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A PHASE II PEDIATRIC STUDY OF A GRAFT-VS.-HOST DISEASE (GVHD) PROPHYLAXIS REGIMEN WITH NO CALCINEURIN INHIBITORS AFTER DAY +60 POST FIRST ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANT FOR HEMATOLOGICAL MALIGNANCIES

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Protocol Summary

Protocol MNEMONIC and Title: CNI60 - A Phase II Pediatric Study of a Graft-vs.-host disease (GVHD) prophylaxis regimen with no calcineurin inhibitors after day +60 post first allogeneic hematopoietic cell transplant for hematological malignancies.
Principal Investigator: Ashok Srinivasan, MD
Brief Overview: The primary objective of the study is to estimate the incidence of severe acute Graft-vs.-Host disease (saGVHD) using a prophylaxis regimen with no calcineurin inhibitors after day +60 post a first allogeneic Human Leukocyte antigen (HLA)-matched sibling or unrelated donor Hematopoietic cell transplant (MSD/MUD HCT) for hematological malignancies. Secondary objectives are focused on determining the cumulative incidence of relapse, non-relapse mortality (NRM), chronic GVHD, and overall survival (OS). Exploratory objectives are focused on evaluating the pharmacokinetic/pharmacodynamic (PK/PD) profiles of ruxolitinib, fludarabine, and rabbit anti-thymocyte globulin (rATG), and to assess immune reconstitution in HCT recipients. The central hypothesis of the study is that it may be possible to safely stop calcineurin inhibitors (CNIs) early on day +60 post allogeneic hematopoietic cell transplantation by adding ruxolitinib after engraftment. This may decrease the incidence of saGVHD within the first 100 days post-transplant while reducing toxicity from prolonged use of CNIs. We expect that the performed PK/PD tests will have a broad impact on other transplant protocols that utilize these agents. Finally, we expect that the performed immune reconstitution studies will inform future transplant studies.
Intervention: The primary interventions are allogeneic progenitor cell transplantation for treatment of hematologic malignancy and subsequent administration of selected agents for prevention of GVHD.
Brief Outline of Treatment Plan: Children 12 years and older will be eligible for the study**.** Only bone marrow donors will be used for this trial. Human leukocyte antigen (HLA) identical sibling donors will be considered first followed by histocompatible relatives or unrelated donors matched at 12 of 12 HLA alleles. We propose to employ two preparative regimens based on the underlying hematological malignancy. For hematological malignancies of the lymphoid lineage we will use a standard preparative regimen consisting of Total Body Irradiation and cyclophosphamide (TBI/Cy), unless TBI is contraindicated. For myeloid malignancies we will use thiotepa, busulfan, and fludarabine (TBF), a preparative regimen that has been associated with a reduced risk of relapse and trend for improved survival with comparable NRM in comparison to busulfan and cyclophosphamide (BuCy), our current regimen. All HCT recipients will receive cyclosporine in combination with methotrexate and ruxolitinib as

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GVHD prophylaxis. Recipients of MUD HCT will receive rATG for additional immunosuppression as is standard for unrelated donor transplants
Study Design: Phase II
Sample Size: We anticipate 32 enrollees to yield 30 evaluable research participants.
Data Management: Data management and statistical analysis will be provided locally by the Department of Bone Marrow Transplantation and Cellular Therapy and the Department of Biostatistics at St. Jude Children's Research Hospital
Human Subjects: The risks to participants are primarily related to the conditioning regimen and post-transplant complications. The proposed regimen and donor cells may induce serious and possibly fatal disorders such as GVHD, veno-occlusive disease, and post-transplant lymphoproliferative disease. Because of the required conditioning, recipients may be at high-risk for serious and possibly life-threatening infection, bleeding, and anemia. Adverse events will be treated, monitored, and reported as per the Department of Bone Marrow Transplantation and Cellular therapy SOP for recipients of allogeneic HCT.

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1.0 OBJECTIVES

1.1 Primary objectives

- To estimate the incidence of severe acute GVHD (saGVHD) using a prophylaxis regimen with no calcineurin inhibitors after day +60 post first allogeneic Human Leukocyte antigen (HLA)-matched sibling or unrelated donor HCT for hematological malignancies.

