

This is a non randomized, non blinded, phase II clinical trial in which eligible patients (see section 7.2 and 7.3) will be enrolled to receive one of the two treatments based on the availability of the donor stem cell source as outlined in section 7.4. Up to 20 pediatric and AYA patients (Age 0-25) will be enrolled for each treatment group. Assessment of the tolerability will be conducted in each treatment group.

Figure 1: A Pilot Study of Allogeneic Hematopoietic Stem Cell Transplantation for Pediatric and Adolescent-Young-Adult Patients (age 0-25) with High Risk Solid Tumors



VP-16: Etoposide CSA: Cyclosporine MMF: Mycophenolate Mofetil ATG: Anti-Thymocyte Globulin

5.0 Definitions

5.1 **Engraftment:** Engraftment is defined as recovery of blood counts (neutrophil and platelet engraftment, as defined in 5.1.1) with cells of donor origin, documented by either bone marrow or peripheral blood chimerism assays after hematopoietic cell transplant.

5.1.1 Neutrophil engraftment. Neutrophil engraftment is defined as the first day of three consecutive days when the absolute neutrophil count (ANC) is $\geq$500/mm^3.

Platelet engraftment. Platelet engraftment is defined as the first of three consecutive days when the platelet count is $\geq$20 K/uL (and the patient has not received a platelet transfusion for seven days preceding the first day).

5.1.2. Donor chimerism. Donor cells in either peripheral blood or bone marrow, as documented by fluorescent *in situ* hybridization (FISH), for sex-mismatched donor/recipients) or short-tandem repeat testing (STR) for sex-matched donor recipient pairs.

5.2 Immunological function

5.2.1 T lymphocyte recovery. T lymphocyte recovery is defined as absolute number of donor-derived CD3+ T lymphocytes $\geq$100/mm^3.

5.2.2. Mitogen-specific T lymphocyte function recovery. Mitogen-specific T lymphocyte function is defined as proliferative responses to phytohemagglutinin (PHA) $\geq$25,000 CPM of titrated thymidine incorporation.

5.2.3 Antigen-specific B lymphocyte function recovery. Antigen-specific B lymphocyte function is defined as normal titers of specific antibody to immunization with tetanus toxoid (TET) and not receiving IVIG for at least 3 months.

5.3 Toxicity

5.3.1 Acute conditioning regimen related toxicity is defined according Bearman Regimen-Related Toxicities Scale) Grade III or higher of the following organs are to be documented:

5.3.1.1 Hepatic

5.3.1.2 Pulmonary

5.3.1.3 Renal

5.3.1.4 Cardiac

5.3.1.5 Bladder

5.3.1.6 Central Nervous System

5.3.1.7 Mucosal (stomatitis)

5.3.1.8 Gastrointestinal

5.4 Acute and Chronic Graft Versus Host Disease

Acute Graft-Versus-Host Disease (aGVHD): Acute graft-versus-host disease usually develops within the first three months after transplantation and may appear as a characteristic dermatitis which may be accompanied by cholestasis and/or enteritis. Staging of acute GVHD will follow the Glucksberg staging criteria.

Chronic Graft-Versus-Host Disease: Chronic graft-versus-host disease is typically a late complication of HCT characterized by a connective-tissue disease-like syndrome and usually, but not always, occurring greater than 100 days following transplantation. Occasionally, its onset may closely follow aGVHD (known as progressive presentation of cGVHD).
Initial symptoms of cGVHD frequently include nausea and anorexia with ocular and oral sicca. Rash characteristically appears with pigmentary changes progressing to sclerosis and contractures. Other organs may be involved. Symptoms may mimic those seen in patients with scleroderma and other autoimmune disorders. Chronic GVHD will be assessed by the criteria set up by the National Institutes of Health Consensus Development Project on criteria for clinical trials in cGVHD. The following organs are evaluated for grading of cGVHD:

5.4.1 Skin: Assessment of skin for erythematous maculopapular rash, scleroderma, hypertrophy or atrophic changes of skin, hyperpigmentation or hypopigmentation, lichen planus or lichenoid reactions

5.4.2 Joints and fascia: Tightness, contractures and decrease range of motion

5.4.3 Eyes: Xerophthalmia and photophobia.

5.4.4 Mucosa: Erythema, lichenoid changes, and ulcers of mucosal surfaces of the mouth, vagina.

5.4.5 Gastrointestinal: Anorexia, nausea, vomiting, abdominal pain and weight loss may be manifestations of GI involvement. Esophageal stenosis and dysmotility may also be a GVHD presentation.

5.4.6 Liver function abnormalities with cholestasis and fibrosis, resulting in elevated serum levels of bilirubin, alkaline phosphatase, and hepatic transaminases and transferases.

5.4.7 Obliterative bronchiolitis (OB) may occur as a pulmonary manifestation of cGVHD.

5.4.8 Genital tract: Vaginal scarring, labial agglutination, phimosis or urethral scarring.

5.5 Sinusoidal Obstructive Syndrome (SOS) will be diagnosed and graded according to Pediatric and AYA modified EBMT Consensus Criteria

5.6 Chronic toxicities

Chronic toxicities are defined as those occurring at least 100 days post HCT and will be monitored and graded using the Common Terminology Criteria (CTCAE) version 5.0. The CTCAE v5.0 will be used as it allows standardized reporting of adverse events through the use of assigned uniformed terminology and severity grading. The following specific toxicities that represent common chronic effects related to HCT will also be monitored:

5.6.1 Chronic GVHD (see above 5.4).

5.6.2 Hypogammaglobinemia (see B lymphocyte recovery above 5.2).

5.6.3 Learning disability and/or neurocognitive dysfunction. Determined by both school performance and appropriate battery of age-appropriate neurocognitive tests.

5.7 EVALUATION OF RESPONSE

This study will use the revised (RECIST 1.1) Response Evaluation Criteria in Solid Tumors[3]. PET scan, bone marrow examinations, and/or tumor specific markers may be used as described below.

5.8 Response criteria

5.8.1 **Complete Response (CR**): disappearance of all lesions. For lymph nodes reduction of the short axis to 10 mm or less, and no new lesions

5.8.2 **Partial Response (PR):** At least a 30% decrease in the total tumor burden. Reference is disease measurement prior to starting treatment, and no new lesions

5.8.3 **Progressive Disease (PD):** At least 25% increase in the total tumor burden. Reference is disease measurement prior to starting treatment.

5.8.4 **Stable Disease (SD**): Changes that do not meet definition of CR, PR, or PD.

5.9 Response Criteria for Solid Tumors with FDG-PET positive lesions

A 'positive' FDG-PET scan lesion is one that is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image. Patients without measurable disease with only FDG-PET positive lesions will have disease evaluated by FDG-PET response.

FDG-PET Response

Complete response: resolution of all FDG-PET positive lesions

Incomplete response: resolution of at least one positive FDG-PET lesions with persistence of other FDG-PET lesions

Stable Disease: No change in FDG-PET scan in number of positive lesions

Progressive disease Development of new FDG-PET positive lesion and/or massive unequivocal increase of FDG-PET lesions.

5.9.2 Response Criteria for tumors with only bone marrow metastasis or elevated tumor markers.

Patients without measurable disease with only bone marrow metastasis or elevated tumor marker will have disease evaluated by bone marrow or tumor marker response.

Bone marrow metastasis is defined by the presence of metastatic disease identified on a bone marrow aspirate or biopsy sample prior to initiation of therapy

Elevated tumor marker is defined by a level above upper limit of normal for age and sex

5.9.3 Tumor Marker or Bone Marrow Response

Complete response: resolution of bone marrow metastasis and/or normalization of tumor markers.

Incomplete response/stable disease: does not meet definition of complete response nor progressive disease.

Progressive disease: For patients with no measurable disease at baseline will only contribute to overall progression disease if develops new measurable disease that can be used to compare subsequent responses

6.0 Background

Allogeneic HCT/Immunotherapy for high-grade solid tumors:

Relapsed, and/or metastatic solid tumors are associated with a 5-year survival as low as <35%. Outcomes for these patients have failed to significantly improve over the past two decades despite the use of novel agents, increased chemotherapy dose intensity, new chemotherapy combinations and high dose chemotherapy requiring autologous stem cell rescue. The addition of novel agents such as cixutumumab and temozolamide to an already dose intensified regimen for newly diagnosed patients with rhabdomyosarcoma metastatic at sites other than regional lymph nodes, resulted in a 3 year event free survival (EFS) of 16% and 18% and OS of 47 and 33% respectively[4].The EURO-E.W.I.N.G 99 and Ewing-2008 trials compared the effect of consolidation therapy with combined high dose chemotherapy- Busulfan/Melphalan (BuMel) followed by autologous stem cell transplantation versus standard chemotherapy- vincristine/dactinomycin/ifosfamide (VAI)for patients with localized high risk Ewing sarcoma. Three year EFS rates for VAI and BuMel followed by autologous stem cell transplantation were 56.7% and 69% respectively with this benefit being sustained at 8 years where the EFS was 47.1% vs 60.7% respectively[5]. While The International Society of Pediatric Oncology European Neuroblastoma (SIOPEN) group added a combination of

topotecan, vincristine, and doxorubicin to patients who failed induction therapy with only 6.4% of patients achieving clinical remission.[6]

With approximately 25% of children and young adults presenting with metastatic disease, the development of new modalities of cancer therapy including hematopoietic stem cell therapy (HCT) for these tumors is therefore essential to the improvement of patient outcomes

