UNIVERSITY OF MINNESOTA BONE MARROW TRANSPLANTATION PROTOCOL

HEMATOPOIETIC CELL TRANSPLANTATION IN CHILDREN WITH JUVENILE MYELOMONOCYTIC LEUKEMIA

MT1999-20

Revised January 3, 2022

Study Committee

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DATE	PROTOCOL CHANGE
2/4/2005	Section 5.1.2.5: values for creatinine clearance added to eligibility criteria.
5/4/2006	MMF replaces methylprednisone as GVHD prophylaxis in cord blood recipients
07/15/2008	Revised for study reapplication, increased enrollment goal to 20

02/16/2009	Remove Scott Baker as co-investigator. Update registration, DSMP, time to keep study records, add adverse event reporting section per CPRC annual review.
01/08/2015	Protocol: MMF replaces methylprednisone (MP) as GVHD prophylaxis for all cell sources Add retinoic acid to begin on day 60 through 1 year post transplant (mentioned in the consent form, but not in the protocol) Add ATG to the preparative regimen in section 4.1, currently only in section 7.4 Update seizure prophylaxis to levetiracetam (Keppra) Update adverse event reporting section to current IRB and MCC requirements Both treatment consent forms: Delete pregnancy language – JMML occurs only in very young children (< 4 years of age) while retaining the risks of future fertility issues Correct Busulfan dosing schedule Add ATG treatment details Add risks of retinoic acid Update both treatment consents to match protocol and current template language Bone marrow as donor consent only (additional change): Replace MP with MMF as GVHD prophylaxis
10/03/2017	Protocol Revised ATG dosing to current standard of care Tacrolimus replaces cyclosporineas GVHD prophylaxis Updated eligibility to current definition of JMML Updated toxicity information to current tables Consents Updated HRPP contact information Updated GVHD drug information
10/19/2021	Per IRB request, updated section 9.3 to match study accrual period in section 9.1
1/3/2022	Updated section 9.1 and 9.3 to match consent Section 4 - updated all chemotherapy dosing instructions to match current standard of care and Section 7 - removed redundancies Removed Appendix 1 – eligibility checklists are housed in Oncore Additional standard template language added to clinical follow up Corrected link to DSMP plan

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1.0 TITLE

Hematopoietic cell transplantation in children with juvenile myelomonocytic leukemia.

2.0 OBJECTIVES

2.1 Primary Objective

To determine the probability of long-term disease-free survival in patients with JMML treated with busulfan (BU), cyclophosphamide (CY) and melphalan (L-PAM) followed by hematopoietic cell transplantation.

2.2 Secondary Objectives

To determine:

1. probability of engraftment
2. incidence and severity of acute and chronic graft-versus-host disease (GVHD)
3. incidence of regimen-related toxicity
4. incidence of relapse

3.0 BACKGROUND AND RATIONALE

JMML is a rare myeloproliferative disease of very young children (age <4 years), accounting for less than 2% of childhood leukemias. Approximately 40 cases occur annually in the US, corresponding to an incidence of 0.61 cases per year per million children.[1] Few treatment approaches other than HCT have been shown to be beneficial for children with JMML with median survival of 1-2 years commonly reported.[1-3] Intensive chemotherapy may prolong survival but has not been shown to be curative.[4,5] Recent studies suggest that isotretinoin may benefit a proportion of children with JMML; however, its potential to affect long term survival is unclear.[6,7] To date, long-term DFS has only been achieved using HCT.[8-21]

