St. Jude

MITREL – NCT02433483

Initial version, dated: 01-22-2015. Resubmitted to CT-SRC 02-18-2015 and 03-20-2015 (IRB approved: 04-22-2015).
Activation date: 05-20-2015
Amendment 1.0, dated 10-21-2015. Resubmitted to CT-SRC 11-11-2015. Resubmitted to IRB on 11-30-15. (IRB approved: 12-01-2015)
Activation date: 12-16-2015

A PHASE II STUDY OF MICROTRANSPLANTATION IN PATIENTS WITH REFRACTORY OR RELAPSED HEMATOLOGIC MALIGNANCIES

Principal Investigator
Jeffrey E. Rubnitz, MD, PhD[1]

Co-Principal Investigator
Ewelina Mamcarz, MD[2]

Co-Investigators

Stanley Pounds, PhD[3]

Departments of [1]Oncology, [2]Bone Marrow Transplantation & Cellular Therapy, and [3]Biostatistics

St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, Tennessee 38105-3678
Telephone: (901) 595-3300

Protocol Summary

MITREL, A Phase II Study of Microtransplantation in Patients with Refractory or Relapsed Hematologic Malignancies
Principal Investigator: Jeffrey Rubnitz, MD, PhD
Sponsor/IND-holder: St. Jude Children's Research Hospital; non-IND study.
Brief overview: Pilot study to explore the safety, feasibility, and efficacy of chemotherapy plus GCSF-mobilized Hematopoietic Progenitor Cell, Apheresis (HPC-A) in pediatric patients with relapsed or refractory acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).
Intervention: Interventional, primary therapeutic Drug: Cytarabine (Ara-C) Drug: (Donor) Filgrastim (G-CSF) Procedure: Intrathecal chemotherapy with methotrexate, cytarabine and hydrocortisone Procedure: GCSF-mobilized donor Hematopoietic Progenitor Cell, Apheresis (HPC-A) infusion.
Brief outline of treatment plan: Patients will receive standard chemotherapy followed by infusion of donor peripheral blood mononuclear cells 2 days after the completion of chemotherapy. Patients who have at least a partial response are eligible to receive a second cycle. Diagnostic lumbar puncture and intrathecal (IT) chemotherapy will be given prior to cycle 1. Patients without evidence of central nervous system (CNS) leukemia will receive no further IT therapy during cycle 1. Patients with CNS disease will receive weekly IT therapy (age-adjusted methotrexate, hydrocortisone, and cytarabine) until the cerebrospinal fluid (CSF) becomes free of leukemia (minimum of 4 doses). Bone marrow aspiration (BMA) and biopsy to assess response will be performed on approximately day 29 of therapy.
Study design: Simon 2-stage design.
Sample size: Maximum of 19 AML/MDS patients and 19 donors
Data management: Data management and statistical analysis will be provided by the Cancer Center Clinical Trials, Leukemia/Lymphoma Division, and Biostatistics Department at St. Jude Children's Research Hospital.
Human subjects: The risks to subject will be related to the toxicity of high-dose cytarabine and to the infusion of HLA-mismatched GCSF-mobilized Hematopoietic Progenitor Cell, Apheresis (HPC-A). Patients will be informed of all risks of drugs and procedures during informed consent. Adverse events will be monitored, reported, and treated appropriately.

TABLE OF CONTENTS

APPENDICES

1.0 OBJECTIVES

1.1 Primary Objectives

1.1.1 To assess the safety and feasibility of standard chemotherapy plus GCSF-mobilized Hematopoietic Progenitor Cell, Apheresis (HPC-A) in pediatric patients with relapsed or refractory acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).

1.1.2 To estimate the response rates to standard chemotherapy plus GCSF-mobilized HPC-A in pediatric patients with relapsed or refractory hematologic malignancies.

1.2 Secondary Objectives

1.2.1 To describe the event-free and overall survival of patients treated with standard chemotherapy plus GCSF-mobilized HPC-A.

1.2.2 To estimate the time to neutrophil and platelet recovery after treatment with standard chemotherapy plus GCSF-mobilized HPC-A.

1.2.3 To determine the cumulative incidence of acute and chronic graft-versus-host disease (GVHD).

1.3 Exploratory Objectives

1.3.1 To characterize donor chimerism and microchimerism.

1.3.2 To explore associations between dose of donor T cells, donor KIR type, and response to therapy.

1.3.3 To explore associations between levels of PR1+ and WT1+ HLA-A*02:01 CD8 T cells in peripheral blood and response to therapy.

2.0 BACKGROUND AND RATIONALE

2.1 Background

The outcome for children with relapsed or refractory leukemia is very poor, necessitating the development of novel salvage regimens. Allogeneic hematopoietic stem cell transplantation (HSCT) reduces the risk of relapse and is commonly used in the treatment of patients with high-risk or relapsed leukemia. In contrast to chemotherapy alone, the beneficial effects of HSCT are primarily due to cellular reactivity, resulting in graft-versus-leukemia and graft-versus-host effects. The potent effects of the immune system at controlling or eliminating leukemia were first recognized over 35 years ago and have been extensively reviewed.[1] The eradication of leukemia in irradiated mice that received

allogeneic marrow transplants was the first demonstration of the antileukemic effects of alloreactivity.[2] In the 1970s, many investigators demonstrated the important graft-versus-leukemia (GVL) effects of donor T-cells in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT).[3-5] The anti-leukemia effects of alloreactive donor T lymphocytes are likely greatest in the setting of donor-recipient HLA mismatch (e.g., haploidentical HSCT), in which they recognize differences in major histocompatibility antigens. Subsequently, donor lymphocyte infusions given after HSCT were shown to have anti-leukemic effects that could enhance the GVL effects of the original graft, and are commonly used today.[6,7] Although early studies suggested that GVL effects were due to donor-derived T cells, more recent studies demonstrated the contributions of donor-derived, KIR-mismatched natural killer cells.[8-13]

The safety and efficacy of infusions of HLA-mismatched peripheral blood stem cells (PBSCs), also referred to as Hematopoietic Progenitor Cell, Apheresis (HPC-A), has been demonstrated in several studies. In one study, 58 adults (ages 60-88 years) with acute myeloid leukemia (AML) were randomized to receive mitoxantrone and cytarabine alone (n=28) or mitoxantrone and cytarabine followed by infusion of GCSF-mobilized PBSCs (n=30).[14] The complete remission rates (80% vs. 43%) and 2-year disease-free survival estimates (39% vs. 10%) were significantly higher, and time to count recovery was shorter, in patients who received cell infusions. No graft-versus-host disease (GVHD) was observed. In another study, 101 patients with AML in first remission received 3 cycles of high-dose cytarabine following by infusions of GCSF-mobilized HLA-mismatched PBSCs as post-remission therapy.[15] The therapy was well tolerated and no GVHD was observed. Despite the high doses of cytarabine (2.5 g/m^2 every 12 hours x 6 doses), the time to neutrophil recovery was remarkably short, with median times of 8, 9, and 12 days after courses 1, 2, and 3. Platelet recovery was also brisk, occurring at median times of 11, 12, and 14 days. Of the 101 patients, 97 had no mixed donor or full donor chimerism, whereas 4 patients had low levels of mixed chimerism that persisted for less than 2 weeks. Among 23 patients who were evaluable for microchimerism testing, 20 had detectable donor microchimerism (range, 7×10^{-6} to 0.46) that peaked at 1-2 weeks after microtransplantation. Leukemia-free and overall survival rates were excellent, and were higher in patients who received greater than 1.1×10^8/kg donor CD3+ T cells compared to those who received lower doses of donor T cells (LFS: 76% vs. 50%; OS: 82% vs. 55%). Multivariable analysis revealed that the dose of donor T cells was significantly associated with better leukemia-free and overall survival.

2.2 Rationale for This Study

Based on the encouraging results described above, we propose to test the safety and activity of microtransplantation in pediatric patients with relapsed AML or MDS. The outcome for patients with relapsed AML is poor, with survival rates less than 40%.[16] The development of new agents has been slow and treatment options are therefore limited. Microtransplantation appears to be safe, even in elderly patients, and may be beneficial. The results of this study may lead to the incorporation of microtransplantation into the upfront therapy for patients with high-risk disease.

2.3 Rationale for Amendment 1.0

The first two subjects enrolled on MITREL experienced complete donor engraftment, an unanticipated event that led to the voluntary suspension of accrual. The main purpose of Amendment 1.0 is to reduce risks to subjects by reducing the potential for engraftment.

The major changes include the following:

1. The removal of fludarabine from the conditioning regimen. Fludarabine is known to be more immunosuppressive than cytarabine and likely contributed to the lack of graft rejection observed in the first two patients. The fludarabine/cytarabine regimen has been replaced by cytarabine alone based on the trial reported by Guo et al,[15] which used a dose of 2.5 g/m^2/dose given every 12 hours for 6 doses. The dose used in Amendment 1.0 is slightly lower (2 g/m^2/dose given every 12 hours for 6 doses), as there is no evidence that increasing the dose of cytarabine beyond 2 g/m^2 provides any clinical or pharmacological benefit.
2. The dose of infused cells has been changed from "the minimum cell dose that will be infused is 1 x 10^8 total nucleated cells (TNC)/kg and the maximum is 10 x 10^8 TNC/kg" to "the minimum cell dose that will be infused is 1 x 10^8 total nucleated cells (TNC)/kg and the maximum is 5 x 10^8 TNC/kg" based on the report by Guo et al,[15] in which a maximum of 5.6 x 10^8 cells/kg were safely infused.
3. The addition of engraftment to the stopping rules.
4. The stipulation that the first 6 patients will be enrolled sequentially, rather than simultaneously.