The ability to rescue a patient with hematopoietic progenitor cells allows the use of very high-doses of chemotherapy that could overcome resistance. Autologous transplants have been viewed more favorably because the high morbidity/mortality due to GVHD with allogeneic transplants. The research, however shows inconclusive data to support high-dose chemotherapy with autologous stem-cell rescue and as mentioned above, finds about 20-30% overall and event free survival [7, 8]. With autologous HCT, adequate collection depends, on the bone marrow reserve after chemo and/or radiotherapy, and bone/bone marrow involvement with disease. Marrow that has been irradiated may be unable to mobilize sufficiently to provide the increased number of cells needed for collection. Furthermore, if disease has disseminated to the bones and bone marrow, subsequent fibrosis may reduce the marrow's efficacy. Finally, disseminated bone marrow disease may increase the risk of potential contamination of an autologous stem cell collection. For these patients, it becomes unlikely to obtain an adequate autologous stem cell source. At present, it is not possible to separate a malignant cell from a peripheral stem cell harvest leaving the potential risk of re-infusing malignant cells after the high-dose therapy leading to disease recurrence. Therefore, these patients are not ideal candidates for marrow-ablative therapy with autologous HCT

This paradigm is shifting with new advancements in allogeneic transplant management [9]. There is evidence to suggest that allogeneic immune effect can be seen in solid tumors [10-14]. A group in Spain performed allogeneic HCT among 6 patients with a variety of high-risk solid tumors (neuroblastoma, Ewing sarcoma, desmoplastic tumor, nasopharyngeal carcinoma, and embryonal rhabdomyosarcoma) and found response among all 6 patients, with 3 developing complete response (CR), 2 developing partial response, and 1 patient having stable disease for greater than 9 months. Three patients remained alive in CR at a median follow-up of 15 (4-65) months[15]. Lang *et al* reported allogeneic HCT for treatment of solid tumors. Four patients with neuroblastoma (NB), a patient with rhabdomyosarcoma (RMS), and a patient with Ewing sarcoma (EWS) were included in this study. All patients entering transplant had significant tumor burden. All patients had primary engraftment at a median of 11 days. One patient had secondary graft failure. Acute GVHD grade II was seen in 4 patients while 2 experienced limited cGVHD; no TRM occurred. Four patients died from progression while two are alive, with an overall median survival of 6 (2-11) months [16].

GvT effect in allogeneic transplants is clearly a benefit over autologous transplants. There is emerging clinical evidence which suggests a clinically meaningful GvT effect following allogeneic

HCT in Ewing sarcoma and rhabdomyosarcoma [17-24].

Donor T lymphocytes in the allograft, reactive to host histocompatibility antigens, can directly kill human cancer cells in vitro and in vivo. This can occur even after donor T lymphocytes are rejected and suggests that host lymphocytes might mediate the anti- tumor effect of donor allogeneic lymphocytes[25].

Donor lymphocyte infusion (DLI) has been reported to induce GvT after allogeneic HCT in a patient with multiply-relapsed rhabdomyosarcoma to sustain remission. At 31 months post- transplant, this patient remained disease-free[24]. In a case-report, Donker *et al* reported a patient with stage IV rhabdomyosarcoma who at 4 years post-allogeneic transplant remains in CR[23]. Baird *et al* reported a clinical trial of 23 sarcoma patients who underwent reduced intensity HCT [26] (44). All patients had successful donor engraftment. Five of 23 patients remained alive (OS of 30% at 3 years), including 3 of 7 (42%) transplanted without gross disease (median survival 14.5 versus 29.0 months from HCT). Koscielniak *et al* reported long-term remission in a patient with Ewing sarcoma after HCT[21]. Capitini *et al* reported response of metastatic Ewing sarcoma after allogeneic HCT[18]. Hosono *et al* reported clinically significant GvT effect in a patient with pulmonary Ewing sarcoma who failed high-dose chemo with autologous transplantation[19].

Additionally, tumors have evolved sophisticated mechanisms to avoid immune detection. They may down-regulate expression of MHC class I and other proteins involved in antigen presentation.[27-29] Tumors can also decrease, or shed, expression of proteins that are recognized by the immune system (immunoediting) and they can by-pass death mechanisms by elevating expression levels of survival factors, such as anti-apoptotic proteins (survivin, BCL-X_L), metastatic proteins (VEGF, MMPs) and proliferation factors (EGFR, c-Myc). The transcription factor STAT3 is upregulated in a number of tumors and controls expression of some of these genes.[30] Tumor cells adapt to immune recognition by down regulating expression of antigens, and can also adapt to chemotherapy by increasing expression of adenosine-triphosphate binding cassette (ABC) pumps to actively secrete intracellular drugs.[31] However chemotherapies have the potential to enhance cancer vaccine-induced immune responses by lowering the defenses of the tumor[32], targeting the immune system to reduce tumor-induced immune suppressive cells and/or targeting the tumor to increase immunogenicity (increase MHC or antigen expression) and/or directly stimulating effector response by activating T cells.

Some chemotherapy agents/combinations, such as fludarabine,[33] may induce significant and relevant T cell depletion and seems to generate a micro milieu suitable for subsequent T cell activation. Hence, high-dose chemotherapy may lower the defences of the tumor and allow a suitable micro milieu for subsequent allogeneic donor immune-surveillance. Effective combination treatments hinge on our understanding of the role of the immune system in tumor rejection. Combining chemotherapies and immunotherapies effectively to attack the tumor from multiple sides may enhance our ability to quickly and thoroughly eliminate enhance our ability

to quickly and thoroughly eliminate cancer.[34]

Cord blood (CB) natural killer (NK) cells represent another promising effector cell immunotherapy due to their recognition of malignant cells without the need for a known target and their potential to expand exponentially.[35] Natural killer cells have documented activity against medulloblastoma.[36, 37]However, complete elimination of tumor by autologous NK cells is unlikely as the inhibitory signals from the tumor generally render their own NK cells incapable of inducing potent cytolytic activity. Although most NK cell clinical trials have used allogeneic peripheral blood (PB) as a cell source [38], in vitro studies suggest that umbilical cord blood (CB) NK cells may possess better cytolytic ability.[39, 40] The use of cord blood as a source of allogeneic NK cells is also advantageous because: (a) they can be ex vivo expanded to clinically useful cell numbers; and (b) they allow a higher chance of identifying HLA-compatible and KIR-mismatched products because of their immediate availability in established cord blood banks. Such a readily available "off-the-shelf" source of NK cells greatly enhances the feasibility of using these cells as therapy.[35] In this study, for patients lacking matched related donors, cord blood graft(s) or matched unrelated donor (MUD) grafts will be used as an alternative donor source. This study may inform the design of future pivotal studies regarding use of HLA-compatible and KIR-mismatched products as well as CB derived NK cells in combination with HCT.

Finally more precise patient selection, pre-transplant work-up, the use of reduced intensity preparative regimens and better supportive care medications to aide with side-effects/GVHD has led to improved overall survival post HCT.[41-44]

Taken altogether, the main advantages of the proposed approach can be summarized as follows:

- Overcome the challenges in autologous bone morrow/PBSC collection in patients who are heavily pre- treated and/or have disseminated disease within the bone/bone marrow.
- Use of radiation sparing thiotepa based regimen
- Eliminate the risk of autologous graft contamination with tumor cells.
- Provide GvT to eliminate residual disease after conditioning chemotherapy.

The main potential risks are GVHD, risk for post-HCT infections and delayed immune recovery. The use of allografting with the proposed regimen combines the benefits of high dose chemotherapy and an immune approach to disease therapy

7.0 Patient (Recipient) and Donor Selection:

7.1 Recipient inclusion criteria

7.1.1 Pathological criteria, including malignant recurrent/refractory solid tumors. This would include:

7.1.1.1 ***Ewing's sarcoma with the following translocations*:***
EWS-FLI1
EWS-ERG
EWS-ETV1
EWS-ETV4
EWS-FEV
FUS-ERG
FUS-FEV

7.1.1.2 ***Ewing-like sarcoma with the following translocations*:***
EWS-NFATC2
EWS-POU5F1
EWS-SMARCA5
EWIS-PATZ1
EWS-SP3
CIC-DUX4
BCOR-CCNB3

**Additional translocations may be considered based on changes in classification and discussion with the PI*

7.1.1.3 ***Peripheral PNET***

7.1.1.4 ***Malignant peripheral nerve sheath tumor, neurofibrosarcoma***

7.1.1.5 ***Rhabdomyosarcoma***

7.1.1.6 ***Neuroblastoma (*patients who are ineligible for tandem autologous transplant or who are at least 3 months post autologous HCT**)

7.1.1.7 ***Desmoplastic small round cell tumor (DSRCT)- both new diagnoses as well as recurrent/refractory disease***

7.1.1.8 ***Synovial Sarcoma***

7.1.2 Extent of disease eligibility: Patients must have chemo-responsive disease, defined as; 30% or greater decrease in the tumor target lesions when compared to its pre-treatment evaluation. Patients with complete response will be eligible to participate.

7.1.3 Available suitable HCT donor as defined below.

7.1.4 Eligible patients must have adequate organ function to tolerate the chemotherapy conditioning regimen and the HCT, as measured by:

7.1.4.1 Renal: creatinine clearance or GFR ≥50 ml/min/1.73m^2, and not requiring dialysis

7.1.4.2 Pulmonary: Diffusing Capacity of lung for carbon monoxide (DLCO) (corrected for hemoglobin) ≥ 50% predicted. If unable to perform pulmonary function tests, then O2 saturation ≥ 92% in room air.