Aricò et al (1997) reviewed the outcome of 91 patients with JMML treated with HCT in 16 different reports.[3] Thirty-eight patients (41%) were still alive at time of reporting, including 30 of the 60 (50%) patients who received grafts from HLA-matched or one-antigen mismatched familial donors, 2 of 12 (17%) with mismatched donors, and 6 of 19 (32%) with matched unrelated donors. Transplant results reported from the Fred Hutchinson Cancer Center (Sanders et al, 1988) and recently updated (Smith et al, 1994) include 27 children with JMML treated with preparative regimens that included dimethylbusulfan, busulfan, cyclophosphamide, and/or TBI followed by allogeneic HCT.[8-10] Unmodified marrow was obtained from HLA-identical siblings (n=10), family members differing by 1, 2, or 3 antigens (n=14), or HLA minor-mismatched unrelated donors (n=3). Graft-versus-host disease (GVHD) prophylactic regimens included the use of methotrexate (MTX), cyclosporine (CSA), anti-thymocyte globulin (ATG), steroids, and anti-T cell antibodies. Acute GVHD (grades IIIV) occurred in 50% of recipients of matched sibling donor HCT and in 76% of recipients of mismatched or unrelated grafts. Six patients died within the first three months from graft rejection (n=1), fungal infection (n=1), or interstitial pneumonia (n=4). Two patients developed secondary malignancies, one with myelodysplasia at one year, and one with a fatal chondrosarcoma at 17 years.

Recurrent leukemia occurred in ten patients from 1.6 to 56 (median 2.8) months and nine have died. Seven patients underwent a second transplant for relapse of disease or graft rejection. None of these patients survived. While the numbers of patients are small, there was no apparent association between the type of preparative regimen, degree of HLA disparity, pre-transplant chemotherapy or pre-transplant splenectomy and survival. Five patients survive disease-free (DFS) from 2.7-10 (median 7.3) years. Cumulative incidence estimates of non-relapse mortality and relapse at five years are 44% and 49%, respectively, with a Kaplan-Meier estimate of DFS at five years of 25%.

Six bone marrow transplant recipients have been reported by Bunin et al (1992).[12] These children received T-cell depleted bone marrow grafts (using T10B9 monoclonal antibody plus complement) from HLA-mismatched related donors or HLA-matched unrelated marrow donors after a preparative regimen of busulfan, cytosine arabinoside, cyclophosphamide, methylprednisolone and hyperfractionated TBI. GVHD prophylaxis was with CSA. All patients engrafted with acute GVHD $\leq$ grade II in all patients. Two patients died of infection with one patient relapsing on day +939. Three of six patients were disease-free survivors at days 180+, 1610+, and 2400+ at the time of the report.

Twelve children with JMML, 5 of whom also had Monosomy 7, underwent transplantation in Paris.[16] Seven patients were recipients of matched sibling marrow, 4 from mismatched related donors and one from a matched unrelated donor. Conditioning regimens were different for all patients, consisting of chemotherapy +/- TBI. In the matched sibling recipients, five engrafted after chemotherapy conditioning. Two patients failed to engraft, one having received chemotherapy alone, the other having had chemotherapy and TBI as conditioning. Long-term remission was achieved in three patients and two others are in remission 6 and 11 months post HCT. In contrast, HCT with marrow from mismatched related donors failed in five patients because of graft failure in 3 patients and relapse in two. One patient engrafted well after receiving a chemotherapy regimen and a matched unrelated donor transplant and remains in remission.

The European Working Group on Myelodysplastic Syndrome in Childhood (EWOG-MDS) report on 43 children with JMML who underwent HCT.[20] Eight of these patients also had Monosomy 7. Twenty-two patients were given TBI and chemotherapy, which included cyclophosphamide. Twenty one patients were conditioned with busulfan in combination with cytoxan alone, or with etoposide, or melphalan. Six of the total 43 patients (14%), 5 of whom received HCT from an alternative donor, failed to engraft. GVHD prophylaxis consisted of CSA alone, MTX alone, both of these agents, or both agents with anti-thymocyte globulin or monoclonal antibodies. One child received T-cell depleted marrow. Grade II to IV acute GVHD occurred in eight of 24 patients (33%) transplanted from HLA-identical siblings or one-antigen-mismatched relatives and in eight of 13 patients (62%) given HCT from other HLA-partially matched family donors or unrelated donors. Transplant related mortality was 20% for the entire group, 9% for children given HCT from HLA-identical/one-antigendisparate relatives, and 46% for patients transplanted using other HLA-partially matched family donors or mismatched unrelated donors. Twenty-two (58%) children relapsed, at a median of 3.5 months after HCT. The 5 year Kaplan-Meier DFS for the entire cohort of patients was 31 □ 7%. The actuarial probability of DFS for children transplanted from HLA-partially matched family donors or unrelated volunteers was 22 +/- 10% whereas the DFS for children given HCT from an

HLAidentical sibling or one-antigen disparate relative was 38 +/- 10%. A shorter interval between diagnosis and HCT was associated with improved DFS probability. Patients who received chemotherapy alone preparative regimen had a better EFS compared to those treated with a TBI containing regimen (48 ± 11% vs 15 ± 8%, p <.05).