1.2 Secondary objective

1.2.1 Determine the cumulative incidence of relapse, NRM, chronic GVHD, and OS in study participants at one year post-transplant.

1.3 Exploratory objectives

1.3.1 To evaluate the pharmacokinetic/pharmacodynamic (PK/PD) profiles of ruxolitinib, fludarabine, and rATG.

1.3.2 To assess immune reconstitution in study participants within the first year post-HCT.

2.0 BACKGROUND AND RATIONALE

2.1 Hypothesis to be tested in this study

While outcomes of MSD/MUD HCTs have improved, severe acute GVHD (saGVHD), defined as grade II-IV GVHD, remains a significant clinical problem. CNIs (for example, cyclosporine (CsA) or tacrolimus) in combination with methotrexate are commonly used for GVHD prophylaxis. However, their use can be associated with significant side effects, and are only effective in a subset of patients. Further, CNIs inhibit the graft-vs.-leukemia (GVL) effect increasing the risk of relapse which occurs in a quarter of recipients and is the most common cause of death post-HCT for children with hematological malignancies.

The central hypothesis is that it may be possible to safely stop CNIs early on day +60 post allogeneic HCT by adding ruxolitinib after engraftment on day +40. CNIs are currently stopped on day +100. Early discontinuation of CsA will help decrease toxicities. Replacement with ruxolitinib may decrease the incidence of saGVHD within the first 100 days post-transplant as ruxolitinib has potent effects in treating GVHD. Ruxolitinib also does not impair the GVL effect; this may decrease the risk of relapse.

Ruxolitinib is approved for treatment of saGVHD in children 12 years of age and older, hence only children in this age group will be eligible to participate in the study.

2.2 HCT donor types and conditioning regimens

2.2.1 Matched sibling and matched unrelated donor HCT for hematological malignancies

Most patients referred for allogeneic HCT lack an HLA-MSD, thus an alternative HLA-MUD must be identified. As advances in supportive care and donor selection have improved, HCT outcomes using MUD have been

compared to those using MSD. A single center retrospective analysis of 87 children with ALL in second complete remission (CR2) showed comparable outcomes with MSD and MUD HCT.[1] Transplant outcomes for children with T-ALL in CR2 as reported by the Center for International Blood and Marrow Transplant Research (CIBMTR) showed no difference in relapse, GVHD or disease-free survival rates based on graft source.[2] A multi-center retrospective analysis from Europe analyzing the influence of stem cell source on transplant outcomes for children with AML showed no difference in relapse, GVHD, NRM or OS based on donor source.[3] The addition of rATG to GVHD prophylaxis with MUD donors has resulted in comparable GVHD rates between the 2 groups.[4] Hence MSD and MUD HCT will be analyzed together in this study.

2.2.2 Total body irradiation/cyclophosphamide preparative regimen for ALL

A CIBMTR analysis of children with ALL receiving MSD HCT compared outcomes with TBI/Cy (451 patients) and busulfan/cyclophosphamide (BuCy; 176 patients). The 3-year probability of leukemia-free survival was significantly higher in the TBI/Cy group. The risk of relapse was similar in the 2 groups, but NRM was higher in children receiving BuCy.[5] Similar inferior outcomes of BuCy conditioning compared to TBI/Cy were reported in a prospective trial conducted by the Pediatric Blood and Marrow Transplant Consortium (PBMTC).[6] A prospective phase III, multicenter, randomized controlled trial from Europe was stopped prematurely owing to significantly improved OS with TBI-based compared to chemotherapy-based conditioning in 417 children with high risk ALL.[7] Hence, we selected TBI/Cy for children with lymphoid malignancies.