7.1.4.3 Bilirubin ≤3x upper limit of normal (ULN) and ALT and AST ≤ 5x for age (with the exception of isolated hyperbilirubinemia due to Gilbert's syndrome).

7.1.4.4 Patients will be 0 – 25 years of age.

7.1.5 Non-English speakers will be eligible to participate in this study

7.2 Recipient exclusion criteria

7.2.1 Lack of histocompatible suitable related donor/ graft source as defined in 7.4

7.2.2 End-organ failure that precludes the ability to tolerate the transplant procedure, including conditioning regimen

7.2.3 Renal failure requiring dialysis

7.2.4 Congenital heart disease resulting in congestive heart failure

7.2.5 Ventilatory failure: requires invasive mechanical ventilation

7.2.6 HIV infection

7.2.7 Uncontrolled bacterial, viral, or fungal infections (currently taking medication yet clinical symptoms progress); stable, controlled disease with treatment is not an exclusion criteria

7.2.8 A female of reproductive potential who is pregnant, planning to become pregnant during the study, or is nursing a child

7.2.9 Any patient who does not fulfill the inclusion criteria listed above

7.2.10 Patient, parent, guardian or LAR unable/unwilling to provide consent and when indicated, assent

7.3 Vulnerable populations

a. There will be pediatric patients as the patients may experience prolongation of life. Adequate safeguards as outlined in the HRP-416 checklist will be followed to protect their rights and welfare.

b. When possible, the research will be explained to the child compatible with the child's understanding.

- Any person that has not achieved the age of 18 is considered a child.
- For research conducted in the state, HRP-013 - SOP - LARs, Children, and Guardians to be aware of which individuals in the state meet the definition of "children" will be followed.
- Research will not be conducted outside of the state.
- Parental permission will be obtained from one or both parents as determined by the IRB.
- In the event a LAR is used, we will follow consent SOP-04_Informed Consent Process and HRP-013_SOP Legally authorized representative.
- Assent will be obtained per the SOP-04_informed consent process.
- Assent will be documented per the SOP-04_informed consent process.

c. This research may involve adults unable to consent as this vulnerable population may experience prolongation of life. Adequate safeguards as outlined in the HRP-417 checklist will be followed to protect their rights and welfare.

- For this population this will include:
  - when appropriate, the IRB would allow a legally authorized representative or family member to sign proxy informed consent, if that person is authorized to make decisions about medical care for the subject. The investigator should document in the medical and research record who is legally authorized to make medical decisions.
- -The investigator will provide detailed information of the risks and benefits of the research to the proxy and the subject (if the subject can possibly understand). The protocol should be based on good science and sound research design with the potential for significant beneficial results
- Patients have medical decision-making capacity if they can demonstrate understanding of the situation, appreciation of the consequences of their decision, and reasoning in their thought process, and if they can communicate their wishes.
- For patients of who carry a diagnosis of a genetic condition leading to a lack of decision making capacity (e.g. Trisomy 21), no further evaluation will be required. For patients without a known diagnosis, further evaluation will be made based on the patient's history including possible evaluation by neurology or neuro-psychology to determine decision-making capacity. A determination of lack of decision-making capacity shall be made after an appropriate medical evaluation that concludes there is little or no likelihood that the participant will regain decision-making capacity in a reasonable period of time.
- Physician will do the following:
  (1) assessment of any language or communication barriers interfering with the patient's understanding
  (2) identification and treatment of any reversible causes of incapacity
  (3) a directed interview to assess the elements of consent
- For research conducted in the state, HRP-013 - SOP - LARs, Children, and Guardians to be aware of which individuals in the state meet the definition of "legally authorized representative" will be followed.
- Research will not be conducted outside of the state.
- Assent will be required of some of the subjects. Assent will be obtained on subjects that have the capacity to provide assent.
- Documentation of assent will be documented in the note documenting the consent process.
- Subjects will be particularly closely monitored.
- Subjects will be withdrawn if they appear to be unduly distressed.

7.4 Donor Selection

HCT will be done using stem cell sources **in the following order of preference:**

7.4.1 Matched related donor bone marrow (10 of 10 HLA alleles [HLA-A, B, C, DR, and DQ]. Matched related donor PBSC is allowed only if collection of BM is not available

or refused by parents/donor.

7.4.2 Umbilical Cord Blood (UCB)

Related

7.4.2.1 Single Matched allogeneic umbilical cord blood (UCB): (Single Cord) Related

- High-resolution matching at A, B, DRB1 (minimum 4/6)
- Patients must have one CB unit available which is matched with the patient at 4/6, 5/6, or 6/6 HLA class I (serological) and II (molecular) antigens. The cord must contain a minimum of 2.5 x $10e^7$ total nucleated cells/Kg recipient body weight (pre-thaw), with optimal goal of 4.0 x $10e^7$ total nucleated cells/Kg

7.4.2.2 Double Matched allogeneic umbilical cord blood (UCB): (Double Cord) Related

- High-resolution matching at A, B, DRB1 (minimum 4/6)
- Patients who lack an appropriately matched single cord unit), with an optimal dose of 4.0 x $10e^7$ total nucleated cells/Kg recipient body weight (pre-thaw) as defined above (7.3.2.1), may receive two CB units which are matched with the patient at 4/6, 5/6, or 6/6 HLA class I (serological) and II (molecular) antigens. Each cord must contain at least 1.5 x $10e^7$ total nucleated cells/Kg recipient body weight (pre-thaw) to achieve a cumulative cell dose of at least 3.0 x $10e^7$ with the goal to be above 4.0 x $10e^7$ total nucleated cells/Kg

Unrelated

7.4.2.3 Single Matched allogeneic umbilical cord blood (UCB): (Single Cord) Unrelated

- High-resolution matching at A, B, DRB1 (minimum 4/6)
- Patients must have one CB unit available which is matched with the patient at 4/6, 5/6, or 6/6 HLA class I (serological) and II (molecular) antigens. The cord must contain a minimum of 2.5 x $10e^7$ total nucleated cells/Kg recipient body weight (pre-thaw), with optimal goal of 4.0 x $10e^7$ total nucleated cells/Kg

7.4.2.4 Double Matched allogeneic umbilical cord blood (UCB): (Double Cord) Unrelated

- High-resolution matching at A, B, DRB1 (minimum 4/6)
- Patients who lack an appropriately matched single cord unit), with

an optimal dose of 4.0 x $10e^7$ total nucleated cells/Kg recipient body weight (pre-thaw) as defined above (7.3.2.3), may receive two CB units which are matched with the patient at 4/6, 5/6, or 6/6 HLA class I (serological) and II (molecular) antigens. Each cord must contain at least 1.5 x $10e^7$ total nucleated cells/Kg recipient body weight (pre-thaw) to achieve a cumulative cell dose of at least 3.0 x $10e^7$ with the goal to be above 4.0 x $10e^7$ total nucleated cells/Kg

7.4.3 Matched unrelated donor bone marrow (10 of 10 HLA alleles [HLA-A, B, C, DR, and DQ]. Matched unrelated donor PBSC is allowed only if collection of BM is not available or refused by parents/donor.

8.0 Enrollment on the Study:

Principal investigators or designee will review each patient's eligibility prior to patient enrollment. Patients are considered enrolled on this study after all of the following: (i) patient, and/or parent(s) or legal guardian (s) consent (and assent when appropriate) for the patient to undergo the transplant procedure under this study protocol, (ii) patient meets all inclusion/exclusion criteria, and patient subsequently undergoes HCT under this study.

9.0 Treatment Regimen and Procedure:

Potential subjects will initially undergo screening for eligibility and a request for financial clearance will be submitted, once obtained, a written informed consent and assent (when appropriate) for HCT on this protocol will be obtained by the principal investigator or study designees prior to initiation of the conditioning regimen.

9.1 TREATMENT SCHEMA

Day	Conditioning Regimen	GVHD Prophylaxis
-8	Thiotepa 300 mg/m^2 IV (10 mg/kg for < 12 Kg) Etoposide 100 mg/m^2 IV (3.3 mg/kg for < 12 Kg)	
-7	Thiotepa 300 mg/m^2 IV (10 mg/kg for < 12 Kg), Etoposide 100 mg/m^2 IV (3.3 mg/kg for < 12 Kg)	

-6	Thiotepa 300 mg/m^2 IV (10 mg/kg for < 12 Kg), Etoposide 100 mg/m^2 IV (3.3 mg/kg for < 12 Kg)	
-5	Melphalan 70 mg/m^2 IV (2.3 mg/kg for < 12 Kg), Fludarabine 30 mg/m^2	
-4	Melphalan 70 mg/m^2 IV (2.3 mg/kg for < 12 Kg), Fludarabine 30 mg/m^2 (UCB and MUD: rATG 1.25 mg/kg)	
-3	Fludarabine 30 mg/m^2 IV (UCB and MUD: rATG 1.75 mg/kg)	
-2	Rest	Start Tacrolimus[1] 0.05 mg/kg
-1	Rest	
0	HSC Infusion	Start MMF[2] 15 mg/kg q8hr

[1]Cyclosporine (CSA) may be substituted for Tacrolimus:

- Age ≤ 6 years old: (CSA) 6 mg/kg IV QD in divided doses (e.g. 2 mg/kg q8hrs).
- Age > 6 years old: (CSA) 3 mg/kg IV QD in divided doses (1.5mg/kg q12hrs)

[2]MMF first dose 4-6 hours following HSC infusion

9.2 HSC processing and administration will be performed per institutional SOP.

9.3 Graft-Versus-Host Disease prophylaxis and treatment

9.3.1 GVHD prophylaxis. All patients will receive a calcineurin inhibitor per institutional practice to prevent GVHD.

9.3.1.1 Calcineurin inhibitor: The patient will receive a calcineurin inhibitor (CI), either tacrolimus or cyclosporine (CSA), starting on Day -2 sufficient to maintain a serum trough level of approximately 7-12 ng/dl for tacrolimus, or 150-300 ng/dl for CSA.