2.4 Rationale for Exploratory Studies

In addition to the well-characterized GVL effects described above, there is emerging evidence that graft rejection mediated by host-versus-graft responses may possess antileukemic effects.[17] Although the mechanism is not fully understood, it appears that host CD4+ and CD8+ T cells are involved.[18,19]

In the present trial, we will explore the potential roles of the patient and donor immune systems by measuring patient and donor-derived PR1+ and WT1+ CD8+ T cell counts at various time points after infusion of the HPC-A product.[20,21] Associations between levels and origins (patient versus donor) of these T cells and response to therapy may provide clues regarding the mechanism of action of this treatment. Similarly, we will explore associations between donor T cell dose and response, as well as associations between donor KIR type and response, the results of which may suggest that donor T cells, donor NK cells, or both, are important mediators of the graft versus leukemia effect. Although any conclusions will be limited by the small numbers of patients enrolled in this trial, these analyses may help direct future studies.

3.0 ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

According to institutional and NIH policy, the study will accession research participants regardless of gender and ethnic background. Institutional experience confirms broad representation in this regard.

3.1 Inclusion Criteria

3.1.1 Participants must have a diagnosis of AML or MDS and must have disease that has relapsed or is refractory to chemotherapy, or that has relapsed after HSCT.

a) Refractory disease is defined as persistent disease after at least two courses of induction chemotherapy.
b) Patients with AML must have ≥ 5% leukemic blasts in the bone marrow or have converted from negative MRD status to positive MRD status in the bone marrow as assessed by flow cytometry. If an adequate bone marrow sample cannot be obtained, patients may be enrolled if there is unequivocal evidence of leukemia in the peripheral blood.

3.1.2 Participant is ≤ 21 years of age (i.e., has not reached 22nd birthday).

3.1.3 Adequate organ function defined as the following:

- Total bilirubin ≤ ULN for age, or if total bilirubin is > ULN, direct bilirubin is ≤ 1.5 mg/dL
- AST (SGOT)/ALT (SGPT) < 5 x ULN
- Calculated creatinine clearance > 50 ml/min/1.73m^2 as calculated by the Schwartz formula for estimated glomerular filtration rate
- Left ventricular ejection fraction ≥ 40% or shortening fraction ≥ 25%.

3.1.4 Has an available HPC-A donor.

3.1.5 Performance status: Lansky ≥ 50 for patients who are ≤ 16 years old and Karnofsky ≥ 50% for patients who are > 16 years old (Appendix I)

3.1.6 Does not have an uncontrolled infection requiring parenteral antibiotics, antivirals, or antifungals within one week prior to first dose. Infections controlled on concurrent anti-microbial agents are acceptable, and anti-microbial prophylaxis per institutional guidelines is acceptable.

3.1.7 Patient has fully recovered from the acute effects of all prior therapy and must meet the following criteria.

- At least 14 days must have elapsed since the completion of myelosuppressive therapy.

- At least 24 hours must have elapsed since the completion of low-dose or non-myelosuppressive therapy, such as hydroxyurea or low-dose cytarabine (up to 200 mg/m^2/day).
- At least 30 days must have elapsed since the use of investigational agents.
- For patients who have received prior HSCT, there can be no evidence of GVHD and greater than 60 days must have elapsed since the HSCT. Patients cannot be receiving therapy, including steroids, for GVHD. All such medications must be discontinued at least 72 hours prior to enrollment.

3.1.8 Post-menarchal female has had negative serum pregnancy test within 7 days prior to enrollment.

3.1.9 Male or female of reproductive potential has agreed to use effective contraception for the duration of study participation.

3.1.10 Not breastfeeding

3.2 Inclusion Criteria - HPC-A Cell Donor

3.2.1 At least 18 years of age.

3.2.2 Family member (first degree relative).

3.2.3 Not pregnant as confirmed by negative serum or urine pregnancy test within 7 days prior to enrollment (if female).

3.2.4 Not breast feeding.

3.2.5 Meets donation eligibility requirements as outlined by 21 CFR 1271.

3.3 Enrollment On Study at St. Jude

A member of the study team will confirm potential participant eligibility as defined in Section 3.1-3.2, complete and sign the Participant Eligibility Checklist. The study team will enter the eligibility checklist information into the Patient Protocol Manager (PPM) system. Eligibility will be reviewed, and a research participant-specific consent form and assent document (where applicable) will be generated. The complete signed consent/assent form(s) must be faxed or emailed to the CPDMO at [redacted] to complete the enrollment process.

The CPDMO is staffed 7:30 am-5:00 pm CST, Monday through Friday. A staff member is on call Saturday, Sunday, and holidays from 8:00 am to 5:00 pm. Enrollments may be requested during weekends or holidays by calling the CPDMO "On Call" cell phone [redacted] or referencing the "On Call Schedule" on the intranet).

4.0 TREATMENT PLAN

4.1 Overview

Patients will receive chemotherapy followed by infusion of donor HPC-A. All patients will be transferred to the BMT service prior to the infusion of HPC-A. The first 6 patients enrolled on the trial will remain inpatient until the time of graft rejection.

4.2 Treatment Administration

General assumptions regarding chemotherapy administration for all treatment phases:

- The timing and duration for administration for all commercially available agents are provided in the treatment phase sections as guidelines only. Variations in the timing and duration of chemotherapy infusions according to institutional practice or variations based on patient care needs are acceptable, as long as the treating investigator and/or PI determines that there was no impact on patient safety. These variations will not be considered protocol deviations, as long as the total dose is given within 10% of protocol specified dose.
- The term “day” does not refer to an absolute calendar day. It refers to a general 24-hour period as per St. Jude Nursing P&P.
- Medication dosing may be modified for research recipients based upon actual body weight or adjusted ideal body weight when clinically indicated. Criteria for medication calculations based on body weight/body surface area can be found in any version of the St. Jude Formulary. Medication doses may be rounded to the nearest integer or to the nearest appropriate quantity when clinically or pharmaceutically indicated as per the M.D. and Pharm.D.

4.2.1 Intrathecal Treatment

Diagnostic lumbar puncture and intrathecal (IT) chemotherapy will be given prior to each cycle of therapy. Patients with no evidence of CNS disease (CNS1: no leukemic blast cells on CSF cytospin) will receive no further IT therapy during cycle 1. Patients with < 5 leukocytes per µl of CSF and the presence of leukemic blast cells on CSF cytospin (CNS2) will receive weekly intrathecal therapy until the CSF is free of blast cells. Patients with overt CNS leukemia (CNS3: ≥ 5 leukocytes per µl of CSF and the presence of leukemic blast cells on CSF cytospin) will receive weekly intrathecal therapy until the CSF is free of blast cells (minimum number of doses, 4).

Patient Age	Methotrexate	Hydrocortisone	Cytarabine	Volume
< 1 year	6 mg	12 mg	18 mg	6 ml
1-2 years	8 mg	16 mg	24 mg	8 ml
2-3 years	10 mg	20 mg	30 mg	10 ml
> 3 years	12 mg	24 mg	36 mg	12 ml

Leucovorin rescue (5 mg/m^2/dose, max 5 mg) PO will be given at 24 and 30 hours after each triple intrathecal treatment. Follow plasma methotrexate levels (starting 24 hours after intrathecal therapy and until level becomes undetectable) in patients with renal dysfunction or extra fluid in third space, and rescue with leucovorin according to PharmD recommendation.

4.2.2 Cycle 1

Drug/Procedure	Dose	Route	No. doses	Schedule
Cytarabine	2 g/m^2/dose	IV over 3 hours Q12 hours	6	Days -4 through -2
Donor HPC-A infusion			1	Day 0 (± 1 day)

All participants should undergo response evaluations at the time of count recovery (ANC >0.3 x 10^9/L and platelet count >30 x 10^9/L) after each course of therapy. If counts have not recovered by day 29 of cycle 1, bone marrow aspiration (BMA) should be performed to evaluate for persistent leukemia. In addition to bone marrow aspiration, bone marrow biopsy should be considered to evaluate cellularity if clinically indicated. In cases with hypocellular marrows (<10 % cellularity), repeat bone marrow examination should be considered if feasible. If multiple bone marrow examinations are performed after a course of therapy, the last examination will be used to classify the response to that course.

Participants who have at least a partial response to Cycle 1 are eligible to receive Cycle 2.

4.2.3 Cycle 2

Cycle 2 may begin after count recovery from Cycle 1 (WBC $\geq 1000/mm^3$, ANC $\geq 500/mm^3$ and platelet count $\geq 30 \times 10^9/L$) and is identical to Cycle 1

Bone marrow aspirate and MRD will be performed following count recovery or on day 29 (whichever comes first): ANC $\geq 300/mm^3$ and platelet count $\geq 30 \times 10^9/L$.

4.2.4 Cellular Infusion Procedures and Monitoring

For the proper infusion procedures and monitoring of the HPC product, refer to BMT&CT SOP 40.02 "Hematopoietic Progenitor Cell Infusion – FRESH (Allogeneic): IV Push and IV Drip" or SOP 40.03 "Hematopoietic Progenitor Cell Infusion – FROZEN: IV Push". All relevant SOPs can be found on the BMT&CT Clinical Transplant Program intranet page:
http://home.web.stjude.org/bone_marrow/clinicalHome.shtml

During the cellular infusions, monitoring of vital signs, breath sounds, heart rate, pulse oximetry, and I/O will be done per the established nursing procedure, as well as appropriate Department of BMT&CT SOPs, and then documented on the Cellular Product Infusion Record. If a reaction is suspected at any time during the infusion, the nurse will stop the infusion and notify the attending physician but will not discard the product until physician orders are given. Proper documentation (symptoms of patient, vital signs, actions taken, outcome, and follow-up) will be completed in the Cellular Product Infusion Record.

The HPC-A infusion may be delayed by approximately 24 hours in order to accommodate stem cell collection with the donor, the Blood Donor Center and/or HAL as well as the research participant clinical condition.