At the University of Minnesota, seven children, 11 months to 5.9 years of age, with JMML underwent allogeneic HCT over the past 14 years.[21] All patients had active disease at time of transplant despite chemotherapy in 4 patients and chemotherapy and splenectomy in one patient prior to conditioning. Patients received related or unrelated bone marrow or umbilical cord blood (UCB) after a preparative regimen of cyclophosphamide and TBI, with the addition of busulfan in two cases. Six patients engrafted without difficulty; primary graft failure occurred in one patient who required two further HCT to achieve engraftment. Six patients relapsed post HCT, at a median of 67 days, four of whom subsequently died. One relapsed patient achieved a second sustained remission after CSA was abruptly withdrawn to induce graft-versus-leukemia (GVL). Two patients are in remission more than 2 years and 2.5 years after HCT.

As JMML affects only very young children, preparative regimens which avoided radiation-induced long term sequelae, such as growth retardation and neuropsychological impairment were developed. A conditioning regimen consisting of three alkylating agents, busulfan, cyclophosphamide and melphalan has been shown to be effective in patients with JMML. Results from the European Group (EWOG-MDS) showed that in the group of children given BMT from an HLA-identical sibling or one-antigen-disparate related donor, patients given busulfan had a lower risk of relapse (38 +/- 13% vs 78 +/- 17%), which resulted in a significantly better EFS in comparison to those treated with TBI

(62% vs 11%).[20]

The levels of oral busulfan have been shown to be quite variable, presumably due to marked differences in absorption and/or clearance.[22] In addition, there is some suggestion that increased plasma levels may relate to enhanced toxicity, while inadequate levels may be associated with graft failure.[23] The recent availability of an intravenous form of the drug may result in decreased variation of the pharmacokinetics of the drug.[24] The drug has been tested in children using a dose of 0.8 mg/kg; however, it was determined that in children less than 4 years of age the clearance was more rapid and in this population a dose of 1.0 mg/kg will be used.[25]

In summary, HCT offers the only possibility of a cure for patients with JMML. To prevent relapse, abrupt withdrawal of CSA, inducing GVL has resulted in sustained second remissions in a few patients with relapsed JMML after HCT.[21,26-28] Scheduled early tapering of CSA after HCT may promote GVL and prevent early relapse.

4.0 TREATMENT SCHEMA

This is a phase II single arm trial to evaluate the efficacy of busulfan, cyclophosphamide and melphalan and allogeneic hematopoietic cell transplantation in the treatment of JMML. This study will not be able to determine if pre-transplant chemotherapy or splenectomy is efficacious, nor will it

identify a superior stem cell source. The intent of this proposal is to provide a common protocol that will use unmanipulated allogeneic hematopoietic stem cells from related and unrelated donors after a common preparative regimen. Results will be compared to prior reports using cyclophosphamide and total body irradiation after which the probability of engraftment, toxicity, and event free survival are 50-97%, 9-28% and 38-50% after sibling donor BMT and 50-65%, 46-67%, and 17-22% after 2- to 3-antigen disparate relative or unrelated donor BMT.[10,16,19]

4.1 Preparative Therapies

The preparative cytoreductive therapy will be identical for all patients. Toxicities related to each regimen are defined in Section 9.0.