2.2.3 Thiotepa, busulfan and fludarabine (TBF) preparative regimen for AML

The combination of intravenous fludarabine and busulfan (FluBu) is considered to be the standard conditioning regimen for older patients with AML.[8] A prospective multicenter randomized study by the Italian Bone Marrow Transplant Group (GITMO), comparing FluBu with BuCy in older patients with AML in remission, showed the cumulative incidence of relapse at 5 years to be 38% in both groups. However, NRM was reduced to 8% in the FluBu arm compared to 17% with BuCy.[9] PBMTC reported favorable results with a FluBu regimen with a NRM of 11% and relapse rate of 43%.[10]

Bartelink et al. in a retrospective European study found FluBu to be a less toxic regimen for children with AML with lower rates of infection, lung injury, veno-occlusive disease, and shorter duration of neutropenia.[11] Historically, our rates of NRM and relapse with the BuCy regimen using a bone marrow graft are 11% and 28%, respectively.

A modified FluBu regimen has been evaluated in which one day of busulfan was omitted, and thiotepa was added (TBF).[12] In a European multicenter retrospective comparison of FluBu and TBF in adults with AML in remission, TBF reduced the risk of relapse, and there was a trend for improved survival with comparable NRM.[13] Augmentation of thiotepa to myeloablative FluBu for

adults and children with AML reduced relapse rates from 46% to 14% without additional toxicity in a single-center retrospective study.[14]

Thus, we selected this conditioning regimen for children with myeloid or undifferentiated leukemia. Our hypothesis is that substituting FluBu for BuCy will decrease the NRM, and adding thiotepa will decrease relapse rates. Busulfan targeting will continue to be in the myeloablative range.

2.3 Graft-vs.-host disease

2.3.1 GVHD after myeloablative conditioning for hematological malignancies and prophylactic regimens

While HCT is a curative modality for children with certain hematological malignancies, acute GVHD limits its efficacy ultimately preventing broader application.[15] This life-threatening complication occurs when immunocompetent T-cells in the graft recognize the recipient (host) as foreign and mount an immune response to eliminate foreign antigen-bearing cells. Acute GVHD prophylaxis with CsA, and methotrexate is used in the majority of patients receiving allogeneic HCT based on a randomized trial showing efficacy.[16] Addition of pan T-cell depleting reagent rATG to the standard prophylaxis further decreased risk of acute GVHD in a subset of patients.[17]

2.3.2 Limitations of calcineurin inhibitors for GVHD prophylaxis

A third of our patients still develop acute GVHD with CNI-containing regimens and a fifth have saGVHD. Furthermore, some patients have contraindications to the use of CNIs, such as renal/hepatic dysfunction, veno-occlusive disease, thrombotic microangiopathy,[18] and posterior reversible encephalopathy syndrome.[19] CNI replaced by other agents for GVHD prophylaxis such as sirolimus in these situations increases the risk of veno-occlusive disease.[20] CNIs increase the risk for viral reactivation post -HCT.[21] This results in significant morbidity and mortality.

CNIs are metabolized by CYP 3A 4/5 and are also a substrate of P-glycoprotein. Hence interactions with azoles which are commonly used for antifungal prophylaxis, may result in erratic levels. Further, its pharmacokinetics are complex and highly variable resulting in wide inter-and-intra-patient variability. Variability in serum concentrations have been associated with increased risk for GVHD and CNI-therapy-related toxicities. CNIs are highly lipophilic, hence dosing in obese patients risks renal dysfunction owing to high levels or increased risk of acute GVHD with subtherapeutic levels Thus, CNIs are not ideal agents to prevent GVHD, and there is a need to establish more effective and better tolerated GVHD prophylactic regimens.

2.4 Relapse following HCT and strategies to decrease relapse

CNIs can also inhibit the Graft-vs.-Leukemia (GVL) activity of the transplanted immune cells, contributing to relapse which occurs in a fourth of our patients. The outcome after relapse is poor.[22] A second allogeneic HCT is a treatment option. Second transplantations are, however, more problematic than first ones owing to increased

NRM with cumulative organ toxicity and an even higher risk of a subsequent relapse. Hence GVHD prophylactic agents which do not inhibit the GVL effect are desirable.