4.3 Mobilization and Apheresis of Donor HPC-A

4.3.1 Donor Selection

If more than one family member donor is acceptable, then donor selection will be based on the preference of the primary attending. Factors in selection will include donor-recipient matching of CMV serology, donor-recipient red blood cell compatibility, degree of HLA matching, size of the potential donor, previous use as a donor, presence of donor-specific antibody, and overall health of the potential donor.

Donor eligibility for cell collection will be determined through the guidelines outlined in 21 CFR 1271 and the Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps). Potential donors will undergo an initial screening process that will include at least a complete physical exam, history and testing for relevant communicable diseases. Physical exams to evaluate donor candidacy will be conducted by a non-Department of BMT&CT physician

(St. Jude or non-St. Jude). For subsequent therapeutic cell collection procedures, if a complete screening procedure has been performed within the previous 6 months, an abbreviated donor screening procedure may be used for these repeat donations. The abbreviated screening procedure must determine and document any changes in the donor's medical history since the previous donation that would make the donor ineligible, including changes in relevant social behavior.

If a donor is determined to be ineligible, the donor is not automatically excluded. Part 21 CFR 1271.65 allows use of ineligible donors who are first or second degree blood relatives. In this situation, the physician will follow BMT&CT SOP 30.08.00, "Statement of Acknowledgment and Consent to Receive Stem Cells or Bone Marrow from a Donor with Abnormal Findings." Recipients or their legal guardians will be informed of the use of an ineligible donor.

4.3.2 Donor Mobilization and Graft Collections

G-CSF mobilized HPC-A products will be used for this study. Donors will undergo a standard hematopoietic stem cell mobilization regimen consisting of 5 days of G-CSF given subcutaneously at 10 μg/kg. HPC-A will be collected by leukapheresis on day 6. The dose of G-CSF may require modification based on the donor's white blood cell (WBC) count. If the donor's WBC count is > 75 x 10^6/ml, the dose of G-CSF will be reduced. The guidelines for dose modification can be found in the St. Jude Children's Research Hospital Department of BMT&CT SOP 30.06.00 "The practice for the evaluation, preparation and care of allogeneic and autologous donors mobilized with growth factor."

4.3.3 Quality Assurance for Cellular Products

The Department of Therapeutic Production and Quality has established an independent division of Quality Assurance (QA). This group is responsible for the management of Quality Control, Quality Assurance and Quality Improvement processes for the Human Applications Laboratory. Production and QA Systems include Standard Operation Procedures (SOPs) for production and quality processes, Documentation of Donor Eligibility, Documentation of processes captured in Batch Records, In-process quality control testing including sterility, Release Specifications established for all products, Out of Specification Reporting and Investigation Process, Authorization by QA for the release of all products after review of records and release specification test results, Product labeling procedures with multiple person review, Variance Management Process, Personnel Competence and Proficiency Program, and Inventory control and documentation of product history through patient infusion.

Test results that are out of specification for products that are needed on a clinically urgent basis will be evaluated by the laboratory medical director. The patient's physician or attending transplant physician will be informed of the test result prior to infusion of the product. Notification regarding positive sterility results before or after infusion will be given to the primary attending physician and patient and/or parent/guardian. Notification

to the FDA and St. Jude IRB will be given and will include testing results or adverse events and any required intervention. An investigation following TPQ/HAL SOPs will be completed, reviewed by TPQ Quality Assurance and outcomes of the investigation reported.

4.3.4 Dose of Cellular Product

Each donor will undergo leukapheresis one time. Based on current BMT&CT protocols and common clinical practice, the minimum cell dose that will be infused is 1 x 10^8 total nucleated cells (TNC)/kg and the maximum is 5 x 10^8 TNC/kg. In addition, the maximum number of CD3+ cells infused is 2 x 10^8 cells/kg. In general, patients will receive 2 cycles of therapy on the MITREL study. Thus, if the apheresis product contains at least 2 x 10^8 TNC/kg, the product will be split into 2 equal aliquots; one aliquot will be given after cycle 1 and the other will be frozen and given after cycle 2, with a maximum dose of 5 x 10^8 TNC/kg for each infusion. In the unlikely event that the apheresis product contains less than 1 x 10^8 TNC/kg, the product will not be infused. If the product contains 1-2 x 10^8 TNC/kg, the entire product will be given after cycle1 and the patient will not receive cycle 2. Guidelines for product infusion:

- < 1 x 10^8 TNC/kg: HPC-A not infused
- 1-2 x 10^8 TNC/kg: entire HPC-A infused after cycle 1
- ≥ 2 x 10^8 TNC/kg: 50% of HPC-A infused after each cycle (max dose, 5 x 10^8 TNC/kg per infusion)

4.4 Definitions of Unacceptable Toxicities

Toxicities will be graded according to the CTEP Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Grade 3 and 4 hematologic toxicities will be monitored, but will not be considered unacceptable toxicity except as described below. GVHD will be assessed and graded according to the Children's Oncology Group (COG) Stem Cell Committee Consensus Guidelines for Establishing Organ Stage and Overall Grade of Acute Graft Versus Host Disease (GVHD) – see Appendix II.

Death from causes other than leukemia progression, ≥ Grade 3 acute GVHD that is associated with detectable MITREL donor chimerism, and persistent engraftment defined as > 5% donor chimerism at the time of count recovery (ANC > 0.3 x 10^9/L and platelet count > 30 x 10^9/L) will be considered unacceptable toxicities. Acute GVHD that is not associated with detectable MITREL donor chimerism, but that is only associated with detectable donor chimerism from a previous HSCT, will be recorded but will not be considered an unacceptable toxicity. As described in Section 12.1, six patients will be enrolled and evaluated for toxicity. If two or more patients experience unacceptable toxicity during or after completion of two cycles of therapy, then the cohort will close due to intolerability.

4.5 Concomitant Therapy and Supportive Care

Antineoplastic therapy: Concurrent cancer therapy, including chemotherapy, immunotherapy, or biologic therapy is prohibited.

Investigational agents: No investigational agents may be given while the patient is on protocol therapy.

Growth factors: The routine use of filgrastim (G-CSF, Neupogen®) or other growth factors is discouraged, but may be used in cases of documented infection or sepsis during periods of neutropenia, according to institutional practice.

Conjunctivitis prophylaxis: Dexamethasone or prednisolone ophthalmic solution or artificial tears (e.g., hydroxymethylcellulose, hypromellose, polyvinyl alcohol), 2 drops in each eye every 4 hours while awake, should be used during cytarabine administration and for 24 hours after completion to prevent conjunctival irritation.

Prophylaxis and treatment of metabolic derangement: Care should be taken to prevent hyperuricemia and hyperphosphatemia in participants with large tumor burdens. Such participants should receive IV hydration at 1500-3000 mL/m^2/day before the initiation of therapy, oral phosphate binders, and recombinant urate oxidase or allopurinol as needed.

Prophylaxis for *Pneumocystis jiroveci* pneumonia: All participants should receive TMP/SMZ (trimethoprim 150 mg/m^2/day in 2 divided doses on Monday, Tuesday, and Wednesday of each week). For those who cannot tolerate TMP/SMZ, monthly pentamidine may be substituted. Other options include atovoquone or dapsone. Please consult with clinical pharmacists and local institutional guidelines.

Prophylaxis for fungal infections: Because patients with relapsed leukemia are at high risk for fungal infections, all participants should receive antifungal prophylaxis according to institutional guidelines. Prophylaxis with voriconazole, posaconazole, micafungin, or caspofungin is acceptable.

Prophylaxis for bacterial infections: Because patients with relapsed leukemia are also at high risk for bacterial infections, all participants should receive antibiotic prophylaxis according to institutional guidelines. Prophylactic antibiotics should be started when the ANC $\leq$ 1000 and falling or predicted to fall and should be continued until the ANC $\geq$ 100 and rising. The recommended prophylactic regimen is intravenous vancomycin plus oral ciprofloxacin. An acceptable alternative regimen is oral or intravenous levofloxacin.

Management of febrile neutropenia: Participants with fever (defined as a single oral temperature $\geq$ 38.3° C (101°F) or temperature of $\geq$ 38.0° C (100.4° F) sustained over a one hour period and neutropenia (defined as ANC < 500 cells/µL) should be given IV antibiotics immediately..

5.0 DRUG/DEVICE/BIOLOGIC AGENT INFORMATION

5.1 Cytarabine (Cytosine arabinoside, Ara-C, Cytosar®)

Source and pharmacology: Cytarabine is a deoxycytidine analogue. It must be tri-phosphorylated to its active form, ARA-CTP, by deoxycytidine kinase and other nucleotide kinases. Ara-CTP inhibits DNA polymerase. In addition, ara-CTP is incorporated into DNA as a false base, causing inhibition of DNA synthesis. It is cell cycle, S phase specific. Cytarabine does penetrate the blood brain barrier. It is converted to its inactive form, uracil arabinoside, by pyrimidine nucleoside deaminase. Approximately 80% of the dose is recovered in the urine, mostly as uracil arabinoside (ara-U).

Formulation and stability: Cytarabine is available in multi-dose vials containing 100, 500, 1000 and 2000mg of lyophilized drug. Intact vials can be stored at room temperature. *For IV use,* either sterile water for injection or bacteriostatic water for injection can be used to reconstitute the lyophilized drug. *For intrathecal use,* only sterile water for injection should be used for reconstitution. The 100 and 500 mg vials are reconstituted with 2 and 10 ml respectively resulting in a final concentration of 50 mg/ml. The 1000 and 2000 mg vials are reconstituted with 20 ml and 40 ml respectively resulting in a final concentration of 50 mg/ml. After reconstitution, the drug is stable for 8 days at room temperature.

Supplier: Commercially available.