4.1.1 Design/Therapy Plan

Busulfan (intravenous) **dose**, **administration** and **pharmacokinetic monitoring** to be administered per institutional standard of care (SOC).
Cyclophosphamide 60 mg/kg/day IV x 2 days (administered with Mesna 60 mg/kg/day)
Melphalan 140 mg/m^2 IV x 1 day
ATG 30 mg/kg/day IV over 4-6 hours every 24 hours (UCB recipients only)

Days Prior to BMT	-7	-6	-5	-4	-3	-2	-1	0	+1
Busulfan IV daily*	●	●	●	●					
Cyclophosphamide 60 mg/kg/day** Mesna 60 mg/kg/day					●	●			
Melphalan 140 mg/m^2 ***							●		
ATG 30 mg/kg/day IV over 4-6 hours every 24 hours (UCB recipients only)					●	●	●		
Stem Cell Infusion								●	

* Model-based dosing utilizing Bayesian methodology and therapeutic drug monitoring per Institutional SOC
** Infusion over 2 hours.
*** Infusion over 15 minutes.

4.1.2 Busulfan

Busulfan is to be given as a 3 hour intravenous infusion. As seizures have occurred following high dose busulfan, all patients will be treated with levetiracetam (Keppra) per institutional guidelines.. Busulfan area under the curve (AUC) analyses will be calculated in-house per

University of Minnesota BMT SOC guidelines. Busulfan therapeutic drug monitoring (TDM) will be used to target a cumulative exposure for the entire course cAUC=68-76 mg*hr/L (target 72 mg*hr/L). Results of TDM performed with the first dose will inform subsequent dosing. Refer to busulfan SOC for sample collection process, busulfan results reporting and TDM procedures.

4.1.3 Cyclophosphamide

Cyclophosphamide is to be given as a 2 hour intravenous infusion. Mesna 60 mg/kg and IV hydration will be administered per institutional guidelines. Cyclophosphamide dosing is calculated based on actual weight (ABW) unless ABW is >150% above Ideal Body Weight (IBW) per institutional guidelines.

4.1.4 Melphalan

Melphalan is to be given as a 15 minute infusion on day -1. For children < 10 kg, melphalan dosing will be 4.67 mg/kg IV x 1 dose.

4.1.5 Antithymocyte Globulin - equine (ATG)

ATG 30 mg/kg/day IV over 4-6 hours every 24 hours on day -3, -2 and -1 to umbilical cord blood (UCB) recipients only.

Pre-medications will be administered 30 minutes prior to each dose of ATG including:
• acetaminophen (10 mg/kg orally; maximum dose 500 mg)
• diphenhydramine (1 mg/kg IV or PO; maximum dose 50 mg)
• methylprednisolone (1 mg/kg IV; maximum dose 125 mg)

4.2 Hematopoietic Stem Cell Source

Acceptable stem cell sources include: HLA-matched related or unrelated donor bone marrow (6/6 or 5/6 antigen match) and related or unrelated donor umbilical cord blood (6/6, 5/6 or 4/6 match). HLAmatch is determined by serology for class I antigens (HLA A, B) and high resolution DNA typing for class II (DRB1) unless parental typing is available (DR is satisfactory). The stem cell source will not be manipulated (T-cell depletion, CD34+ cell selection, etc.). Cord blood units selected for transplantation as per institutional guidelines.

4.3 Graft-Versus-Host Disease Prophylaxis

GVHD prophylaxis will be as per University of Minnesota GVHD prophylaxis protocols.

All patients (regardless of allograft source) will receive tacrolimus therapy beginning on day –3.

Tacrolimus dosing will be monitored and altered as clinically appropriate per institutional pharmacy guidelines. Dose adjustments will be made on the basis of toxicity and/or low tacrolimus levels.

Tacrolimus taper begins at day +100 or 1 month after control of GVHD. Taper to zero by 10% weekly dose reduction over approximately 10 weeks.

All patients will begin mycophenolate mofetil (MMF) on day -3. Patients ≥ 40 kilograms will receive MMF at the dose of 3 grams/day divided into 2 or 3 doses (every 12 or 8 hours). Pediatric patient (<40 kilograms) will receive MMF at the dose of 15 mg/kg three times a day.

Patients will be eligible for MMF dosing and pharmacokinetics studies.