2.5 Can Cyclosporine A be weaned early to reduce toxicity without increasing risk for GVHD?

In a retrospective study by Storb et al. 103 adults with hematological malignancies were transplanted with MSD (92 patients), or mis-matched related donors (11 patients).[23] GVHD prophylaxis was with CsA and methotrexate. By day **+60** patients who never had acute GVHD, or whose acute GVHD had resolved, were randomized to have CsA stopped (52 patients) or continued for 180 days (51 patients). With a median follow up of 9.3 years after transplant, patients in whom CsA was discontinued on day +60 had a significantly more rapid onset, *but not a significantly higher overall incidence of chronic GVHD* than those in whom the drug was stopped on day +180 (p = 0.26). NRM was comparable in both arms. They concluded that *CsA can safely be discontinued in patients who never had evidence of acute grade II-IV GVHD by day +60.* Patients who have acute grade II-IV GVHD prior to day +60 on our study *will not be eligible for early CsA wean* (Sec 5.1).

It must be noted that this study was performed more than 30 years ago and in an adult population at high risk for chronic GVHD (43-54%) compared to 4% in our historical cohort of children. GVHD rates have decreased substantially in the past 2 decades.[24] Further, all our patients will be fully matched at a much higher level of resolution.

In a retrospective study by Zhao et al. 10 adults with hematological malignancies who were intolerant to CNIs after allogeneic HCT *received ruxolitinib to replace CNI as GVHD prophylaxis*. Reasons for CNI replacement included thrombotic microangiopathy, renal dysfunction, hepatic injury and CNI-induced pain syndrome. The median interval from HCT to ruxolitinib was 35 days (range 28-45 days). After ruxolitinib replacement, toxicities resolved rapidly, and only 1 patient developed grade II skin GVHD within 100 days.[25]

2.6 Ruxolitinib for treatment of GVHD

The Janus Kinase (JAK)-signal transducer and activator of transcription (STAT) pathway is now recognized as an evolutionary conserved signaling pathway employed by diverse cytokines, interferons, growth factors and related molecules.[26]

JAK kinases have several domains. Ruxolitinib competitively inhibits the ATP-binding site of the kinase domain of JAK1 and JAK2. This was the first Jakinib approved by the FDA in 2011 for the treatment of myelofibrosis. It is an oral, reversible, class I inhibitor. Peak plasma concentrations are achieved within one hour after administration and decline in a monophasic or biphasic manner with a mean terminal half-life of 2.3 hours. Ruxolitinib selectively inhibits JAK1 and JAK2 with IC_{50} values of 3.3 and 2.8 nM, respectively.[27]

2.6.1 Ruxolitinib for treatment of GVHD in adults

saGVHD is a life-threatening complication after allogeneic HCT. Glucocorticoids are the choice for induction of remission. Patients with glucocorticoid-refractory (SR) aGVHD have long-term survival rates of only

5% to 30%.[28] While SR aGVHD is usually severe in degree, not all cases of saGVHD are steroid-refractory. Cytokines play a major role in aGVHD and a common denominator of many cytokines is the downstream activation of JAK1/2. Inhibition of JAK1/2 was tested as an approach to interfere with aGVHD in different mouse models and found to have activity.[29-31] Based on a retrospective multicenter survey where ruxolitinib showed promise in patients who had failed multiple previous therapies for SR aGVHD,[32] prospective trials were initiated including a single-arm phase II study (REACH1) showing activity of ruxolitinib *leading to FDA approval for SR aGVHD*.[33, 34] To clarify the question of whether ruxolitinib was superior to best available therapy (BAT), a multicenter randomized phase III trial was performed (REACH2).[35] The primary study objective was met: the ORR at day 28 was statistically significantly higher with ruxolitinib than with BAT (62% vs 39%; odds ratio, 2.64; 95% CI, 1.65-4.22; P < .001). Ruxolitinib is now being considered the gold standard in SR aGVHD.[36]

2.6.2 Ruxolitinib for treatment of GVHD in children

Ruxolitinib use in children was associated with an ORR of 45% in a retrospective study of 11 children with SR aGVHD. Ruxolitinib was administered orally at 5 mg twice daily for children ≥25 kg or 2.5 mg twice daily if <25 kg, and escalated weekly as tolerated to a maximum of 10 mg twice daily. Adverse events included transaminitis, neutropenia, and thrombocytopenia which were reversible with discontinuation of therapy.[37] Infectious complications were observed, and all were successfully treated with antimicrobial therapy.