Toxicity: Myelosuppression is the dose limiting adverse effect, with leukopenia and thrombocytopenia being predominant. Other adverse effects reported commonly include nausea and vomiting (may be severe at high doses), diarrhea, mucositis, anorexia, alopecia, skin rash and liver dysfunction. A flu-like syndrome characterized by fever, muscle and bone aches is common. Less common side effects include allergic reactions and cellulitis at the injection site. High doses of cytarabine can cause conjunctivitis, hepatitis, and a group of CNS symptoms including somnolence, peripheral neuropathy, ataxia, and personality changes. CNS symptoms are usually reversible and are more common in patients who have received previous cranial irradiation. In addition, a syndrome of sudden respiratory distress progressing to pulmonary edema has occurred.

Guidelines for administration: Intrathecal and intravenous. See treatment administration sections.

5.2 G-CSF (Filgrastim) (Neupogen®)

Source and pharmacology: G-CSF (granulocytic colony stimulating factor), is a biosynthetic hematopoietic agent that is made using recombinant DNA technology in cultures of *Escherichia coli.* G-CSF stimulates production, maturation and activation of neutrophils. In addition, endogenous G-CSF enhances certain functions of mature neutrophils, including phagocytosis, chemotaxis and antibody--dependent cellular cytotoxicity.

Formulation and stability: G-CSF is supplied in vials containing 300 mcg and 480 mcg of G-CSF at a concentration of 300 mcg/ml. The intact vials should be stored under refrigeration. The vials can be left out of refrigeration for 24 hours, but should be discarded if left at room temperature for longer periods of time. G-CSF can be drawn up into tuberculin syringes for administration and stored under refrigeration for up to 7 days prior to usage. G-CSF can be further diluted for IV infusion in 5% dextrose. Do not dilute in saline-precipitate may form. If the final concentration of this product is < 15 mcg/ml, it is recommended that albumin be added to a final concentration of 2mg/ml (0.2%) to minimize adsorption of the drug to infusion containers and equipment.

Supplier: Commercially available

Toxicity: G-CSF causes marked leukocytosis. Adverse reactions reported commonly include bone pain, thrombocytopenia, diarrhea, nausea, rash, alopecia, fever, anorexia and pain or bruising at the injection site. Allergic reactions, MI, atrial fibrillation, and splenomegaly have been reported rarely. G-CSF is contraindicated in participants with allergy to E. coli derived products.

Dosage and route of administration: Donors: 10 mcg/kg/day for standard hematopoietic stem cell mobilization. See Section 4.3.2.

5.3 Intrathecal Triples

(ITMHA, methotrexate/hydrocortisone/cytarabine)

Source and pharmacology**:** The intrathecal route of administration of a drug produces more consistent CSF drug concentrations at relatively smaller doses because of the volume difference between the CSF and blood compartments (140 mL vs. 3500 mL in an adult). (The CSF volume of children after the first 3 years is equivalent to that of an adult). Drug half-lives are longer as well because clearance is related to flow rather than metabolism or protein binding. Intrathecal methotrexate has a biphasic elimination curve from the CSF with a t½ of 4.5 and 14 hours respectively. Following IT injection of cytarabine the elimination of the drug from the CSF is biphasic with a t½ of 1 and 3.4 hours respectively which is 8-fold longer than the clearance from plasma. The elimination of hydrocortisone is similarly prolonged.

Formulation and stability: Methotrexate 25 mg/mL preservative free 2 mL vial or methotrexate 20 mg preservative free sterile powder for injection vial. Cytarabine 100 mg preservative free sterile powder for injection. Hydrocortisone sodium succinate100 mg vial sterile powder for injection.

Toxicity: Nausea, vomiting, fever, headache.

Guidelines for administration: Intrathecal. See Section 4.2.1.

6.0 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS

All entry/eligibility studies must be performed within 2 weeks prior to study enrollment (unless otherwise specified). Reasonable adjustments can be made in the schedule to allow for weekends or holidays.

6.1 Pre-Treatment Clinical Evaluations

- Physical exam, height, weight, BSA
- CBC with differential
- Chemistry profile: glucose, electrolytes, BUN, creatinine, LDH, uric acid, bilirubin, SGOT, SGPT, calcium, phosphorous, magnesium, total protein and albumin
- Coagulation screen
- HLA typing, if not done previously
- Chest x-ray, as clinically indicated
- EKG, echocardiogram
- Bone marrow evaluation for morphology, immunophenotyping, cytogenetics, molecular diagnosis, and minimal residual disease (MRD). Morphologic examination and MRD are required for all patients. Immunophenotyping, cytogenetic analysis, and molecular analysis should be performed as clinically indicated (e.g., if not done at the time of relapse). If an adequate bone marrow specimen cannot be obtained, all diagnostic studies may be performed on blood rather than bone marrow.
- Lumbar puncture with CSF cell count and cytology.
- Serum pregnancy test of females of childbearing potential

6.2 Evaluations During Study Treatment

STUDIES TO BE OBTAINED	Timing
Physical exam with vital signs	Weekly
Height, weight, BSA	Weekly
CBC with differential	Weekly
Chemistry profile[1]	Weekly
Bone marrow evaluation[2]	Day 29 of each cycle[2]
Lumbar puncture with CSF cell count and cytology	Prior to each cycle and with every dose of IT therapy
Serum pregnancy test of females of childbearing potential	Prior to each cycle
Microchimerism (blood)	Weekly follow infusion of HPC-A
Microchimerism (bone marrow)	Day 29 of each cycle[2]
Detection of PR1+ and WT1+ HLA-A*02:01 CD8 T cells (blood)	Days 15 and 29 of each cycle

[1]Chemistry profile: glucose, electrolytes, BUN, creatinine, LDH, uric acid, bilirubin, SGOT, SGPT, calcium, phosphorous, magnesium, total protein and albumin
[2]Bone marrow evaluation for morphology and minimal residual disease (MRD) should be performed at the time of count recovery (ANC > 0.3 x 10^9/L and platelet count > 30 x 10^9/L) after each course of therapy. If counts have not recovered by day 29, bone marrow evaluation should be performed to evaluate for persistent leukemia.

Obtain other studies as needed for good patient care.

6.3 After Completion of Therapy

When a participant discontinues the study, a final visit will be conducted. Following discontinuation of the study treatment, the participant will be treated according to the investigator's discretion. If a participant discontinues from the study due to an adverse event considered related to study treatment, a follow-up visit should be conducted no later than 30 days after the last dose of protocol therapy. Safety assessments are recommended at least every 30 days, until all toxicities resolve, return to baseline or become clinically satisfactory, stable, or are considered irreversible.

6.4 Exploratory Research Studies

Dr. Shurtleff's laboratory will use a quantitative PCR assay (sensitivity of 0.008%) to measure donor chimerism following infusion of HPC-A.[22] Patients who have HLA-A*02:01 or whose donors have HLA-A*02:01 will be monitored for PR1+ and WT1+ HLA-A*02:01 CD8 T cells in peripheral blood before and after microtransplantation.[20,21]

7.0 EVALUATION CRITERIA

7.1 Response Criteria

Because morphologic examination of the bone marrow during periods of hematopoietic recovery after intensive chemotherapy may be unreliable, bone marrow response will be based on blast percentage by flow cytometry. Blast percentages determined by morphology will be used in cases that are not evaluable by flow cytometry.

Note that the following criteria apply to patients who have ≥ 5% blasts at the time of enrollment.

7.1.1 Complete remission (CR)

- Bone marrow with < 5% blasts confirmed by flow cytometry
- ANC ≥ 500/µL and platelets ≥ 75,000/µL without transfusions
- No evidence of extramedullary disease

7.1.2 Complete remission with incomplete blood count recovery (CRi)

- Bone marrow with < 5% blasts confirmed by flow cytometry
- ANC < 500/µL or platelets < 75,000/µL without transfusions
- No evidence of extramedullary disease

7.1.3 Partial response (PR)

- Bone marrow with 5% to 25% blasts by flow cytometry and a decrease of at least 50% in blast percentage
- No evidence of extramedullary disease

7.1.4 No response (NR)
Participant fails to qualify for any of the categories listed above

7.1.5 Relapse
Subsequent appearance, after achievement of CR, of ≥ 5% blasts in the bone marrow with confirmation by flow cytometry or the development of extramedullary disease after achievement of CR

For patients who have < 5% blasts at the time of enrollment, "therapeutic success" is defined as ≥ 10-fold decrease in MRD level.

7.2 Toxicity Evaluation Criteria

Common Terminology Criteria for Adverse Events v4 (CTCAE): This study will utilize the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the current version of the CTCAE can be downloaded from the Cancer Therapy Evaluation Program (CTEP) home page (http://ctep.info.nih.gov). Additionally, toxicities will be reported on the appropriate data collection screens.

GVHD will be assessed and graded according to the Children's Oncology Group (COG) Stem Cell Committee Consensus Guidelines for Establishing Organ Stage and Overall Grade of Acute Graft Versus Host Disease (GVHD) – see Appendix II.

8.0 OFF THERAPY AND OFF-STUDY CRITERIA

8.1 Off-Therapy Criteria

- No response to therapy
- Relapse
- Second malignancy
- Treatment with other antineoplastic therapy or hematopoietic stem cell transplantation
- Development of unacceptable toxicity
- Refusal of further protocol therapy by participant, parent, or guardian
- Completion of protocol therapy

8.2 Off-Study Criteria (Recipient)

- Death
- Lost to follow up
- Withdrawal of consent

8.3 Off-Study Criteria (Donor)

- Donors will be considered off study 7 days after apheresis

9.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

9.1 Reporting Adverse Experiences and Deaths to St. Jude IRB

Only "unanticipated problems involving risks to participants or others" referred to hereafter as "unanticipated problems" are required to be reported to the St. Jude IRB promptly, but in no event later than 10 working days after the investigator first learns of the unanticipated problem. Regardless of whether the event is internal or external (for example, an IND safety report by the sponsor pursuant to 21 CFR 312.32), only adverse events that constitute unanticipated problems are reportable to the St. Jude IRB. As further described in the definition of unanticipated problem, this includes any event that in the PI's opinion was:

- Unexpected (in terms of nature, severity, or frequency) given (1) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, as well as other relevant information available about the research; (2) the observed rate of occurrence (compared to a credible baseline for comparison); and (3) the characteristics of the subject population being studied; and

- Related or possibly related to participation in the research; and
- Serious; or if not serious suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unrelated, expected deaths do not require reporting to the IRB. Though death is "serious", the event must meet the other two requirements of "related or possibly related" and "unexpected/unanticipated" to be considered reportable.