Use IV route between days –3 and +5, then, if tolerated, may change to PO between days +6 and +30.

Stop MMF at day +30 *or* 7 days after engraftment, whichever day is later, if no acute GVHD. Engraftment is defined as the 1st day of 3 consecutive days of absolute neutrophil count [ANC] ≥ 0.5 x 10^9 /L.

4.4 Retinoic Acid

Retinoic acid 100 mg/m2/day starts at the day 60 visit and continues daily until 1 year posttransplant.

4.5 Supportive Care

Growth Factors: As the malignant cell population of JMML is known to be hypersensitive to GM-CSF, this cytokine will not be used in this protocol. Patients with ANC <0.2 x 10^8 /L on day 21 may receive G-CSF at 5µg/kg/day.

5.0 ELIGIBILITY AND EXCLUSION CRITERIA

5.1 Inclusion Criteria

A diagnosis of JMML requires the presence of all of the following Category 1 criteria:

- Peripheral blood monocyte count of >1000/microL
- Blast percentage in peripheral blood and bone marrow <20 percent
- Splenomegaly
- Absence of t(9;22) *BCR-ABL1* fusion gene

In addition, the patient must have at least one of the following Category 2 criteria:

- Somatic mutation of *PTPN11, KRAS,* or *NRAS*
- Clinical diagnosis of neurofibromatosis-1 or *NF1* mutation

●Germline *CBL* mutation or loss of heterozygosity of *CBL*
●Monosomy 7

If none of the Category 2 criteria are met, the child must have at least two of the following Category 3 criteria:
●Clonal cytogenetic abnormality other than monosomy 7
●White blood cell >10,000/microL
●Increased fetal hemoglobin (HbF) for age
●Circulating myeloid precursors ●GM-CSF hypersensitivity

5.1.1 Adequate major organ function including:

5.1.1.1 Cardiac: ejection fraction ≥45%
5.1.1.2 Hepatic: no clinical evidence of hepatic failure
5.1.1.3 Karnofsky performance status ≥70% or Lansky score ≥50%
5.1.1.4 GFR>40

5.1.3 Voluntary written consent signed before performance of any study-related procedure not part of normal medical care.

5.2 Exclusion Criteria

5.2.1 Active uncontrolled infection within one week of HCT.

6.0 REQUIRED OBSERVATIONS

6.1 Patient Pre-study Assessment

6.1.1 Past Medical History
A complete medical history with full details of the patient at time of diagnosis.

6.1.2 Physical Examination

6.1.3 Investigations

6.1.3.1 Complete blood count with differential, platelet count, serum electrolytes, liver and renal function tests

6.1.3.2 Bone marrow aspiration and biopsy;

6.1.3.3 Chest radiograph and other radiographic studies (as clinically indicated)

6.1.3.4 Electrocardiogram; echocardiography with measurement of the left ventricular ejection fraction (LVEF)

6.1.3.5 Viral titers as per institutional guidelines

6.2 Patient Evaluation During Therapy Until Engraftment - follow-up evaluations may be altered as clinically appropriate without being considered a deviation

6.2.1 Routine practices at the University of Minnesota will be maintained.

6.2.2 Bone marrow aspirate, biopsy, and chimerism studies on day 21.

6.3 Patient Evaluation on Day 60, 100 and 180, and then annually for 2 years- follow-up evaluations may be altered as clinically appropriate without being considered a deviation