In another retrospective study including 13 children with SR aGVHD, ORR was 77%. Dosing being similar, only one patient developed thrombocytopenia which was reversible.[38] Cytomegalovirus reactivation was successfully treated without discontinuation of ruxolitinib. Responders included those with sa gut GVHD suggesting good absorption and bioavailability in the setting of diarrhea and gastrointestinal bleeding.

A multicenter, retrospective study of 29 children who received ruxolitinib for SR aGVHD reported an ORR of 72.4%.[39] No cytopenias requiring ruxolitnib dose adjustment occurred and viral infections were noted in 41.4% of patients. The authors reported a wide range for ruxolitinib dosing, however, they did not find a correlation with dose and response in their study.[39]

In a prospective study of 17 children with SR aGVHD the ORR was 75%. Dosage was 10 mg twice daily for children >40 kg and 0.15 mg/kg twice daily for children weighing <40 kg.[40] More than half the patients had hematologic toxicity with this higher dosing regimen. A phase III trial of ruxolitinib for SR aGVHD in children is ongoing (NCT03491215). *Ruxolitinib is currently FDA approved for treatment of SR aGVHD in children 12 years of age and older. We will use the dose recommended for treatment (i.e., 5 mg twice daily).*

2.7 Ruxolitinib for prophylaxis of GVHD

2.7.1 Biology

Ruxolitinib prevents GVHD via suppression of inflammatory cytokine production. In murine GVHD models of fully MHC-mismatched HCT, TNF-α and IL-12 were significantly reduced in mice treated with ruxolitinib compared with vehicle.[41] Ruxolitinib also impairs differentiation of $CD4^+$ T cells into IFN-γ and IL-17A producing cells,[41] modulates chemokine receptor expression,[29] increases $FoxP3^+$ regulatory T cells,[28] and promotes Th2 differentiation. All these immunologic effects of ruxolitinib reduce GVHD, while preserving the GVL effect.[28,29]

2.7.2 Clinical experience

In a prospective study by Zhang et al. after alternative donor HCT for hematological malignancies, early administration of ruxolitinib as GVHD prophylaxis after engraftment in an experimental group of 41 adults significantly decreased the incidence of aGVHD compared to a control group of 16 patients who received post-transplant cyclophosphamide, Fresnius ATG, CNI and mycophenolate mofetil for prophylaxis. There was no adverse effect on hematopoiesis.[42]

In the REACH-1 phase II study investigating ruxolitinib for treatment of SR aGVHD, approximately half the patients were rescued, however, a substantial proportion of patients had limited benefit from ruxolitinib treatment, showing no clinical response and a very bad outcome. Our hypothesis is that early "prophylactic" use of ruxolitinib will target kinase activity and block the inflammatory cascade leading to T-cell activation and GVHD.

2.8 Ruxolitinib for treatment of refractory heme-malignancies

Oncogenic IL-7Rα gain of function mutations are found in 9% of individuals with T-ALL. This drives constitutive signaling via JAK1 and promotes cell transformation and tumor formation.[43] In vitro, treating a transformed cell line with ruxolitinib inhibited ligand-independent signaling and induced cell death. Transfer of the transformed cell line into mice resulted in aggressive leukemia. Mice treated with ruxolitinib showed a potent therapeutic benefit with reduction of leukemic burden and extension of survival.[44] In a phase II study of ruxolitinib in 18 adults with relapsed/refractory leukemias, ruxolitinib showed modest antileukemic activity with 3 of the patients with post-myeloproliferative neoplasms showing a significant response.[45] A GVHD prophylactic agent which has anti-leukemic activity may thus be more effective in preventing relapse than one which inhibits GVL activity of the transplanted immune cells.

2.9 Rationale for early discontinuation of CsA and replacement with ruxolitinib

The rationale for early discontinuation of CsA by day +60 rather than the standard practice of discontinuation by day +100 is supported by the retrospective study by Storb et al. who concluded that early withdrawal of CsA was safe.