Tacrolimus will begin on Day –2 pre-HCT at a starting dose of 0.05 mg/ kg/ day as a continuous infusion.

Cyclosporine (CSA) may be substituted for Tacrolimus:

- Age ≤ 6 years old: 6 mg/kg IV QD in divided doses (e.g. 2 mg/kg q8hrs)
- Age > 6 years old: 3 mg/kg IV QD in divided doses (1.5 mg/kg q12hrs)

Tapering schedule for Calcineurin inhibitors (if no active GVHD): Recipients of Matched (10/10 allele matched) related Donors

- Start taper at Day +60 at approximately 20% of the original therapeutic dose/week to be and complete by Day +100.

Recipients of all other donors

- Start taper at Day +100 at approximately 10% of the original therapeutic dose/week to be completed by Day +180.

9.3.1.2 Mycophenolate mofetil (MMF)

MMF will be given at 15 mg/kg q 8 hours (45 mg/kg/day; max.3g/day) PO, or IV if indicated (i.e. first dose 4-6 hours following stem cell infusion) to Day +40 post-transplant. MMF will be given until Day +40 post-transplant and then tapered by 10% per week to be discontinued by Day +90.

Taper for both Tacrolimus (CSA)/MMF may be halted or modified as clinically indicated or with the development of any evidence of GVHD at the evaluation of the treating physician.

For patients with suspected recurrence a more rapid taper of immunosuppression can be done at the discretion of the treating physician.

9.3.1.3 Rabbit Anti-thymocyte globulin (rATG)

rATG will be given on days -3 and -4 to patients receiving umbilical cord blood transplants.

9.3.2 GVHD treatment: If clinical GVHD develops, then further immunosuppressive treatment may be required. The individual aspects of each case would affect the choice of immunosuppression. Therefore, the method of immunosuppression, if necessary, would be decided on a case-by-case basis, using standard treatments as prescribed by the treating physician and in consultation with the study chair.

9.4 Duration of participation on the study

Patients should be followed and remain in the hospital until there is evidence of engraftment of donor cells and there is recovery from the side effects of the transplant procedure, per institutional guidelines. Monitoring parameters will be performed weekly until day 42 or longer, if the engraftment process is delayed. If the patient is engrafted and stable enough to be discharged from the inpatient facility, patients will be followed as outpatients at regular intervals. Study assessment data will be collected around the weeks of Day +100, +180, +270 and +365 post-transplant (please refer to Section 11.3). Subject participation will continue for 1 year post- HCT under this study. Patients are considered off-study only if death occurs, they withdraw consent/assent, and/or are confirmed lost to follow-up after a 12- month period of effort to locate the patient.

10.0 Drug Formulation and Administration

10.1 Fludarabine (FLUDARA®)

10.1.1 Source and Pharmacology

Supplier: Berlex Laboratories, Inc. FLUDARA FOR INJECTION contains fludarabine phosphate, a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-ß-D-arabinofuranosyladenine (Ara-A) that is relatively resistant to deamination by adenosine deaminase. The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2- fluoro-9-(5-O-phosphono-ß-D-arabinofuranosyl). Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

10.1.2 Formulation and Stability

Each vial of sterile lyophilized solid cake contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Reconstitution with 2 mL of Sterile Water for Injection USP results in a solution containing 25 mg/mL of fludarabine phosphate intended for intravenous administration. FLUDARA FOR INJECTION is supplied in a clear glass single dose vial (6 mL capacity) and packaged in a single dose vial carton in a shelf pack of five.

10.1.3 Solution Preparation

FLUDARA should be prepared for parenteral use by aseptically adding Sterile Water for

Injection USP. When reconstituted with 2 mL of Sterile Water for Injection, USP, the solid cake should fully dissolve in 15 seconds or less; each mL of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The pH range for the final product is 7.2-8.2. In clinical studies, the product has been diluted in 100 cc or 125 cc of 5% Dextrose Injection USP or 0.9% Sodium Chloride USP.

10.1.4 Storage and Stability

FLUDARA is supplied as a white, lyophilized solid cake. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Store under refrigeration, between 2°-8° C (36°-46° F).

10.1.5 Administration:

FLUDARA will be administered by the central venous catheter over 1 hour, daily for 3 days. Patients will receive antiemetics prior to administration of this chemotherapy.

10.2 **Thiotepa**

10.2.1 Source and Pharmacology

Thiotepa is a cytotoxic agent of the polyfunctional type, related chemically and pharmacologically to nitrogen mustard. The radiomimetic action of thiotepa is believed to occur through the release of ethylenimine radicals which, like irradiation, disrupt the bonds of DNA. One of the principal bond disruptions is initiated by alkylation of guanine at the N-7 position, which severs the linkage between the purine base and the sugar and liberates alkylated guanines. Thiotepa is desulfurated by cytochrome P-450 enzymes such as 2B1 and 2C11 which catalyze the conversion of thiotepa to tepa.

Tepa is less toxic than thiotepa and has been demonstrated to produce alkali-labile sites in DNA, rather than cross-links. These findings indicate that tepa reacts differently from thiotepa and produces monofunctional alkylation of DNA. A second metabolite of thiotepa, a mercapturic acid conjugate, is formed via glutathione conjugation. Monochlorotepa is the third metabolite found in the urine.

10.2.2 How supplied and storage

Thiotepa for Injection USP, for single use only, is available in vials containing 15 mg of nonpyrogenic, sterile lyophilized powder. Store in a refrigerator at 2° to 8°C (36° to 46°F). PROTECT FROM LIGHT AT ALL TIMES.

10.2.3 Dosage and Administration

Reconstitute Thiotepa for Injection with 1.5 mL of Sterile Water for Injection resulting in a

drug concentration of approximately 10 mg/mL. (As per manufacturer's information: Actual content per vial 15.6 mg; withdrawable amount 14.7 mg/1.4 mL; approximate reconstituted concentration: 10.4 mg/mL). When reconstituted with Sterile Water for Injection, solutions of thiotepa should be stored at refrigerated temperatures 2°-8°C (36°-46°F) and used within 8 hours. The reconstituted solution is hypotonic. For administration by intermittent or continuous infusion, the reconstitution solution should be further diluted with Sodium Chloride Injection or 5% Dextrose Injection to a concentration of between 1-5 mg/mL. Thiotepa 1 mg/mL in 0.9% sodium chloride is nearly isotonic. Reconstituted solutions further diluted with Sodium Chloride Injection should be used immediately. In order to eliminate haze, filter solutions through a 0.22 micron filter [Polysulfone membrane (Gelman's Sterile Aerodisc®, Single Use) or triton- free mixed ester of cellulose/PVC (Millipore's MILLEX®-GSFilter Unit)] prior to administration. Filtering does not alter solution potency. Reconstituted solutions should be clear. Solutions that remain opaque or precipitate after filtration should not be used.

10.3 Antithymocyte Globulin (Rabbit) (rATG)

10.3.1 Source & Pharmacology:

Antithymocyte globulin (rabbit) is a polyclonal antibody which appears to cause immunosuppression by acting on T-cell surface antigens and depleting CD4 lymphocytes. Its onset of action (T-cell depletion) occurs within 24 hours with a half-life of 2-3 days. Its resulting lymphopenia may persist for up to 1 year.

10.3.2 How Supplied

rATG for injection is available in sterile multiple dose vials of 25mgs each. Its pH: 6.5 to 7.2 when reconstituted and it contains glycine, mannitol and sodium chloride.

10.3.3 Dosage and Administration

Store intact vial at 2°C to 8°C (36°F to 46°F); do not freeze. Protect from light.
Allow vials to reach room temperature, then reconstitute each vial with 5 mL sterile water for injection (SWFI). Rotate vial gently until dissolved; resulting concentration is 5 mg/mL of thymoglobulin. Further dilute dose to a final concentration of 0.5 mg/mL (e.g., one vial [25 mg] in 50 mL saline or dextrose); in adults, total volume is usually 50 to 500 mL depending on total number of vials needed per dose.
Mix by gently inverting infusion bag once or twice. Reconstituted product is stable for up to 24 hours at room temperature; however, the product contains no preservative and room temperature storage is not recommended. Immediate use is recommended after reconstitution and preparation for infusion in D5W or NS.

Infuse the first dose over at least 6 hours in adults and 6-12 hours in pediatric patients; subsequent doses may be infused over at least 4 hours.
Infuse through a high-flow vein (central line). Administer through an in-line 0.22 micron filter. Premedication with corticosteroids, acetaminophen, and/or an antihistamine 1 hour prior to infusion may reduce the incidence and severity of infusion-related reactions.
Reducing the infusion rate may minimize infusion reactions.
No dosing adjustments are required for renal or hepatic impairment

10.4 Etoposide (VP-16)

10.4.1Source and Pharmacology

Etoposide is a semisynthetic derivative of podophyllotoxin that forms a complex with topoisomerase II and DNA which results in single and double strand DNA breaks. Its main effect appears to be in the S and G2 phase of the cell cycle. The initial t½ is 1.5 hours and the mean terminal half-life is 4 to 11 hours. It is primarily excreted in the urine. In children, approximately 55% of the dose is excreted in the urine as etoposide in 24 hours. The mean renal clearance of etoposide is 7 to 10 mL/min/m² or about 35% of the total body clearance over a dose range of 80 to 600 mg/m².