Deaths meeting reporting requirements are to be reported immediately to the St. Jude IRB, but in no event later than 48 hours after the investigator first learns of the death. The following definitions apply with respect to reporting adverse experiences:

Serious Adverse Event: Any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include: any substantial disruption of the ability to conduct normal life functions, allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse), a congenital anomaly/birth defect, secondary or concurrent cancer, medication overdose, or is any medical event which requires treatment to prevent any of the medical outcomes previously listed.

Unexpected Adverse Event:

- Any adverse event for which the specificity or severity is not consistent with the protocol-related documents, including the applicable investigator brochure, IRB approved consent form, Investigational New Drug (IND) or Investigational Device Exemption (IDE) application, or other relevant sources of information, such as product labeling and package inserts; or if it does appear in such documents, an event in which the specificity, severity or duration is not consistent with the risk information included therein; or
- The observed rate of occurrence is a clinically significant increase in the expected rate (based on a credible baseline rate for comparison); or

The occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Internal events: Events experienced by a research participant enrolled at a site under the jurisdiction of St. Jude IRB for either multicenter or single-center research projects.

Unanticipated Problem Involving Risks to Subjects or Others: An unanticipated problem involving risks to subjects or others is an event which was not expected to occur and which increases the degree of risk posed to research participants.

Such events, in general, meet all of the following criteria:

- unexpected;
- related or possibly related to participation in the research; and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. An unanticipated problem involving risk to subjects or others may exist even when actual harm does not occur to any participant.

Although some adverse events will qualify as unanticipated problems involving risks to subjects or others, some will not; and there may be other unanticipated problems that go beyond the definitions of serious and/or unexpected adverse events. Examples of unanticipated problems involving risks to subjects or others include:

- Improperly staging a participant's tumor resulting in the participant being assigned to an incorrect arm of the research study;
- The theft of a research computer containing confidential subject information (breach of confidentiality); and
- The contamination of a study drug.

Unanticipated problems generally will warrant consideration of substantive changes in the research protocol or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of subjects or others.

9.2 Recording and Reporting AEs and SAEs

Adverse events (AEs) will be evaluated and documented by the clinical staff and investigators throughout inpatient hospitalizations and each outpatient visit. CRAs are responsible for reviewing documentation related to AEs and entering directly into CRIS protocol-specific database. The data to be recorded are 1) the event description, 2) the NCI CTCAE v4.0 code and grade, 3) the onset date, 4) the resolution date (or ongoing), 4) action taken for event, 5) patient outcome 6) relationship of AE to protocol treatment/interventions, 7) if AE was expected or unexpected, and 8) comments, if applicable. AEs that are classified as serious, unexpected, and at least possibly related will be notated as such in the database as "Reportable Events".

Attribution of an Adverse Event

Not related - The lack of a temporal relationship of the event to study treatment makes a causal relationship not reasonably possible, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation.

Unlikely related - The temporal relationship of the event to study treatment makes a causal relationship reasonably unlikely, and other drugs, therapeutic interventions or underlying conditions may not provide sufficient explanation for the observed event. Possibly related – The temporal relationship of the event to study treatment makes a causal relationship reasonably possible, and the event is more likely explained by exposure to the study treatment than by other other drugs, therapeutic interventions or underlying conditions.

For the purpose of safety analyses, all AE's that are classified as unlikely or possible will be considered treatment-related events.

These events will be reported expeditiously to the St. Jude IRB within the timeframes as described above. Cumulative summary of Grades 3-5 events will be reported as part of the progress reports to IRB at the time of continuing review. Specific data entry instructions for AEs and other protocol-related data will be documented in protocol-specific data entry guidelines, which will be developed and maintained by study team and clinical research informatics.

Patients with abnormal blood counts due to bone marrow involvement by disease (i.e. all leukemia patients and lymphoma patients with bone marrow involvement) will be considered non-evaluable for hematological toxicities.

The study team will meet regularly to discuss AEs (and other study progress as required by institutional DSMP). The PI will review Adverse Event reports generated from the research database, and corrections will be made if applicable. Once the information is final the PI will sign and date reports, to acknowledge his/her review and approval of the AE as entered in the research database.

10.0 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

10.1 Data Collection

Electronic case report forms (e-CRFs) will be completed by the clinical trials staff from the Cancer Center Comprehensive Center, Hematological Malignancies Program. Data will be entered from record directly into a secure CRIS database, developed and maintained by St. Jude Clinical Research Informatics.

Data Management will be supervised by the Director of Clinical Trials Management, and Manager of Clinical Research Operations for the Hematological Malignancies Program, working with Dr. Rubnitz or his designee. All protocol-specific data and all grade 3-5

adverse events will be recorded by the clinical research associates into the CRIS database, ideally within 2-4 weeks of completion of study phase. All questions will be directed to the attending physician and/or PI and reviewed at regularly-scheduled working meetings. The attending physicians (or their designees) are responsible for keeping up-to-date roadmaps in the patient's primary SJCRH medical chart. Regular (at least monthly) summaries of toxicity and protocol events will be generated for the PI and the department of Biostatistics to review.

10.2 Study Monitoring

Monitoring of this protocol is considered to be in the moderate-risk category. The Monitoring Plan is outlined in a separate document from this protocol, but has been submitted for review and approval by the Clinical Trials Scientific Review Committee and the Institutional Review Board.

The study team will hold monthly meetings and review case histories or quality summaries on active participants.

Source document verification of eligibility and informed consent for 100% of St. Jude participants will be performed by the Eligibility Coordinators within 5 working days of completion of enrollment.

The Clinical Research Monitor will monitor applicable essential regulatory documentation and review the timeliness of serious adverse event reporting (type, grade, attribution, duration, timeliness and appropriateness) for selected study participants *semi-annually* and track accrual continuously. The monitor will verify those data points relating to the primary study objective for a certain number of study enrollees as specified in the Moderate Risk monitoring plan checklist for this study. Protocol compliance monitoring will include participant status, safety assessments, eligibility, the informed consent process, participant protocol status, off-study, and off-therapy criteria. The Monitor will generate a formal report which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC). Monitoring may be conducted more frequently if deemed necessary by the CPDMO or the IMC.

Continuing reviews by the IRB and CT-SRC will occur at least annually. In addition, SAE reports in TRACKS (Total Research and Knowledge System) are reviewed in a timely manner by the IRB/ OHSP.

Source document verification of eligibility for all SJCRH cases will be performed within two weeks of completion of enrollment. This will include verification of appropriate documentation of consent. Monitoring of timeliness of serious adverse event reporting will be done as events are reported in TRACKS.

10.3 Confidentiality

Study numbers will be used in place of an identifier such as a medical record number. No research participant names will be recorded on the data collection forms. The list containing the study number and the medical record number will be maintained in a locked file and will be destroyed after all data have been analyzed. The medical records of study participants may be reviewed by the St. Jude IRB, FDA, and clinical research monitors.

11.0 STATISTICAL CONSIDERATIONS

11.1 Study Design

The primary objectives of this protocol are to evaluate the tolerability and response rate of microtransplantation in patients with relapsed or refractory AML or MDS

We will use a cohort of six patients to evaluate the tolerability of the treatment regimen. If two or more patients experience unacceptable toxicity as defined in Section 4.4, then the trial will close due to intolerability (section 4.4). The first six patients will be enrolled sequentially; a patient may not be enrolled until the previous patient has reached day +28 after the infusion of HPC-A.

We will use a Simon minimax two-stage phase II design[23] to evaluate efficacy. All patients (including those used in evaluation of tolerability) will be counted towards this two-stage design. For implementing this design, we define a therapeutic success as follows. For patients with fewer than 5% blasts at enrollment, therapeutic success is defined as ≥ 10-fold decrease in level of minimal residual disease (MRD) after one or two cycles of therapy. A therapeutic success for patients with greater than 5% leukemic blasts in the marrow at the time of enrollment is defined as achieving CR or CRi (as defined in section 8.1) after one or two cycles of therapy. Any patient who dies before achieving therapeutic success will be counted as a failure in terms of efficacy. All patients who receive at least one dose of protocol chemotherapy will be counted as a failure or success in terms of efficacy for this phase II design. Only subjects who withdraw or die prior to receiving the first dose of protocol chemotherapy will be considered inevaluable and replaced. Also, the evaluation of tolerability described above and this phase II design will be performed concurrently, i.e., the first enrollees will be counted for both tolerability and efficacy.

The first stage will enroll nine patients. If there are four or more therapeutic successes among the first nine patients, then ten additional patients will be enrolled. If there are eleven or more therapeutic successes among the total of 19 patients, then the therapy will be considered worthy of further investigation according to the statistical design. The design has 80% power at the 10% level to detect a desirable success rate of 65% against the null hypothesis of an unacceptable success rate of 40%.