The rationale for early discontinuation of CsA after engraftment and replacement with ruxolitinib is supported by the retrospective study by Zhao et al. and the prospective study by Zhang et al. detailed above where this practice allowed resolution of toxicities due to CsA without increasing risk of GVHD.

Our hypothesis is that early discontinuation of CsA will preserve renal function, decrease risk of viral reactivation, thrombotic microangiopathy, veno-occlusive disease, and inhibition of the GVL effect. Further, patients will receive standard GVHD prophylaxis early after HCT with close monitoring of CsA levels early after transplant. Optimum CsA levels early after transplantation has been shown to be important in reducing the risk of GVHD.[46] Replacement with ruxolitinib on day +40 will remove its potential for serious hematopoietic toxicity such as delayed engraftment; preservation of the GVL effect and activity against ALL may improve outcomes.

2.10 Rationale for PK/PD profiling

2.10.1 Ruxolitinib profiling

Pharmacodynamics of ruxolitinib will be evaluated by inhibition of pSTAT 3 and/or 5. Studies in healthy adult volunteers and children with solid tumors have shown good correlation with ruxolitinib plasma concentrations and inhibition of pSTAT 3 and/or 5.[47, 48] However, no PK/PD data exist for children post-HCT and there is a need to perform the proposed studies to help define an optimal exposure range to prevent GVHD and minimize hematologic toxicity as well as correlate exposure with pSTAT 3 and/or 5 inhibition.

2.10.2 rATG profiling

rATG has been incorporated into conditioning regimens to prevent GVHD after HCT.[49-51] Pre-treatment with rATG in a phase III, multicenter, open-label, randomized controlled trial in adults undergoing HCT led to a significant proportion of patients having freedom from immunosuppressive treatment, including steroid usage, at 12 months.[52] rATG is recommended for addition to preparative HCT regimens when using unrelated donors.[52] However, the use of weight-based dosing may result in rATG overexposure in heavier patients in the setting of lymphopenia resulting in higher NRM and lower OS in adults.[53]

rATG-induced T-cell depletion is unpredictable and may contribute to delayed immune reconstitution and increase both relapse and NRM.[54] The optimum range of rATG exposure after HCT with a non-myeloablative conditioning regimen in a retrospective adult study was determined to be 60-95 AU per day/mL. rATG exposure after HCT was shown to be the best predictor for 5-year OS. Below-optimum exposure increased NRM, whereas above optimum exposure led to higher relapse-related mortality.[54] Lower rATG exposure prior to HCT has also been shown to be associated with inferior relapse-free survival, whereas lower rATG exposure after HCT was associated with increased acute GVHD in adults undergoing myeloablative allogeneic HCT.[49] These studies suggest that both patient weight and absolute lymphocyte count (ALC) prior to rATG infusion may affect rATG PK and exposure.[49,54]

An association was found between rATG exposure and CD4+ immune reconstitution in a multicenter retrospective PD analysis in children.[50] rATG exposure after HCT was predictive of successful immune reconstitution at day +100 with exposure exceeding 100 AU per day/mL noted to have decreased immune reconstitution in recipients of a bone marrow or peripheral blood HCT. This finding is consistent with the optimal range suggested in the previously described retrospective adult study. Pre-transplant rATG exposure was associated with both acute and chronic GVHD as well as graft failure with improved outcomes with an exposure of at least 40 AU per day/mL.[50] A population PK model of rATG in children receiving allogeneic HCT revealed that a cumulative dosing regimen of 10 mg/kg resulted in higher exposure in children with a higher bodyweight and/or lower ALC pre-rATG infusion, which was consistent with adult PK studies.[51]

We will prospectively study rATG PK/PD to characterize exposure in our patient population to correlate with transplant-related outcomes (e.g. GVHD, immune reconstitution, relapse, NRM) and to identify patient-specific parameters that affect exposure and may be used to inform individualized or optimized dosing in future protocols.