10.4.2 How Supplied

Etoposide for Injection is available in sterile multiple dose vials. The pH of the clear, nearly colorless to yellow liquid is 3 to 4. Each mL contains 20 mg etoposide, 2 mg citric acid, 30mg benzyl alcohol, 80 mg modified polysorbate 80/tween 80, 650 mg polyethylene glycol 300, and 30.5 percent (v/v) alcohol. Vial headspace contains nitrogen. Unopened vials of Etoposide are stable until expiration date on package at room temperature (25°C).

10.4.3 Dosage and Administration

Etoposide will be given through an IV. Preparation, administration, and monitoring will be according to standard practice procedures.

10.5 Melphalan

10.5.1 Source and Pharmacology

Melphalan is a derivative of nitrogen mustard, it is a bifunctional alkylating agent which causes miscoding, cross-linkage of DNA and single-strand breakage of DNA. It inhibits glycolysis, respiration and protein synthesis. It is not phase specific. After IV administration the μ t½ is 8 minutes and the b t½ is 2 hours. Oral absorption is erratic, but averages 60% of IV dose. Initial half-life after oral dose is 90 minutes. Dosage may need reduction in patients with renal impairment

10.5.2 How Supplied

For injection, it is supplied as 50mg sterile, lyophilized powder with 10ml special diluent for reconstitution. After reconstitution to 5 mg/mL, shake until clear solution obtained.

10.5.3 Dosage and Administration

Prior to administration, further dilute the solution with 0.9% sodium chloride to a concentration no greater than 0.45 mg/mL. Intact packages and reconstituted solutions should be stored at room temperature (15°-30°C), protected from light. The manufacturer recommends melphalan administration should be completed within 60 minutes of reconstitution. IV infusion over 20 minutes and good IV hydration with 3000 mL/m^2 of D5W 1/2 normal saline 24 hours after melphalan infusion. Lasix may also be given to maintain urine output.

10.6 Cyclosporine A (cyclosporine, CYA, CSA, Sandimmune®, Neoral®, Gengraf®)

10.6.1Source and Pharmacology

Cyclosporine (CSA) is a lipophilic fungal peptide consisting of 11 amino acids. CSA is a potent immunosuppressive agent, which prolongs survival of allogeneic transplants involving skin, heart, kidney, pancreas, bone marrow, small intestine, and lung. CSA binds to a cellular protein, cyclophilin, which, like tacrolimus, then binds to calcineurin. The inhibition of calcineurin inhibits the transcription of IL-2, the action of which stimulates the proliferation of activated T-lymphocytes. The terminal half-life of CSA is approximately 19 hours (ranges from 10-27 hours). Ninety-nine percent of CSA is metabolized. Elimination is primarily biliary with approximately 6% excreted in the urine. In the circulation, CSA is mainly bound to high, low, or very low density lipoproteins and to chylomicrons. Only a small fraction circulates unbound. The volume of distribution varies from 3.5 L/kg to 13 L/kg, with higher concentrations of drug found in the liver, lymphocytes, kidney, heart, lung, pancreas, fat, neural, and muscle cells. CSA clearance rates have been shown to be higher in pediatric patients and for patients <25 years old. The absorption of CSA from the gastrointestinal tract is incomplete and variable, exhibiting large intra- and inter-patient variability. Drugs that stimulate or inhibit hepatic P-450 enzymes will alter clearance of CSA; thus, close attention to potential drug interactions is crucial.

10.6.2 Formulation, Stability and Storage

IV formulation: Cyclosporine (Sandimmune®) is available as a (50 mg/mL) 5 mL ampoule containing 650 mg polyoxyethylated castor oil (cremophor) and 32.9% alcohol. It should be stored at temperatures below 30°C (86°F) and protected from light.

Oral formulations: Cyclosporine (Sandimmune®) is available as 25mg, 50mg and 100mg

capsules, or in a 100 mg/mL oral solution Inactive ingredients include: Sandimmune® Capsule = Dehydrated alcohol, sorbitol, glycerol, corn oil, gelatin, polyoxyethylated glycolysed glycerides; Sandimmune® Solution = Olive oil, dehydrated alcohol, polyoxyethylated glycolysed glycerides.

Cyclosporine USP modified (microemulsion) (Neoral®, Gengraf®) 25 mg, 100 mg capsule, 100 mg/mL oral solution. Inactive ingredients include: Neoral® capsule and solution = dehydrated alcohol, corn oil, polyoxyl 40 hydrogenated castor oil, α- tocopherol, gelatin, and propylene glycol. Gengraf® Capsule and solution = dehydrated alcohol, gelatin, polyethylene glycol, polyoxyl 35 castor oil, polysorbate 80, propylene glycol, and sorbitan monooleate.

Store capsules in the original unit-dose container at controlled room temperature 15°- 30°C (59°-86°F). Store oral solutions in the original container at controlled room temperature 68°-77°F (20°-25°C). Do not store in the refrigerator. Once opened, the contents must be used within two months. At temperatures below 68°F (20°C), the solution may form a gel; light flocculation or the formation of a light sediment may also occur. There is no impact on product performance or dosing using the syringe provided. Allow to warm to room temperature 77°F (25°C) to reverse these changes.

NOTE: Sandimmune®, Neoral® and Gengraf® ARE NOT BIOEQUIVALENT. Liquid formulations of each trade name are equivalent to capsules of that same trade name. Conversion from one trade name product to another is generally done at a 1:1 ratio, but requires close monitoring. Conversions from IV to PO are usually done at a 1:3 ratio, but should be monitored closely. Adjusting emulsion products to the same trough concentration as other oral products results in greater total exposure to the drug.

10.6.3 Solution Preparation

Dilute IV concentrate 1 mL (50mg) of Cyclosporine for Injection in 20 mL-100 mL 0.9% Sodium Chloride Injection or 5% Dextrose Injection (0.5 - 2.5 mg/mL). Diluted infusion solutions are stable for 24 hours at room temperature under fluorescent light.

10.6.4 Administration

The Cremophor® EL (polyoxyethylated castor oil) contained in concentrate for IV infusion can cause phthalate stripping from PVC. It is highly recommended that glass bottles and non-PVC tubing be used to minimize patient exposure to DEHP. Monitor closely for an acute allergic reaction for the first 30 minutes and at frequent intervals thereafter. Note: Cyclosporine absorbs into plastics and can give falsely high serum or blood concentrations if blood samples are collected from the same line through which Cyclosporine was administered.

Oral: Administer at a consistent time of day and at consistent intervals with regard to meals. Do not use plastic or Styrofoam cups. If diluted with juice or milk, use a glass container and rinse with additional diluent, and then consume to ensure that complete dose has been taken. Do not use water or cleaning agents on the dosing syringe. To improve palatability, mix Sandimmune® with milk, chocolate milk or orange juice; mix Neoral® or Gengraf® with orange juice or apple juice, but NOT milk. After mixed, have patient consume immediately. DO NOT MIX GRAPEFRUIT JUICE with any CSA product.

10.7 Tacrolimus

10.7.1 Source and Pharmacology

Tacrolimus is a macrolide immunosuppressant produced by Streptomyces tsukubaensis. Tacrolimus prolongs the survival of the host and transplanted graft in animal transplant models of liver, kidney, heart, bone marrow, small bowel and pancreas, lung and trachea, skin, cornea, and limb. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKP-12. A complex of tacrolimus- FKBP-12, calcium, calmodulin, and calcineurin is then formed, and the phospatase activity of calcineurin is inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (i.e., immunosuppression).

10.7.2 Formulation and Stability

Prograf injection (tacrolimus injection) 5 mg (for IV infusion only). Supplied as a sterile solution in 1 mL ampoules, containing the equivalent of 5 mg of anhydrous tacrolimus per mL.

10.7.3 Dosage and Administration

The usual dose of the drug is 0.05 mg/kg to 0.1 mg/kg/IV per day to achieve the trough level of 10-15mg/ml. Orally, the patient needs to be started on 0.15 to 0.2 mg/kg q12 hours to achieve the same trough level. The dose may have to be decreased if other nephrotoxic agents are being administered.

10.7.4 Preparation for Administration/Stability

Tacrolimus will be stored in glass or polyethylene containers and should be discarded after 24 hours. The diluted infusion solution should not be stored in a PVC container due to decreased stability and the potential for extraction of phthalates. In situations where more dilute solutions are utilized (e.g., pediatric dosing, etc.), PVC-free tubing should, likewise, be used to minimize the potential for significant drug absorption onto the tubing. Parenteral

drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Due to the chemical instability of tacrolimus in alkaline media, Prograf injection should not be mixed or co-infused with solutions of pH 9 or greater (e.g., Ganciclovir or Acyclovir).

Store between 5° C and 25° C (41° F and 77° F). Prograf capsules (tacrolimus capsules) 5 mg, oblong, grayish/red, branded with white “5 mg” on the capsule cap and containing the equivalent of 5 mg anhydrous tacrolimus.

10.8 Mycophenolate mofetil (MMF, Cellcept® Myfortic®)

10.8.1 Action and Pharmacology

MMF is a potent, selective, uncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Because T- and B- lymphocytes are critically dependent for their proliferation on de novo synthesis of purines, whereas other cell types can utilize salvage pathways, MPA has potent cytostatic effects on lymphocytes. MPA inhibits proliferative responses of T- and B- lymphocytes to both mitogenic and allo-specific stimulation.