The success rate parameters for the two-stage design are aligned with recent published experience of clinical trials.[16,24-27] Clinical trials enrolling relapsed pediatric AML patients report complete response rates ranging from 1/3 (33%) to 8/11 (73%), whereas those enrolling relapsed ALL patients report complete response rates ranging from 14/34 (41%) to 16/22 (73%). The wide variability in response rates on these studies is largely attributable to the heterogeneity of patients with respect to multiple risk factors including duration of previous remission presence of previous relapse. We will use the methods of Jung and Kim[28] and Koyama and Chen[29] to adjust for the two-stage design in estimating the confidence interval for the true success rate. The design was computed with the *ph2simon* function in the *clinfun* R package. The confidence interval for the response rate will be computed with the *twostage.inference* function of the same package.

All patients who receive at least one course of therapy from the treatment regimen will be considered evaluable for both toxicity and efficacy in this design. Any patients who die prior to observing a complete response will be counted as a failure in execution of the two-stage design.

Based on historical data, we anticipate that this study will be able to accrue 19 patients in 3 years.

11.2 Statistical Analysis Plans

Statistical analysis plans for each objective are described separately below.
Objective 1.1.1: To assess the safety and feasibility of standard chemotherapy plus GCSF-mobilized Hematopoietic Progenitor Cell, Apheresis (HPC-A) in pediatric patients with relapsed or refractory AML or MDS. (Statistician: Dr. Stanley Pounds, Investigator: Dr. Jeffrey Rubnitz)

As described above, the study design includes formal stopping criteria to close the study if two or more of the first 6 patients experience unacceptable toxicity as defined in Section 4.4. If this stopping rule is satisfied, then we will report that the protocol treatment has unacceptable toxicity and describe the toxicities observed. Otherwise, we will report descriptive statistics for various categories of toxicities and provide 95% confidence binomial intervals for the proportion of subjects experiencing various categories of toxicity during the first two courses.

Objective 1.1.2: To estimate the response rates to standard chemotherapy plus GCSF-mobilized HPC-A in pediatric patients with relapsed or refractory AML or MDS. (Statistician: Dr. Pounds, Investigator: Dr. Rubnitz).

The proportion of patients experiencing therapeutic success will be reported with a confidence interval that adjusts for the two-stage design as described above in section 12.1.

Objective 1.2.1: To describe the event-free and overall survival of patients treated with standard chemotherapy plus GCSF-mobilized HPC-A.

We will use the Kaplan-Meier method to describe the event-free and overall survival. Event-free survival will be defined as the time from enrollment to death, relapse, or refractory disease with event-free subjects' time censored at the date of last follow-up. Overall survival will be defined as the time from enrollment to death, with living subjects' time censored at the date of last follow-up.

Objective 1.2.2: To estimate the time to neutrophil and platelet recovery after treatment with standard chemotherapy plus GCSF-mobilized HPC-A. (Statistician: Dr. Pounds, Investigator: Dr. Rubnitz)

The time to neutrophil and platelet recovery will be summarized using descriptive statistics. If there are no deaths prior to recovery of neutrophils and platelets, nonparametric confidence intervals for the median time to recovery will be computed by inverting the sign test. Otherwise, we will compute cumulative incidence curves to describe the time to platelet and neutrophil recovery while adjusting for competing events.

Objective 1.2.3: To determine the cumulative incidence of acute and chronic graft-versus-host disease (GVHD). (Statistician: Dr. Pounds, Investigator: Dr. Rubnitz)

We will estimate the cumulative incidence of acute and chronic graft-versus host disease.

Objectives 1.3.1 and 1.3.2: To characterize donor chimerism and microchimerism and levels of PR1+ and WT1+ HLA-A*02:01 CD8 T cells in peripheral blood after microtransplantation(Statistician: Dr. Pounds, Investigator: Dr. Leung)

We will compute descriptive statistics for the donor chimerism and microchimerism percentages at each time point and produce graphics for visualization. Similar exploratory analyses will be performed for levels of PR1+ and WT1+ CD8+ T cells, as well as for associations between donor T cell dose and outcome.

12.0 OBTAINING INFORMED CONSENT

12.1 Consent Prior to Research Interventions

Initially, informed consent will be sought for the institutional banking protocol (TBANK research study), PGEN5, and for other procedures as necessary for standard medical care. During the screening process for eligibility, informed consent for SCREEN protocol OR for MITREL is required before any research tests are performed.

12.2 Consent at Enrollment

The process of informed consent for MITREL will follow institutional policy. The informed consent process is an ongoing one that begins at the time of diagnosis and ends after the completion of therapy. Informed consent should be obtained by the attending physician or his/her designee, in the presence of at least one non-physician witness. Initially, informed consent will be sought for the institutional banking protocol (research

study), blood transfusion and other procedures as necessary. After the diagnosis of relapsed or refractory leukemia is established, we will invite the patient to participate in the MITREL protocol.

Throughout the entire treatment period, participants and their parents receive constant education from health professionals at SJCRH and collaborating sites, and are encouraged to ask questions regarding alternatives and therapy. All families have ready access to chaplains, psychologists, social workers, and the St. Jude ombudsperson for support, in addition to that provided by the primary physician and other clinicians involved in their care.

We will also obtain verbal assent from children 7 to 14 years old and written assent for all participants >14 years of age.

12.3 Consent at Age of Majority

Participants who reach the age of majority while on study will be re-consented for continued participation on MITREL at the time of their next clinic visit after turning 18 year according to Cancer Center and institutional policy.

12.4 Consent When English is Not the Primary Language

When English is not the participant, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information will be documented in the participant's medical record. Either a certified interpreter or the telephone interpreter's service will be used to translate the consent information. The process for obtaining an interpreter and for the appropriate use of an interpreter is outlined on the Interpreter Services, OHSP, and CPDMO websites.

13.0 REFERENCES

1. Ringden O, Karlsson H, Olsson R, et al. The allogeneic graft-versus-cancer effect. *Br J Haematol*. 2009;147:614-33.
2. BARNES DW, CORP MJ, LOUTIT JF, et al. Treatment of murine leukaemia with X rays and homologous bone marrow; preliminary communication. *Br Med J*. 1956;2:626-7.
3. Odom LF, August CS, Githens JH, et al. Remission of relapsed leukaemia during a graft-versus-host reaction. A "graft-versus-leukaemia reaction" in man? *Lancet*. 1978;2:537-40.
4. Weiden PL, Flournoy N, Thomas ED, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med*. 1979;300:1068-73.
5. Weiden PL, Sullivan KM, Flournoy N, et al. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. *N Engl J Med*. 1981;304:1529-33.
6. Kolb HJ, Mittermuller J, Clemm C, et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood*. 1990;76:2462-5.
7. Levine JE, Barrett AJ, Zhang MJ, et al. Donor leukocyte infusions to treat hematologic malignancy relapse following allo-SCT in a pediatric population. *Bone Marrow Transplant*. 2008;42:201-5.
8. Velardi A, Ruggeri L, Mancusi A. Killer-cell immunoglobulin-like receptors reactivity and outcome of stem cell transplant. *Curr Opin Hematol*. 2012;19:319-23.
9. Leung W. Use of NK cell activity in cure by transplant. *Br J Haematol*. 2011;155:14-29.
10. Venstrom JM, Pittari G, Gooley TA, et al. HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1. *N Engl J Med*. 2012;367:805-16.
11. Cooley S, Weisdorf DJ, Guethlein LA, et al. Donor killer cell Ig-like receptor B haplotypes, recipient HLA-C1, and HLA-C mismatch enhance the clinical benefit of unrelated transplantation for acute myelogenous leukemia. *J Immunol*. 2014;192:4592-600.
12. Oevermann L, Michaelis SU, Mezger M, et al. KIR B haplotype donors confer a reduced risk for relapse after haploidentical transplantation in children with ALL. *Blood*. 2014;124:2744-7.
13. Knorr DA, Bachanova V, Verneris MR, et al. Clinical utility of natural killer cells in cancer therapy and transplantation. *Semin Immunol*. 2014;26:161-72.
14. Guo M, Hu KX, Yu CL, et al. Infusion of HLA-mismatched peripheral blood stem cells improves the outcome of chemotherapy for acute myeloid leukemia in elderly patients. *Blood*. 2011;117:936-41.
15. Guo M, Hu KX, Liu GX, et al. HLA-mismatched stem-cell microtransplantation as postremission therapy for acute myeloid leukemia: long-term follow-up. *J Clin Oncol*. 2012;30:4084-90.
16. Kaspers GJ, Zimmermann M, Reinhardt D, et al. Improved outcome in pediatric relapsed acute myeloid leukemia: results of a randomized trial on liposomal daunorubicin by the International BFM Study Group. *J Clin Oncol*. 2013;31:599-607.
17. Dey BR, McAfee S, Colby C, et al. Anti-tumour response despite loss of donor chimaerism in patients treated with non-myeloablative conditioning and allogeneic stem cell transplantation. *Br J Haematol*. 2005;128:351-9.
18. Rubio MT, Saito TI, Kattleman K, et al. Mechanisms of the antitumor responses and host-versus-graft reactions induced by recipient leukocyte infusions in mixed chimeras

prepared with nonmyeloablative conditioning: a critical role for recipient CD4+ T cells and recipient leukocyte infusion-derived IFN-gamma-producing CD8+ T cells. *J Immunol*. 2005;175:665-76.