2.10.3 Fludarabine profiling

Fludarabine is the most frequently used agent in conditioning regimens for allogeneic HCT. Despite the use of body surface area-based dosing, high variability in fludarabine exposure has been demonstrated.[55, 56] A retrospective analysis between fludarabine exposure and clinical outcomes was conducted in a study with adults and children undergoing myeloablative conditioning with a FluBu regimen.[56] The relationship between fludarabine exposure and 2-year EFS was evaluated. The optimal fludarabine exposure was determined to be a cumulative AUC of 20 mg*h/L ± 5 in this analysis. EFS was lower in the overexposed group due to higher NRM associated with impaired immune reconstitution. The risks of graft failure were increased in the underexposed group. No relationship with relapse was found. This analysis suggests individualized fludarabine dosing based on weight and renal function or therapeutic drug monitoring may improve EFS.[55, 56]

A prospective, multicenter study characterizing the PK/PD of fludarabine in children underdoing HCT with a fludarabine containing regimen was conducted.[57] The highest OS and EFS were observed in patients with a cumulative exposure ranging from 15 mg*h/L to 19 mg*h/L, which is consistent with the previously described study.[56, 57]

We will prospectively study fludarabine PK/PD to characterize the exposure in our patient population and correlate exposure with transplant-related outcomes. These evaluations will help validate these relationships and help define the optimal exposure in children undergoing HCT as well as identify patient-specific parameters that affect exposure and may be used to inform individualized or optimized dosing in future protocols. We hypothesize that

survival after HCT may be improved with individualized dosing of fludarabine in future studies.

2.11 Risk-benefit analysis of the study

Early withdrawal of CsA has been shown to be safe and may prevent toxicities from its prolonged use. Adding ruxolitinib on day +40 removes its potential for serious hematopoietic toxicity such as delayed engraftment. *Additionally, ruxolitinib has been found have a good safety profile in the REACH studies and is FDA approved for treatment of SR aGVHD in children 12 years of age and older*. Its potent activity in preventing GVHD may reduce risk of saGVHD. Since it does not impair the GVL effect and has anti-leukemic effect for some heme malignancies, its use early post-HCT may decrease the risk for relapse, the most important cause of death after HCT for hematological malignancies.

2.12 Impact of study on field of HCT

Determining the efficacy and safety of a regimen with CsA, methotrexate and ruxolitinib, with/without rATG as GVHD prophylaxis will pave the way for a future randomized controlled trial comparing this regimen with the standard regimen for GVHD prophylaxis using CsA and methotrexate with or without rATG. In addition, the proposed PK/PD studies for ruxolitinib, fludarabine, and rATG will have a broad impact on other transplant protocols. Individualized dosing may maximize efficacy and minimize adverse effects leading to a decrease in NRM and improved OS. Likewise, we expect that the gained insights from the conducted immune reconstitution studies will inform future transplant studies.

3.0 PARTICIPANT 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. However, pregnant and lactating females are excluded from participation as the short and long-term effects of the preparative agents, cellular infusion, and GVHD prophylaxis on a fetus and a nursing child through breast milk are not entirely known at this time.

3.1 Inclusion criteria

- Diagnosis:
  - Patients with high risk acute lymphoblastic leukemia in first remission. Examples include, but are not limited to, patients with certain leukemic cell cytogenetic findings (e.g. t(9;22) or t(4;11)); delayed response to induction chemotherapy; re-emergence of leukemic blasts by MRD (at any level) in patients previously MRD negative; persistently detectable MRD at lower levels; early T-cell precursor (ETP) ALL.
  - Patients with acute lymphoblastic leukemia beyond first remission.