6.3.1 History and physical examination

6.3.2 GVHD evaluation

6.3.3 Bone marrow aspirate, biopsy, and chimerism studies on days 60, 100, 180 and at 1 year and 2 years

6.3.4 Other studies clinically indicated.

7.0 TOXICITIES AND COMPLICATIONS

7.1 Busulfan

7.1.1 Toxicities:

Busulfan		
Common	**Less Common**	**Rare**

• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • low red blood cell count (anemia) which may cause tiredness, headache, dizziness • hair loss or thinning, including face and body hair (usually grows back after treatment) • long-term or short-term infertility (inability to have children) in men and women	• tiredness (fatigue) • sores in mouth or on lips • fever • nausea • vomiting • rash • loss of appetite • diarrhea • serious infection due to low white blood cell count	• abnormal blood tests results which suggest that the drug is affecting the liver • allergic reaction with hives, itching, headache, coughing, shortness of breath, or swelling of the face, tongue, or throat • scarring of lung tissue, with cough, difficulty breathing, and shortness of breath that may occur after prolonged use, or even months or years after stopping the drug • leukemia (several years after treatment) • darkened skin • heart problems with high-dose treatment, most often in people with thalassemia (a type of genetic anemia that is present at birth)
Busulfan		
Common	**Less Common**	**Rare**
		• problems with the hormone system that cause weakness, tiredness, poor appetite, weight loss, and darker skin • death due lung damage, bone marrow shutdown, or other causes

7.2 Cyclophosphamide (Cytoxan)

7.2.2 Toxicities:

Cyclophosphamide		
Common	**Less Common**	**Rare**

• low white blood cell count with increased risk of infection • hair loss or thinning, including face and body hair (usually grows back after treatment) • nausea • vomiting • loss of appetite • sores in mouth or on lips • bleeding from bladder, with blood in urine • diarrhea • long-term or short-term infertility (inability to have children) in women and men	• low platelet count (mild) with increased risk of bleeding • darkening of nail beds • acne • tiredness • infection • fetal changes if you become pregnant while taking cyclophosphamide	• heart problems with high doses, with chest pain, shortness of breath, or swollen feet • severe allergic reactions • skin rash • scarring of bladder • kidney damage (renal tubular necrosis) which can lead to kidney failure • heart damage, with trouble getting your breath, swelling of feet, rapid weight gain • scarring of lung tissue, with cough and shortness of breath • second cancer, which can happen years after taking this drug • death from infection, bleeding, heart failure, allergic reaction, or other causes

7.3 Melphalan

7.3.1 Toxicities:

Melphalan		
Common	**Less Common**	**Rare**

• nausea (at higher doses) • vomiting (at higher doses) • low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • anemia (low red blood cell count) with symptoms like tiredness, paleness, or trouble catching breath	• short-term or long-term infertility (inability to have children) • weakness	• severe allergic reaction • loss of appetite • scarring (fibrosis) or inflammation of lungs • hair loss, including face and body hair • rash • itching • second type of cancer (may happen years after treatment) • death from lung damage or other causes

7.4 Antithymocyte Globulin

7.4.1 Toxicities:

Anti-Thymocyte globulin (ATG)		
Common	**Less Common**	**Rare**
• fever • chills • leukopenia • pain • headache • abdominal pain • diarrhea • hypertension • nausea • thrombocytopenia • peripheral edema • dyspnea • asthenia • hyperkalemia • tachycardia	• malaise • dizziness	• severe allergic reaction (anaphylaxis)

7.5 Hematopoietic Stem Cell Infusion

7.5.1 Volume overload: This is of particular importance in small recipients who have previously received red cell or platelet transfusions. Diuresis with infusion may be indicated.

Allergic reactions: Chills, fever and hives occasionally occur presumably due to antigenic plasma components. These reactions are rarely severe and respond to parenteral ephedrine or antihistamines.

7.6 Retinoic Acid

7.6.1 Daily by mouth beginning day 60 (at the time of the 2 month follow-up visit) and continuing until 1 year post transplant.

7.6.2 Toxicity

Typical retinoid toxicity: (symptoms that are similar to those found in patients taking high doses of vitamin A) Headache, fever, dry skin, dry mucous membranes (mouth, nose), bone pain, nausea and vomiting, rash, mouth sores, itching, sweating, eyesight changes.
Flu-like symptoms: malaise, chills
Bleeding problems
Infections
Swelling of feet or ankles
Pain: bone and joint pain, chest discomfort, abdominal pain