19. Rubio MT, Kim YM, Sachs T, et al. Antitumor effect of donor marrow graft rejection induced by recipient leukocyte infusions in mixed chimeras prepared with nonmyeloablative conditioning: critical role for recipient-derived IFN-gamma. *Blood*. 2003;102:2300-7.
20. Rezvani K, Yong AS, Mielke S, et al. Leukemia-associated antigen-specific T-cell responses following combined PR1 and WT1 peptide vaccination in patients with myeloid malignancies. *Blood*. 2008;111:236-42.
21. Rezvani K, Yong AS, Savani BN, et al. Graft-versus-leukemia effects associated with detectable Wilms tumor-1 specific T lymphocytes after allogeneic stem-cell transplantation for acute lymphoblastic leukemia. *Blood*. 2007;110:1924-32.
22. Alizadeh M, Bernard M, Danic B, et al. Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. *Blood*. 2002;99:4618-25.
23. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials*. 1989;10:1-10.
24. Bhojwani D, Pui CH. Relapsed childhood acute lymphoblastic leukaemia. *Lancet Oncol*. 2013;14:e205-e217.
25. Schrappe M, Hunger SP, Pui CH, et al. Outcomes after induction failure in childhood acute lymphoblastic leukemia. *N Engl J Med*. 2012;366:1371-81.
26. Tallen G, Ratei R, Mann G, et al. Long-term outcome in children with relapsed acute lymphoblastic leukemia after time-point and site-of-relapse stratification and intensified short-course multidrug chemotherapy: results of trial ALL-REZ BFM 90. *J Clin Oncol*. 2010;28:2339-47.
27. Ko RH, Ji L, Barnette P, et al. Outcome of patients treated for relapsed or refractory acute lymphoblastic leukemia: a Therapeutic Advances in Childhood Leukemia Consortium study. *J Clin Oncol*. 2010;28:648-54.
28. Jung SH, Kim KM. On the estimation of the binomial probability in multistage clinical trials. *Stat Med*. 2004;23:881-96.
29. Koyama T, Chen H. Proper inference from Simon's two-stage designs. *Stat Med*. 2008;27:3145-54.

APPENDIX I: PERFORMANCE STATUS CRITERIA

PERFORMANCE STATUS CRITERIA *Karnofsky and Lansky performance scores are intended to be multiples of 10*					
ECOG (Zubrod)		**Karnofsky**		**Lansky**	
Score	**Description**	**Score**	**Description**	**Score**	**Description**
0	Fully active, able to carry on all pre-disease performance without restriction	100	Normal, no complaints, no evidence of disease	100	Fully active, normal
		90	Able to carry on normal activity, minor signs or symptoms of disease	90	Minor restrictions in physically strenuous activity
1	Restricted in physically strenuous activity by ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work	80	Normal activity with effort; some signs or symptoms of disease	80	Active, but tires more quickly
		70	Cares for self; unable to carry on normal activity or do active work	70	Both greater restriction of and less time spent in play activity
2	Ambulatory and capable of self-care but unable to carry out any work activities; up and about more than 50% of waking hours	60	Requires occasional assistance, but is able to care for most of his/her needs	60	Up and around, but minimal active play; keeps busy with quieter activities
		50	Requires considerable assistance and frequent medical care	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	40	Disabled, requires special care and assistance	40	Mostly in bed; participates in quiet activities
		30	Severely disabled, hospitalization indicated; death not imminent	30	In bed; needs assistance even for quiet play
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair	20	Very sick, hospitalization indicated. Death not imminent	20	Often sleeping; play entirely limited to very passive activities
		10	Moribund, fatal processes progressing rapidly	10	No play; does not get out of bed

APPENDIX II: COG STEM CELL COMMITTEE CONSENSUS GUIDELINES FOR ESTABLISHING ORGAN STAGE AND OVERALL GRADE OF ACUTE GRAFT VERSUS HOST DISEASE (GVHD)

Table 1 outlines standard criteria for GVHD organ staging. However, confounding clinical syndromes (such as non-GVHD causes of hyperbilirubinemia) may make staging GVHD in a given organ difficult. In addition, timing of organ specific symptoms affects whether that symptom is more or less likely to be true GVHD. Please refer to ***Tables 2 and 3*** to assist you in deciding whether to attribute these clinical findings to GVHD, especially in situations where a biopsy is not possible. For additional help, please see the text which follows the tables. ***Table 4*** reviews the approach to assessing GVHD as acute, chronic, or the overlap between the two.

Finally, ***engraftment syndrome*** will be reported separately from the GVHD scoring presented below.

Engraftment Syndrome

A clinical syndrome of fever, rash, respiratory distress, and diarrhea has been described, just prior to engraftment in patients undergoing unrelated cord blood and mismatched transplantation. If, in the judgment of the treating physician, a patient experiences this syndrome, details of the event will be recorded in the medical record.

Modified Glucksberg Staging Criteria for Acute Graft versus Host Disease

Table 1: Organ Staging (See tables and text below for details)

Stage	Skin	Liver (bilirubin)	Gut (stool output/day)
0	No GVHD rash	< 2 mg/dL	Adult: < 500 mL/day Child: < 10 mL/kg/day
1	Maculopapular rash < 25% BSA	2-3 mg/dL	Adult: 500-999 mL/day Child: 10-19.9 mL/kg/day. ***Or persistent nausea, vomiting, or anorexia, with a positive upper GI biopsy.***
2	Maculopapular rash 25-50% BSA	3.1-6 mg/dL	Adult: 1000-1500 mL/day Child: 20-30 mL/kg/day
3	Maculopapular rash > 50% BSA	6.1-15 mg/dL	Adult: > 1500 mL/day Child: > 30 mL/kg/day
4	Generalized erythroderma plus bullous formation and desquamation > 5% BSA	>15 mg/dL	Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).

For GI staging: The "adult" stool output values should be used for patients > 50 kg in weight. Use 3 day averages for GI staging based on stool output. If stool and urine are mixed, stool output is presumed to be 50% of total stool/urine mix (see 3.2 below).

For Stage 4 GI: the term "severe abdominal pain" will be defined as:

a) Pain control requiring institution of opioid use, or an increase in on-going opioid use, PLUS
b) Pain that significantly impacts performance status, as determined by the treating MD.

If colon or rectal biopsy is +, but stool output is < 500 mL/day (< 10 mL/kg/day), then consider as GI stage 0.

There is no modification of liver staging for other causes of hyperbilirubinemia.

Overall Clinical Grade (based on the highest stage obtained):
Grade 0: No stage 1-4 of any organ
Grade I: Stage 1-2 skin and no liver or gut involvement
Grade II: Stage 3 skin, or Stage I liver involvement, or Stage l GI
Grade III: Stage 0-3 skin, with Stage 2-3 liver, or Stage 2-3 GI
Grade IV: Stage 4 skin, liver or GI involvement

Table 2 Evaluating Liver GVHD in the Absence of Biopsy Confirmation (See Table 3.0 below)

Establishing liver GVHD with no skin or GI GVHD

No Skin/GI GVHD Day 0-35	Assume no liver GVHD, unless proven by biopsy	
No Skin/GI GVHD Day 36-100	If NO other etiology identified, NO improvement with stopping hepatotoxic medications/TPN: **Stage as liver GVHD**	If other etiology identified or improves with stopping hepatotoxic drugs/TPN: **Do not stage as liver GVHD**

Establishing liver GVHD with skin or GI GVHD and other cause of hyperbilirubinemia

Skin and/or GI GVHD present	Worsening bilirubin level (includes worsening just prior to onset of skin or GI tract GVHD) OR stable elevated bilirubin despite resolution of non-GVHD cause of increased bilirubin: **Stage as liver GVHD**	Stable or improving bilirubin after diagnosis of skin or GI GVHD, irrespective of treatment: **Do not stage as liver GVHD**

Changing liver GVHD stage with other cause of hyperbilirubinemia

Skin and GI GVHD stable, improving, or absent	Liver GVHD staging is carried forward without increase in stage until other disease process resolves (e.g., if TTP is diagnosed in the presence of stage 2 liver GVHD, the liver GVHD stage 2 is carried forward despite rising bilirubin level until TTP is resolved. If there is no liver GVHD – stage 0 – and new onset TTP, the stage 0 is carried forward until TTP is resolved).
Skin and/or GI GVHD worsening	**Liver GVHD is staged according to the Glucksberg criteria. The elevated bill is attributed to GVHD alone**. Thus, when skin or GI GVHD is worsening, there is no downgrading of liver GVHD staging for other causes of hyperbilirubinemia. (e.g., if TTP is diagnosed in the presence of stage 2 liver GVHD and worsening skin or GI GVHD, the liver is staged according to the actual bilirubin level even if some of the rise in bilirubin is attributed to TIP). Similarly, even if there is no liver GVHD at onset of a new process, (such as TPN cholestasis), but skin or GI GVHD worsen during that process, then liver GVHD is diagnosed and staged according to the height of the bilirubin. **There is one exception to this**: the diagnosis of TTP, with high LDH and **unconjugated** bilirubin precludes the diagnosis and staging of new liver GVHD in the absence of a confirmatory liver biopsy.

Table 3 Evaluating GI GVHD in the Absence of Biopsy Confirmation (See Table 4.0 below)

Establishing GI GVHD with new onset diarrhea and no skin or liver GVHD

No skin/liver GVHD Day 0 through engraftment	Assume no GI GVHD, unless proven by biopsy	
No skin/liver GVHD engraftment through Day 100	NO other etiology of diarrhea identified: **Stage as GI GVHD**	Any other etiology of diarrhea identified: **Do not stage as GI GVHD**

Establishing GI GVHD with pre-existing diarrhea and skin or liver GVHD

Skin and/or liver GVHD present	Worsening diarrhea (includes worsening just prior to onset of skin or liver GVHD) OR persistent diarrhea despite resolution of non-GVHD cause: **Stage as GI GVHD**	Improving diarrhea after the diagnosis of skin or liver GVHD (irrespective of treatment) OR persistent diarrhea without resolution of underlying non-GVHD cause: **Do not stage as GI GVHD**

Differentiating Acute GVHD, Chronic GVHD, and Overlap Syndrome:
There is often confusion differentiating acute from chronic GVHD, especially in the setting of reduced intensity transplants, DLI and new prophylactic treatments. The NIH Working Group recently published new classifications for GVHD:

Table 4 Acute GVHD, Chronic GVHD, and Overlap Syndrome

Category	Time of Symptoms after HCT or DLI	Presence of Acute GVHD features	Presence of Chronic GVHD features
Acute GVHD			
Classic acute GVHD	<100 d	Yes	No
Persistent, recurrent, or late-onset acute	>100 d	Yes	No
Chronic GVHD			
Classic chronic GVHD	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

- Scoring of acute GVHD may need to occur past day 100. In particular, patients should continue to be scored for acute GVHD when classic acute GVHD symptoms (maculopapular rash, nausea, vomiting, anorexia, profuse diarrhea - particularly if bloody and ileus) persist past day 100 or if identical symptoms previously scored as acute GVHD resolve and then recur within 30 days during immunosuppression taper but past day 100.