10.8.2 Formulation and Stability

Capsules, 250 mg and 500 mg Store at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F). Dispense in light-resistant containers, such as the manufacturer's original containers. Oral Suspension Supplied as a white to off-white powder blend for constitution to a white to off-white mixed-fruit flavor suspension. Storage: Store dry powder at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F). Store constituted suspension at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F) for up to 60 days. Storage in a refrigerator at 2° to 8°C (36° to 46°F) is acceptable. Do not freeze. Intravenous Supplied in a 20 mL, sterile vial containing the equivalent of 500 mg mycophenolate mofetil as the hydrochloride salt in cartons of 4 vials: Store powder and reconstituted/infusion solutions at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F).

In the setting of marrow transplantation, several etiologies may contribute to alterations in gastrointestinal and hematologic parameters; therefore, MMF dose adjustments are not encouraged.

10.8.3 Administration Guidelines

Initial dosage will be IV or PO with switch to PO when tolerated. Starting doses will be 15 mg/kg/day Q 8 hours (total dose 45 mg/kg/day, maximum 3 g/day; IV same as PO dose) beginning day 0 with the first dose given approximately 4-6 hours after the stem cell infusion.

11.0 Standard Observations

Frequent clinical and laboratory evaluation will be performed to provide appropriate medical care to the patients as per standard practice. For this study's assessment laboratory data will be collected once weekly or as defined below for analysis. Please refer to appendix B for summary of required observations

11.1 Baseline/Pre-Conditioning

Baseline laboratory testing must be done within 7 days prior to the start of the conditioning regimen.

All baseline imaging, cardiac evaluation and pulmonary evaluation must be done within 28 days prior the start of the conditioning regimen. Baseline/pre conditioning evaluation is summarized in Appendix B

11.1.1 History, physical examination, pregnancy test for females of reproductive potential, weight, height, BSA and head circumference (if appropriate for age).

11.1.2 CBC, differential, platelet count, ABO and Rh typing.

11.1.3 Liver function tests (bilirubin, ALT).

11.1.4 Renal function tests (creatinine, BUN, creatinine clearance or GFR).

11.1.5 Cardiac evaluation: echocardiogram, MRI or MUGA with ejection fraction (or shortening fraction) as appropriate.

11.1.6 Pulmonary function evaluation: CT chest and pulmonary function tests (if age appropriate and feasible) or O2 Saturation in room air.

11.1.7 Serology for CMV, Hep. B &C, HSV, VSV, HTLV and HIV (if HIV NAT testing not obtained).

11.1.8 NAT testing for HIV/HCV. (HIV and HCV PCRs may replace serology or NAT testing).

11.1.9 Immune assessment: Immunophenotyping (CD3, CD4, CD8, CD19, CD56, CD4+/CD45RA+, CD4+/CD45RO+), lymphoproliferative responses to

mitogens, Quantitative Immunoglobulins (IgG, IgA, IgM).

11.1.10 Archived blood sample for short tandem repeat (STR) and/or FISH analyses of blood to document a genetic marker that can be used to distinguish donor and recipient cells as per standard of care (as clinically appropriate).

11.1.11 Blood sample for circulating tumor cells prior to the start of conditioning regimen to be sent to Dr.Shulin Li's lab

11.1.12 Karnofsky or Lansky score (age appropriate)

11.1.13 Evaluation of other disease specific manifestations/sites as clinically appropriate which may include: Imaging (e.g. MRI, PET/CT, Bone scan, skeletal surveys); LP/CSF analysis and bone marrow aspirate/biopsy. Bone marrow aspirate and biopsy will be obtained prior to HCT to evaluate for disease by morphology.

11.2 **During Conditioning**

11.2.9 History, physical examination, pregnancy test for females of reproductive potential, weight, height, BSA and head circumference (if appropriate for age).

CBC and serum chemistries, Tacrolimus or CSA level (as clinically appropriate) starting one day after initiation of tacrolimus, until levels are stable on oral dosing or until discharge.

Evaluation required during conditioning is summarized in Appendix B

11.3 Post-Transplant

After transplant, frequent clinical and laboratory evaluation will be performed to provide appropriate medical care to the patients. Post-transplant evaluation summarized in Appendix B The post-transplant data will be captured once a week for the assessment of the study. In the first year, studies scheduled for Days +100, +180, +270 and +365 can be performed at any Day ± 15 of the scheduled test.

11.3.1 Weekly CBC, differential (after WBC >500/mm^3) until ANC = 500/mm^3 for 3 consecutive days;
Post-engraftment: CBC and platelet count weekly until discharge;
Post-discharge: CBC and platelet count weekly until PRBC and platelet transfusion independent and at Days +100, +180, +270, and +365. Differential must be done if WBC < 500/mm^3 at any time post-engraftment and at Days +100, +180, +270, and +365

11.3.2 Donor chimerism study at Day +28 ± 3 days (if engrafted for neutrophils) and at 6 months and at 1 year.

11.3.3 Bone marrow aspirate on Day +42 (+/- 3 days) for patients who do not have an ANC = 500/mm3 by Day +42.

11.3.4 Weekly assessment for toxicity: Mucositis and SOS until Day +30 or until resolution (whichever is longer).

11.3.5 Perform and document: Karnofsky/Lansky performance score, GVHD, history and physical examination weekly for 6 weeks, twice a month until Day +100, then Day +180, +270, and +365.

11.3.6 CBC, renal and liver function tests weekly for 6 weeks; cardiac function tests (echocardiogram), pulmonary assessment, thyroid function tests, height, weight, head circumference (if age appropriate) at one year follow-up as part of the study. However, it is recommended as general part of a general long-term follow-up to assess yearly for a minimum of 4 years.

11.3.7 Immune assessment: Immunophenotyping (CD3, CD4, CD8, CD19, CD56), lymphoproliferative responses to mitogens, quantitative Immunoglobulins (IgG, IgA, IgM) will be monitored at Days +100, +180, +270, +365 post-transplant.

11.3.8 Three months, 6 months, 9 months, and 12 months post-tetanus immunization, lymphoproliferative responses to tetanus and antibody titer to tetanus.

11.3.9 Evaluation of disease specific manifestations/sites *as clinically appropriate/per standard disease assessment* which may include: Imaging (e.g. MRI, PET/CT, Bone scan, skeletal surveys); LP/CSF analysis and bone marrow aspirate/biopsy on at least on Day +100 with subsequent testing on Days +180,+270 and 1 year post-HCT.

12.0 Monitoring

12.1 Supportive Care

Before, during and after the transplant, subjects of this study will require standard supportive care. Programmatic supportive care guidelines will be adhered to, but may vary with the condition or problems of individual patients. Supportive care will include red blood cell and platelet transfusion; antibiotic treatment of bacterial, fungal and viral infections; prevention of bacterial, fungal and viral infections, including Pneumocystis jiroveci; administration of prophylactic and therapeutic intravenous immunoglobulin (IVIG); isolation; treatment of hepatic sinusoidal obstruction syndrome/veno-occlusive disease; and treatment of mucositis. Aggressive supportive care improves outcome, particularly in high-risk patient populations receiving very intensive chemotherapy as incorporated in this protocol. These supportive care guidelines are provided for institutional consideration, in order to optimize patient care as well as to encourage uniformity in the treatment of this study population.

12.2 General Guidelines

12.2.1 Complete blood counts (Hemoglobin, platelet count, WBC and ANC) should be monitored after blood counts drop (i.e. ANC <1000, platelets <50,000), daily for in- patients until blood count recovery commences. Hospitalization is required until the patient achieves and sustains an absolute neutrophil count (ANC) of at least 500/μL for at least five days. Growth factors (e.g. granulocyte colony stimulating factor) can be used at the discretion of the treating physician.

12.2.2 Blood Products

- Irradiated and leukodepleted platelets must be transfused to maintain a platelet count above 20,000/mm^3.
- Irradiated and leukodepleted packed red blood cells must be transfused to maintain a hemoglobin > 8 g/dL.
- All blood products should be leukodepleted and irradiated and/or prepared in a manner to prevent the occurrence of acute graft-versus-host disease with the exception of blood stem cells or bone marrow.

12.2.3 Prophylactic Antimicrobial Therapy is required for viral, fungal and bacterial infections

HSV/VZV Viral prophylaxis with acyclovir IV or Valacyclovir is strongly recommended (dosing per institutional standards). Prophylaxis for cytomegalovirus (CMV) with intravenous ganciclovir (10 mg/kg/day) during conditioning through Day -2 and then high dose acyclovir (1500 mg/m^2/day) is recommended. After engraftment and when patients are able to tolerate oral medications, oral acyclovir or valganciclovir depending on pre-HCT CMV serology may be used for viral prophylaxis; this should be continued for at least one year after HCT.

IV caspofungin (25 mg/m^2 daily for infants, 70 mg/m^2/day on day 1 then 50 mg/m^2/day after for children and adolescents). This may be transitioned to oral posaconazole or voriconazole following engraftment and when clinically appropriate.

IV liposomal amphoterecin B (3-5 mg/kg/day) is strongly recommended as fungal prophylaxis for patients with a history of invasive fungal infection.

Weekly surveillance with viral polymerase chain reactions (PCR) and galactomannans should be performed.

Trimethoprim-sulfamethoxazole (5 mg/kg/day) or pentamidine for pneumocystis jiroveci pneumonia (PJP) prophylaxis should be initiated from admission until Day -2 then restarted at Day +30 (or at the time of engraftment) until one year post-HCT or until appropriate T-cell recovery is achieved.

IVIG replacement per the institutional guidelines.