7.7 Toxicities Associated with GVHD Prophylaxis

Mycophenolate mofetil (MMF)		
Common	**Less Common**	**Rare, but may be serious**
• miscarriage • birth defects • diarrhea • damage to unborn baby • limited effectiveness of birth control • stomach pain • upset stomach • vomiting • headache • tremors • low white blood cell count with increased risk of infection • increased blood cholesterols • swelling of the hands, feet, ankles or lower legs	• anemia • rash • difficulty falling asleep or staying asleep • dizziness • uncontrollable hand shakes	• difficulty breathing • unusual bruising • fast heartbeat • excessive tiredness • weakness • blood in stool • bloody vomit • change in vision • secondary cancers, such as lymphoproliferative disease or lymphoma • Progressive Multifocal Leukoencephalopathy

Tacrolimus (FK506, Prograf®)

Common occurs in more than 20% of patients	**Less Common**	**Rare**
	occurs in 5 to 20% of patients	occurs in fewer than 5% of patients
▪ Kidney problems ▪ Loss of magnesium, calcium, potassium ▪ Cardiovascular: hypertension ▪ Tremors ▪ hyperlipidemia ▪ thrombocytopenia ▪ Infections	▪ Nausea ▪ Vomiting ▪ Liver problems ▪ Changes in how clearly one can think ▪ Insomnia ▪ Unwanted hair growth ▪ Confusion	▪ Seizures ▪ Changes in vision ▪ Dizziness ▪ microangiopathic hemolytic anemia ▪ post-transplant lymphoproliferative disorders

It is very important that grapefruit or drinks with grapefruit juice are not consumed while taking Tacrolimus. Grapefruit has an ingredient called bergamottin, which can affect some of the treatment drugs used in this study. Common soft drinks that have bergamottin are Fresca, Squirt, and Sunny Delight.

8.0 ADVERSE EVENT REPORTING

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 4.0 (CTCAE). A copy of the CTCAE can be downloaded from the CTEP home page (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7.7.1 Definitions

An **adverse event** (AE) is any symptom, sign, illness or experience, regardless of causality, that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

A **serious adverse event** (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in **death**. (death due to disease specifically addressed below) ▪ Is **life-threatening**.
- Requires inpatient **hospitalization or prolongation of existing hospitalization**.

- Results in **persistent or significant disability/incapacity**.
- Is a **congenital anomaly/birth defect**.
- Is an **important medical event**.

An **unexpected adverse experience** is defined as any adverse experience that is neither identified in nature, severity, or frequency of risk in the information provided for IRB review (typically the protocol, investigator's brochure and prescribing information) nor mentioned in the consent form. Unexpected adverse experience and unanticipated adverse experience are **synonymous terms**.

Problems/events that are unanticipated and serious should be reported *only* if in the opinion of the treating investigator they are **possibly, probably or definitely related** to the research treatment.

7.7.2 Adverse Event Documentation

Adverse events occurring after the initiation of any study procedure must be fully recorded in the subject's case record form. Each event should be described in detail along with start and stop dates, severity, relationship to investigational product, action taken and outcome.

All subjects will be monitored through engraftment or the start of a new treatment, which ever occurs earlier, as it is expected that most treatment related adverse events will occur during this period.

After engraftment, follow-up is planned for 2 years (per section 6.3). The investigator is obligated, upon knowledge of, to report any adverse event that requires expedited reporting per section 7.6.3.

7.6.3 Required Reporting (U of MN IRB and Masonic Cancer Center's DSMC)

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ fax numbers	Copy AE to:
U of MN IRB	Events requiring prompt reporting including, but not limited to unanticipated death of a locally enrolled subject(s); new or increased risk; any adverse event that require a change to the protocol or consent form or any protocol deviation that resulting in harm. Refer to http://www.research.umn.edu/irb/guidance/ae.html#.VC7xraI0sh	Within 5 business days of event discovery	Report Form	irb@umn.edu	SAE Coordinator mcc-saes@umn.edu
Cancer Center DSMC	any stopping rule event	upon reporting to database	stopping rule form	SAE Coordinator mccsaes@umn.edu	Not applicable

9.0 EXPERIMENTAL DESIGN AND STATISTICAL CONSIDERATIONS

9.1 Objective and Primary Outcome Variable

Evaluate long-term DFS in JMML using a common preparative regimen. P1 is the product limit estimate of the cumulative proportion of children surviving disease free at 1 year after transplant. Secondary outcome measures are the incidence of neutrophil engraftment, GVHD, regimen-related toxicity, and relapse. The study is planned for 25 years with a total enrollment of 20 patients (about 2 patients per year). Since it will be impossible to reach any definitive conclusions about efficacy or toxicity of the proposed regimen for this rare disease, analysis will be descriptive. The study will be monitored for excessive combined incidence of grade III-IV toxicity or primary graft failure, using standard definitions.