- Those patients being scored as having acute GVHD should NOT have diagnostic or distinctive signs of chronic GVHD.

- **Patients with both acute and chronic symptoms should be diagnosed as having Overlap Syndrome and scored according to their chronic GVHD score**.

Further Explanation of Criteria presented in Tables 2 and 3

1.0 Assessment of Skin GVHD

1.1 Presence or Absence of Skin GVHD: Skin GVHD will be considered present if a rash characteristic of acute GVHD develops after allogeneic marrow transplantation involving more than 25% of the body surface not clearly attributable to causes such as drug administration or infection. The extent of the body surface area involved can be estimated by the "Rule of Nines". In estimating the extent of skin GVHD, the area involved is calculated for individual anatomic areas, such as the arm or leg, and then the total is derived from a simple summation. Areas that are non-blanching should not be considered involved regardless of the overlying color of the rash (red, brown, etc.). Limited distribution erythema (with the exception of palms and soles) in the absence of associated rash elsewhere on the body will not be considered GVHD.

2.0 Assessment of Liver GVHD

2.1 Assessing for the Presence or Absence of Liver GVHD

A. Hyperbilirubinemia (total bilirubin ≥ 2.0 mg/dL) in the **absence** of other signs of acute GVHD in the skin or GI tract:

   i) Day 0-35: If hyperbilirubinemia alone is present with no other signs of acute GVHD in other organ systems, acute GVHD will not be diagnosed based solely on laboratory abnormalities.

      Acute GVHD will be diagnosed if findings on histopathology studies of liver from a biopsy or autopsy are confirmatory.

   ii) Day 35-100: If hyperbilirubinemia (must be conjugated bilirubin) is not improving or is exacerbated (especially if serum alkaline phosphatase is increased), in the absence of acute GVHD in other organ systems, no other etiologies are identified, and does not improve with discontinuation of hepatotoxic drugs, acute GVHD will be diagnosed. However, it is distinctly unusual to develop ascites or a coagulopathy in the early stages of acute GVHD of the liver alone. In the absence of histopathology studies of liver from a biopsy or autopsy specimen, ascites or a coagulopathy secondary to liver dysfunction will be considered to indicate the presence of another disease process (e.g., veno-occlusive disease). Recommended non-invasive studies to define an etiology for hyperbilirubinemia are:

      a. Imaging of liver (CT or ultrasound)
      b. Hepatitis screen (only if ALT is elevated)
      c. PT
      d. Blood cultures
      e. Review of medication list for potentially hepatotoxic drugs
      f. Review of risk factors for viral liver infection (HSV, CMV, VZV, adenovirus, EBV, HBV, and HCV)
      g. Hemolysis screen

B. Pre-existing hyperbilirubinemia clearly attributed to an etiology other than acute GVHD in the presence of signs of acute GVHD in other organ systems.

   i) If pre-existing non-GVHD liver disease (documented clinically, by lab assessment, or by imaging studies) is stable or improving at the onset of signs of acute GVHD in other organs, then acute GVHD of the liver will not be considered to be present unless proven by liver biopsy or autopsy.

   ii) If hyperbilirubinemia worsens several days before or at the time of onset of signs of acute GVHD in other organ systems, GVHD will be considered to be present unless histopathology studies of liver are available and negative on a biopsy during that time interval or autopsy results exclude GVHD.

iii) If hyperbilirubinemia persists and is not improving after resolution of a pre-existing non-GVHD liver disease process (e.g., localized infection of liver, systemic sepsis, biliary tract obstruction) when signs of acute GVHD are present in other organ systems or no other intervening cause has been diagnosed, then acute GVHD will be considered to be present in the absence of a new, clearly identifiable cause of non-GVHD liver disease or unless a liver biopsy or autopsy specimen is negative.

C. Prior acute GVHD in liver with new onset of a disease process that exacerbates pre-existing or recently resolved hyperbilirubinemia:

i) If an etiology other than acute GVHD is clearly identified as causing or exacerbating hyperbilirubinemia and acute liver GVHD has been diagnosed and has been stable, improving, or resolved, then the liver will not be restaged for acute GVHD until the resolution or stabilizing of the concurrent disease process (i.e., the liver stage prior to the onset of the new disease process will be carried forward until the new disease process resolves). Example: Acute GVHD of the liver and gut is diagnosed on day 20. Treatment of acute GVHD results in falling bilirubin levels to liver stage 1. Sepsis or TTP develops with transient worsening of the hyperbilirubinemia. The liver stage is not increased, despite a higher bilirubin level, because the cause of worsening hyperbilirubinemia is attributed to sepsis or TTP.

ii) If an etiology other than acute GVHD is clearly identified as causing or exacerbating hyperbilirubinemia in the presence of already worsening acute liver GVHD **or** GVHD of the skin or GI tract is simultaneously worsening, then the liver GVHD will be staged according to the actual bilirubin level, even though another cause of hyperbilirubinemia is present.

3.0 Assessment of GVHD of the Gastrointestinal Tract

3.1 Assessing for the Presence or Absence of GVHD of the Gastrointestinal Tract

A. Diarrhea (≥500 mL/day in adults or > 10 mL/kg in pediatric patients) in the absence of other signs of acute GVHD in other organ systems

i) Day 0-engraftment: If diarrhea alone is present without other signs of acute GVHD in other organ systems, acute GVHD will not be considered present. Diarrhea will be attributed to acute GVHD if histopathology studies of gastrointestinal tract from a biopsy or autopsy are diagnostic.

ii) Engraftment-day 100: If diarrhea persists and is not improving, is exacerbated, or develops de novo in the absence of acute GVHD in other organ systems, histopathology studies of gut biopsies or from autopsy specimens are not available, and no other etiologies are clearly identified, acute GVHD will be considered to be the cause. A stool specimen should be examined to rule out infectious causes (e.g., rotavirus, adenovirus, and C. difficile toxin). It is recommended, if at all possible, that biopsies be obtained for diagnostic purposes.

B. Pre-existing diarrhea clearly attributed to an etiology other than acute GVHD in the presence of signs of acute GVHD in other organ systems:

   i) If pre-existing diarrhea caused by a process other than GVHD has been documented clinically or by lab assessment and is stable or improving at the onset of signs of acute GVHD in the skin or liver, then acute GVHD of the intestine will not be considered to be present in the absence of biopsy confirmation or autopsy report.

   ii) If diarrhea or gastrointestinal symptoms are already present, but worsen significantly at the time of onset of signs of acute GVHD in the skin or liver, GVHD will be considered present, unless biopsy or autopsy are negative.

   iii) If diarrhea persists after resolution of a pre-existing disease process with signs of acute GVHD present in other organ systems, GVHD will be considered present, unless biopsy or autopsy are negative.

C. Prior or present acute GVHD in other organ systems with new onset of diarrhea:

   If diarrhea is clearly attributable to an etiology other than acute GVHD (e.g., infection) and a history of acute GVHD exists or acute GVHD is present in other organ systems and is stable, then the gastrointestinal tract will not be evaluable for acute GVHD until the resolution or stabilizing of the other disease process (e.g., infection) in the absence of biopsy or autopsy confirmation.

D. Persistent anorexia, nausea or vomiting in the absence of signs of acute GVHD in other organ systems:

   Persistent anorexia, nausea or vomiting in the absence of other known causes of these symptoms will be considered stage I acute GVHD if confirmed by endoscopic biopsy.

   If a biopsy is not possible (e.g. secondary to thrombocytopenia) but the clinical findings are compatible with acute GVHD, then the patient will be treated and recorded as having acute GVHD.

3.2 Staging of the Gastrointestinal Tract for the Severity of Acute GVHD

The severity of gastrointestinal tract GVHD will be staged according to modified Glucksberg criteria. To minimize errors caused by large day-to-day variation, diarrhea volume is measured as an average over 3 days and reported as the volume in milliliters per day. When urinary mixing is noted the stool volume will be considered half of the total volume unless nursing staff is able to give a better estimate from direct observation. Abdominal cramps are considered significant for staging if the severity results in a clinical intervention (e.g. analgesia, fasting, etc.). Blood in the stools is considered significant if the blood is visible or hematochezia/melena is present and not clearly attributed to a cause other than GVHD (e.g., epistaxis/hemorrhoids).

APPENDIX III: TESTS PERFORMED FOR ROUTINE CARE AND FOR RESEARCH

Routine care

- Physical exam, height, weight, BSA
- CBC with differential
- Chemistry profile: glucose, electrolytes, BUN, creatinine, LDH, uric acid, bilirubin, SGOT, SGPT, calcium, phosphorous, magnesium, total protein and albumin
- Coagulation screen
- HLA typing
- Chest x-ray
- EKG, echocardiogram
- Bone marrow evaluation for morphology, immunophenotyping, cytogenetics, molecular diagnosis, and minimal residual disease (MRD).
- Lumbar puncture with CSF cell count and cytology.
- Serum pregnancy test of females of childbearing potential

Research

- Hematopoietic Progenitor Cell, Apheresis (HPC-A) infusion
- Microchimerism studies – blood and bone marrow
- All donor tests/evaluations, GCSF and hematopoietic stem cell mobilization procedure