All antimicrobial prophylaxis medications are strongly recommended to be continued

until patients achieved acceptable evidence of immune reconstitution.

12.2.4 Sinusoidal obstruction syndrome/Veno-occlusive disease prophylaxis: Prophylaxis with oral ursodeoxycholic acid should also be considered from admission until Day +28. Patients who develop SOS/VOD should be promptly started on defibrotide definitive treatment per institutional guidelines.

12.3 Pre-Transplant assessment as outlined in 11.1

12.4 Post-Transplant weekly assessment as outlined in 11.3

12.5 Post-Transplant monthly assessment as outlined in 11.3

13.0 Adverse Event Reporting Requirements

13.1 Assessment of the Adverse Events Severity.

The severity of the adverse events (AEs) will be graded according to the Common Terminology

Criteria v5.0 (CTCAE). Events not included in the CTCAE chart will be scored as follows:

General grading:

Grade 1: Mild: discomfort present with no disruption of daily activity, no treatment required beyond prophylaxis.

Grade 2: Moderate: discomfort present with some disruption of daily activity, require treatment.

Grade 3: Severe: discomfort that interrupts normal daily activity, not responding to first line treatment.

Grade 4: Life Threatening: discomfort that represents immediate risk of death.

13.2 Casualty Assessment.

For the purpose of this study the treatment plan (preparative regimen followed by allogeneic stem cell transplantation) is defined as the “transplant package”; therefore adverse events known to be caused by components of the transplant package and its direct consequences will be scored as definitive related. Adverse events known to be related to drugs used for the treatment of GVHD and infection episodes will be scored as probable related. When the relationship of the adverse event cannot be ruled out with certainty the AE may be considered possible related. Adverse events known to be related to drugs used for supportive treatment will be scored as unrelated.

The principal investigator will be the final arbiter in determining the casualty assessment.

13.3 List of most common expected adverse events.

1. Infections in the presence or absence of neutropenia: fungal, bacterial and or viral infections.
2. Fever: Non-neutropenic or neutropenic without infection
3. Acute graft versus host disease (aGVHD): most commonly manifested by skin rash, diarrhea and abnormal liver function tests could also present with some degree of fever, upper gastrointestinal symptoms (nausea and vomiting) mucositis and eye dryness.
4. Gastrointestinal (GI tract): the GI tract manifestations could be not only due to direct damage from the preparative regiment but also be a manifestation of GVHD or infections. Therefore, the time course and its presentation are crucial when assessing these as adverse events. Nausea/vomiting, mucositis, diarrhea when presented within first 7 to 10 days most likely will be related to the preparative regimen.
5. Skin rash: not related to GVHD could be caused by chemotherapy used for the preparative regimen or antibiotics used a supportive treatment.
6. Transaminitis: liver function test elevation.
7. Pulmonary events: Idiopathic pneumonia syndrome, pneumonia and/or pleural effusions
8. Cytokine Storm/ engraftment syndrome: most likely caused by released cytokines.
9. Hemorrhagic cystitis: not related to chemotherapy agents used in the proposed preparative regimen is most likely caused by viral infection.
10. Thrombotic thrombocytopenic purpura (TTP).
11. Sinusoidal obstruction syndrome
12. Fluid overload due to hydration required for conditioning regimen, blood product transfusions and or IV alimentation
13. Graft failure.
14. Chronic GVHD.
15. For the purpose of this study the following events would not be considered adverse events and would not be recorded in the database:
1. Flu-like symptoms not associated with infection
2. Abnormal laboratory findings considered associated to the original disease
3. Isolated changes in laboratory parameters such as electrolyte, magnesium and metabolic imbalances, uric acid changes, elevations of ALT, AST, LDH and alkaline phosphatase.

13.4 Adverse events considered serious.

1. Prolonged hospitalization due to infections and/or organ failure requiring extensive supportive care (i.e. dialysis, mechanical ventilation).
2. Readmissions from any cause resulting in a prolonged hospitalization (>10 days).

3. Graft Failure/ rejection.

4. Any expected or unexpected event resulting in an irreversible condition and/or leading to death.

13.5 Adverse events data collection.

From the start of preparative regimen up to D+100 the collection of adverse events will reflect the onset and resolution date and maximum grade; beyond this point some events considered related to chronic GVHD or late complications post-transplant may be recorded only with the first date of their awareness with no grade or resolution date. Pre-existing medical conditions will be recorded only if an exacerbation occurs during the active treatment period. Co-morbid events will not be scored separately.

13.6 AE and Protocol Deviations Reporting Requirements.

Adverse events will be reported accordingly to MDACC (HSRM chapter 15.001) and SCT&CT Department (HSRM chapter 15.053) policy and procedures. This study will be conducted in compliance however in the event of any protocol deviations or violations these will be reported accordingly to MDACC (HSRM chapter 25).

14.0 Statistical Considerations

14.1 Overview

This is a pilot study of allogeneic hematopoietic stem cell transplantation in high risk solid tumors. The study plans to enroll a maximum of 20 patients in each of the two groups: 1) Patients with a matched related donor, 2) Patients with a matched allogeneic umbilical cord donor. The primary objective is to assess the tolerability in each treatment group by transplant-related mortality (TRM) at day 30, as well as the rate of grade III or higher organ toxicity (Bearman Regimen-Related Toxicities Scale) attributable to conditioning occurring within 30 days. Secondary objectives are listed in section 2.0. Specifically, the primary endpoints include the TRM at day 30 and the rate of grade III or higher organ toxicity within 30 days. The secondary endpoints include time to platelet and neutrophil engraftment, incidence of aGVHD by day 100, incidence of chronic GVHD at day 100 and one year, rate of grade II organ toxicity through day 100, rate of graft failure (primary and secondary) through day 100, rate of infectious complications through day 100, progression free survival at day 180 and cumulative incidence of relapse, overall survival, and progression-free survival at 100 days and 1 year.

14.2 Statistical Considerations and Analysis Methods

We will use Bayesian monitoring rules to monitor the 30-day TRM rate and stop the trial if there is strong evidence at any time that this rate exceeds 20%. Formally, we will stop accrual if at any time:

Pr (Day 30 TRM> 0.20 | Data) > 0.80. We assume a Beta (0.4, 1.6) prior distribution for the probability of TRM corresponding to a 20% mean, and assume a constant rate of 20% for the standard of care. Stopping boundaries and operating characteristics were generated using the Department of Biostatistics' Bayesian Toxicity Monitoring Shiny version v2.1.1.2 (https://biostatistics.mdanderson.org/shinyapps/BTOX/). Patients will be monitored in cohorts of size 5. The stopping boundaries for this rule corresponding to the probability criterion above are:

No of total Patients (Cohorts of 5)	Discontinuation if No of 30-day TRM is:
5	3-5
10	4-10
15	5-15
20	6-20

Corresponding operating characteristics for toxicity stopping rule for 30-day TRM:

True 30-day TRM Rate	Prob. of Early Stop	Avg. N Patients
.1	.02	20
.15	.09	19
.2	.21	18
.25	.36	16
.3	.53	15
.35	.68	13
.4	.81	11

Similarly for rate of grade III or higher organ toxicity attributable to conditioning occurring within 30 days, we will stop the trial if there is strong evidence at any time that this rate exceeds 30%. Formally, we will stop accrual if at any time: Pr (Day 30 grade III or higher organ toxicity > 0.30 | Data) > 0.80. We assume a Beta (0.6, 1.4) prior distribution for the probability of toxicity corresponding to a 30% mean, and assume a constant rate of 30% for the standard of care. Stopping boundaries are:

No of total Patients (Cohorts of 5)	Discontinuation if No of 30-day grade III or higher organ toxicity is:
5	3-5
10	5-10
15	7-15
20	8-20

Corresponding operating characteristics for toxicity stopping rule for 30-day grade III or higher organ toxicity:

True Grade III or Higher Organ Toxicity Rate	Prob. of Early Stop	Avg. N Patients
.2	.08	19
.25	.15	18
.3	.26	17
.35	.39	15
.4	.53	14
.45	.66	12
.5	.78	10

14.3 Analysis Methods

The proportion of patients with 30-day TRM and the proportion of patients with 30-day grade III or higher organ toxicity will be reported together with the corresponding 95% Bayesian credible interval. Given 20 patients with the stopping criteria thresholds of 20% and 30%, respectively, corresponding 95% Bayesian credible intervals span (.07, .40) and (.13, .51).

The 100-day and 1-year rates of relapse, overall survival (OS) and progression-free survival (PFS) will be calculated and illustrated from the time of transplant by the method of Kaplan and Meier[45]. The same approach will be applied to the time to failure of platelet and neutrophil engraftment. The progression free survival at day 180 will be assessed using the method of Kaplan and Meier.

The 100-day rates of acute and chronic GVHD with the competing risk of relapse will be estimated using the method of Gooley[46]. The same approach will also be applied to 100-day and 1-year chronic GVHD and 30-day TRM. The 100-day rates of grade II organ toxicity, primary and secondary graft failure, and infectious complications will be reported as counts with percentages.
Frequency counts and percentages will also be presented of subjects with serious adverse events and adverse events leading to withdrawal. All other safety parameters will be summarized using descriptive statistics or frequency counts. All time-to-event modeling will include corresponding illustrations. Other statistical approaches might be employed.

15.0 Data and Safety Monitoring Plan

This study is monitored by the MDA DSMC.