9.2 Statistical Analysis

The principal analysis is to compute the 1-year DFS (P1), the standard error (SE), and 95% confidence interval using the product limit (Kaplan Meier survival) estimate at 12 months. Experience shows that about 35% of transplanted patients have long-term DFS, but among those that relapse the median DFS is a very short 2.8 months, the survival curve is therefore expected to have reached a stable plateau by one year post transplant.

The incidence of GVHD, time to engraftment, and relapse will be similarly evaluated. Because death is a competing risk for these outcomes, the cumulative incidence may also be used. The time course of all events may be described using event charts. The analyses can only be descriptive because of the sample size constraints of this rare disease. For example, if the true incidence rate (for a primary or secondary outcome) is about .75, then the observed rate is 90% likely to be in the interval (0.53 0.98). It will therefore not be possible to make definitive comparisons with historical rates observed here and elsewhere for other preparatory regimens.

9.3 Rationale for Sample Size

Based on our past experience we expect to enroll 2 patients per year for a total of 20 patients in 25 years.

9.4 Toxicity and Graft Failure Monitoring

The combined incidence of grade III or IV toxicity and primary graft failure associated with the preparative regimen (Section 7) will be monitored using a stopping rule of R = {2 events in 12 patients, 3/15 or 4 anytime}. This stopping rule has a type I error rate of 0.05 if the true rate is 5%, and a power of 80% if the rate is 25%.[29,30]

9.5 Data Collection

The standard data will be collected, as specified for JMML (CML) patients who receive stem cell transplantation. All patients will be followed until death or loss-to-follow up.

10.0 INFORMED CONSENT

Both the patient and donor are completely evaluated and presented at a conference with family members where the transplant physician outlines the course of therapy. These recommendations are then discussed thoroughly with the patient and family, including the donor (if of appropriate age). The risks of the procedure to the donor are outlined. The marrow graft procedure and rationale as well as alternative forms of therapy are presented as objectively as possible. All the risks and hazards of the procedure are explained to the patient, or in the case of minors, to the patient's responsible family members. Informed consent is obtained using forms approved at the University of Minnesota Committee on the Use of Human Subjects in Research.

11.0 DATA AND SAFETY MONITORING PLAN

This study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Cancer Center's Data & Safety Monitoring Plan, which can be accessed at https://z.umn.edu/dsmp

For the purposes of data and safety monitoring, this study is classified as moderate risk (phase II). Therefore the following requirements will be fulfilled:

- The PI will complete and submit a twice yearly Trial Progress Report to the Masonic Cancer Center Data and Safety Monitoring Council (DSMC) with the understanding more frequent reporting may be required by the DSMC.
- The PI will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the reporting of all adverse events meeting the definition of reportable in section 7.6.3 to the DSMC and the University Of Minnesota IRB.

In addition, at the time of the continuing review with the University Of Minnesota IRB, a copy of the report will be submitted to the Cancer Protocol Review Committee (CPRC).

12.0 REGISTRATION PROCEDURES

Registration will occur after eligibility is confirmed and the patient has signed the informed consent, but before any treatment has been administered. Eligible patients will be invited to participate in the study by their physician, who will explain the details of the trial and obtain informed consent.

Registration with the Clinical Trials Office (CTO)
Upon completion of the screening evaluation, eligibility confirmation and obtaining informed consent, a designated study site staff person will enroll the patient into ONCORE.

Record Retention
The investigator will retain study records, including source data, copies of CRF's and all study correspondence for at least 6 years beyond closure of the IRB file in a secure facility. In addition, the Clinical Trials Office will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records.

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