16.0 Confidentiality of Study Data

Participant confidentiality and privacy is strictly held in trust by the participating investigator, their staff, the safety and oversight monitor(s), and the sponsor(s) and funding agency. This confidentiality is extended to the data being collected as part of this study. Data that could be used to identify a specific study participant will be held in strict confidence within the research team. No personally identifiable information from the study will be released to any unauthorized third party without prior written approval of the sponsor/funding agency, as applicable.

All research activities will be conducted in as private a setting as possible.

Access to Study Records
Study records may be accessed by IRB approved study personnel, or authorized inspectors. The study monitor, other authorized representatives of the sponsor or funding agency, representatives of the Institutional Review Board (IRB), regulatory agencies or representatives from companies or organizations supplying the product, may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

Methods of Storage of Study Records
All data collected from MD Anderson Cancer Center (MDACC) sources will be maintained in REDCap. Study staff will have role based restricted access to REDCap, according to project responsibilities. Only those with data entry permissions can add records. The PI or a delegate will review the conditions under which data will be released to recipient- investigators. Each application for use will need IRB approval and consents, if appropriate. The level of identifiability will determine the process for review and approval as well as the way information is shared.

Any study data or records maintained in paper documents will be stored in the offices of the PI or other delegated study staff, in a locked cabinet or other comparable controlled environment, and will be accessible only to authorized study team members or authorized inspectors.

Duration of Study Record Storage
The study participant's contact information will be securely stored internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor/funding agency requirements.

Sharing of Study Records
There are no plans to share study data with entities external to MD Anderson Cancer Center, aside from authorized inspectors as applicable (i.e. authorized representatives of the sponsor or funding agency, representatives of the Institutional Review Board (IRB), regulatory agencies or representatives from companies or organizations supplying the product). If data will be shared, IRB approval will be sought, and applicable inter-institutional agreements executed, prior to data sharing.

17.0 Informed Consent Process

Each subject / legal representative or proxy consenter will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision. Only if the subject / legal representative or proxy consenter voluntarily agrees to sign the informed consent form and has done so, may he/she enter the study. Additionally, the investigator and other information provider (if any) will personally sign and date the form. The subject / legal

representative or proxy consenter will receive a copy of the signed and dated form. The signed informed consent statement is to remain in the investigator site file or, if locally required, in the patient's note/file of the medical institution. In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or subject's clinical record must clearly show that informed consent was obtained prior to these procedures.

The informed consent form and any other written information provided to subjects / legal representatives or proxy consenters will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol that necessitates a change to the content of the subject information and / or the written informed consent form. The investigator will inform the subject / legal representative or proxy consenter of changes in a timely manner and will ask the subject to confirm his/her participation in the study by signing the revised informed consent form. Any revised written informed consent form and written information must receive the IEC/IRB`s approval / favorable opinion in advance of use.

Consent Process and Documentation

Please select one of the following:

☒ This protocol will follow the SOP 04_Informed Consent Process. SOP 04 has been read by the research staff and investigators.

☐ This protocol will follow SOP 04_Informed Consent Process with the following changes: _____. SOP 04 has been read by the research staff and investigators.

Informed consent may be obtained using the following methods: (check all that apply):

☒ Remote consent

☒ In-person consent

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19.0 Appendix A: Bearman Regimen-Related Toxicities Scale

	Grade I	Grade II	Grade III
Cardiac Toxicity	Mild EKG abnormality, not requiring medical intervention: or noted heart enlargement on CXR with no clinical symptoms	Moderate EKG abnormalities requiring and responding to medical interventions; or requiring continuous monitoring without treatment; or congestive heart failure responsive to digitalis or diuretics.	Severe EKG abnormalities with no or only partial response to medical intervention; or heart failure with no only minor response to medical intervention; or decrease in voltage by more than 50%
Bladder Toxicity	Macroscopic hematuria after 2 days from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection	Macroscopic hematuria after 7 days from last chemotherapy dose not caused by infection; or hematuria after 2 days with subjective symptoms of cystitis not caused by infection	Hemorrhagic cystitis with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomy or other surgical procedure
Renal Toxicity	Increase in creatinine up to twice to baseline value (usually the last recorded before start of conditioning)	Increasing creatinine above twice baseline but not requiring dialysis	Requirement of dialysis
Pulmonary Toxicity	Dyspnea without CXR changes not caused by infection or congestive heart failure; or CXR showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure	CXR with extensive localized infiltrate or moderate interstitial changes combined with dyspnea not caused by infection or CHF, or decrease of PO2 (> 10% from baseline) but not requiring mechanical ventilation or more than 50% O2 on mask and not caused by infection or CHF	Interstitial changes requiring mechanical ventilatory support or > 50% oxygen on mask and not caused by infection or CHF
CNS Toxicity	Somnolence but the patient is easily arousable and oriented after arousal	Somnolence with confusion after arousal; no other new objective CNS symptoms with no loss of consciousness not more easily explained by other medication, bleeding or CNS infection	Seizure sarcoma not explained (documented) by other medication, CNS infection or bleeding
Stomatitis	Pain and/or ulceration not requiring a continuous IV narcotic drug	Pain and/or ulceration requiring a continuous IV narcotic drug	Severe ulceration and/or mucositis requiring preventative intubation; or resulting in documented aspiration pneumonia with or without intubation
GI Toxicity	Watery stools > 500 mLs but < 2000 mLs every day not related to infection	Watery stools > 2000mLs every day not related to infection, or macroscopic hemorrhagic stools with no effect on cardiovascular status not caused by infection; or sub ileus not related to infection	Ileus requiring nasogastric suction and/or surgery and not related to infection; or hemorrhagic enterocolitis affecting cardiovascular status and requiring transfusion
Note: Grade IV regimen–related toxicities defined as fatal toxicity CXR–chest x-ray IV–intravenous			

***Grade IV regimen related toxicity is defined as fatal toxicity**

20.0 APPENDIX B: STANDARD OBSERVATIONS & INTERVENTIONS

Observation/Intervention		Baseline	During Conditioning	Post-Transplant				
				Weekly for 6 weeks	2x's/mo Week 7 - Day+100	Day +180	Day +270	Day +365
History		X	X	X	X	X	X	X
Physical Examination		X	X	X	X	X	X	X
Weight, Height, and BSA		X	X	X	X	X	X	X
Head Circumference (if age appropriate)		X	X	X	X	X	X	X
Karnofsky/Lansky Score (as age appropriate)		X	X	X	X	X	X	X
Pregnancy Test		X						
GvHD Assessment Score				X	X	X	X	X
Serum Chemistries		X	X	X	X	X	X	X
Liver Function Test	Bilirubin	X	X	X	X	X	X	X
	ALT	X	X	X	X	X	X	X
Renal Function Test	Creatinine	X	X	X	X	X	X	X
	BUN	X	X	X	X	X	X	X
	Creatinine Clearance or GFR	X	X	X[a]	X[a]	X[a]	X[a]	X[a]

				Pre engraftment	Post-Engraftment	Post-Discharge	Day			
				Weekly	Weekly	Weekly	+100	+180	+270	+365
CBC/Dif		X	X	X	X	X	X	X	X	X
ABO/Rh		X								
Immune Assessment	Immunophenotyping (CD3, CD4, CD8, CD19, CD56)	X					X	X	X	X
	Lymphoproliferative Responses to Mitogens	X					X	X	X	X
	Quantitative Immunoglobulin (QuIGs = IgG, IgA, IgM)	X					X	X	X	X
	Anti- tetanus antibody titers	X					X	X	X	X
Circulating tumor cells sample		X								
Archived Blood Sample for STR and/or FISH Analyses		X								

Observation/Intervention		Baseline	During Conditioning	1 Year Post-Transplant
Cardiac Evaluation	Echo	X		X
	MRI or MUGA w/ejection fraction (or shortening fraction) as	X		X
Pulmonary Function Evaluation	CT Chest	X		X
	PFT (if age appropriate & feasible) or O2 Saturation in room	X		X
Thyroid Function Tests				X
Serology	CMV	X		
	Hep B&C	X		
	HSV	X		
	VSV	X		
	HTLV 1&2	X		
	HIV (if HIV NAT testing not obtained)	X		
NAT Testing for HIV/HCV		X		

				Day +42	Done as clinically appropriate/per standard disease assessment			
					Day +100[c]	Day +180	Day +270	Day +365
Evaluation of Other Disease Specific Manifestations/Sites[*]	Imaging	X			X	X	X	X
	LP/CSF Analysis	X			X	X	X	X
	Bone Marrow Aspirate/Biop	X		**Aspirate if ANC < 500m³ by Day +42**	X	X	X	X

		Pre-Transplant	Weekly starting 1 day after initiation of tacrolimus/CSA, until stable levels on oral dosing or until discharge													
Tacrolimus or CSA	Level		X					X								
	Taper[c]							**Day +60 to Day +100**				**Day +100 to D+180**				
								20% of original therapeutic dose/wk				**10% of original therapeutic dose/wk**				
Donor Chimerism (D+28 only if engrafted by then)													**X[d]**	**X**	**X**	

				Weekly assessment until Day +30 or until resolution (whichever is longer)
Toxicity	Mucositis			X
	VOD			X

* When applicable to primary disease

a Only if clinically indicated

b Post-discharge: CBC and platelet count weekly until PRBC and platelet transfusion independent and at Days +100, +180, +270, and +365

c If no active GvHD, then begin taper as follows:

Recipients of matched related donors start taper at Day +60 at approximately 20% of the original therapeutic dose/week to be and complete by Day +100.

Recipients of other donors start taper at Day +100 at approximately 10% of the original therapeutic dose/week to be completed by Day +180 as possible.

d Donor chimerism study done on D+28 can be done ±3 days if engrafted for neutrophils