  - Patients with Hodgkin's disease beyond first remission or with refractory disease.
  - Patients with chronic myelogenous leukemia.
  - Patients with primary or secondary myelodysplastic syndrome.
  - Patients with Non-Hodgkin's lymphoma beyond first remission or with refractory disease.
  - Patients with de novo acute myeloid leukemia in or beyond first remission or with relapsed or refractory disease, or myeloid sarcoma (extra-medullary AML).
  - Patients with secondary acute myeloid leukemia.
  - NK cell lymphoblastic leukemia in any CR.
  - Biphenotypic, bilineage, or undifferentiated leukemia.
  - Juvenile Myelomonocytic Leukemia (JMML).
- Patients must have a related or unrelated donor matched at 12 of 12 HLA alleles.
- Patients must have a Karnofsky/Lansky score of 70 or higher.
- Patients must be 12 years of age or older.
- Patients must have a shortening fraction >26% or left ventricular ejection fraction >40%.
- Patients must have bilirubin less than or equal to 2.5 mg/dL and alanine aminotransferase (ALT) less than or equal to 5 times the upper limit of normal.
- Patients must have creatinine clearance, or a glomerular filtration rate (GFR), greater than 70 mL/min/1.73m^2.
- Patients must be free of severe infection that upon determination of principal investigator precludes BMT.
- Patients must have FVC >50% predicted OR, if unable to perform pulmonary function testing, must maintain pulse oximetry oxygen saturation >92% on room air.
- Female patients of childbearing age must have a negative pregnancy test.
- All patient with prior evidence of CNS leukemia must be treated and be in CNS CR.

3.2 Exclusion criteria

- Patients who have undergone prior HCT.
- Patients who have a peripheral blood stem cell graft source.
- Patients who have a non-permissive mismatch at the DPB1 allele.
- Patients who are HIV positive.
- Patients positive for Hepatitis B surface antigen (HBsAg).
- Patients positive for Hepatitis C.
- Patients with latent tuberculosis with positive TB IFN gamma release assay.

3.3 Research participant recruitment and screening

This study will be posted on http://clinicaltrials.gov. Potential transplant recipients are typically referred from their primary clinical service at St. Jude, from the Physician Referral Office for non-St. Jude patients, or are patients currently being treated by the Department of BMTCT. Each patient considered for transplantation is registered on the "Transplant List," an ongoing list used to provide the clinical service with information regarding new patients for consultation and under consideration for transplantation and the status of those undergoing pre-evaluation. The patient remains on this list until a decision has been confirmed for acceptance or rejection based on several factors, primarily completion of prior therapy and/or clinical status. The proposed patient schedules on this form are often subject to change due to various reasons such as family travel issues, donor availability for procurement of progenitor cells, recipient clinical status, etc. This list is a confidential document, updated and maintained by the Department of BMTCT Coordinators.

3.4 Enrollment on study at St. Jude

A member of the study team will confirm potential participant eligibility as defined in Sections 3.1-3.2, and register the participant in OnCore. A research participant-specific consent form and assent document (where applicable) will be generated. The entire signed consent/assent form(s) must be scanned into the Electronic Health Record (EHR) by the study team designee.

4.0 DESIGN AND METHODS

4.1 Design and study overview

This is a phase-II study with development of saGVHD within 100 days post-HCT as the primary endpoint. Proportion (probability) of aGVHD and saGVHD events within 100 days post-HCT, will be estimated. GVHD will be defined using consensus criteria.

5.0 TREATMENT PLAN

5.1 Treatment

Donor Selection

Bone marrow grafts will only be used. The graft will not be T-cell depleted. The donor should be sufficiently healthy not to be at increased risk from the marrow harvest. Therefore donors will undergo evaluation for suitability and eligibility for collection in accordance with departmental guidelines and as outlined in 21 CFR 1271 and agency guidance. HLA-identical sibling donors will be considered first followed by histocompatible relatives or unrelated donors (matched at 12 of 12 HLA-A, B, C and DRB1, DRB3/4/5, and DQB1 alleles). Permissive DPB1 mismatched grafts will be included. Unrelated grafts will be obtained through the NMDP or other cooperative registries.

Preparative Regimens

We have selected TBI/Cy as the backbone conditioning regimen for patients with lymphoid malignancies, and thiotepa, busulfan, and fludarabine (TBF) for patients with myeloid, bi-phenotypic, bilineage or undifferentiated leukemia. In addition, patients will receive the TBF regimen for whom TBI is not appropriate.

TBI/Cy preparative regimen

The TBI/Cy preparative regimen is summarized in **Table 1**. The dose of cyclophosphamide will be 60 mg/kg/day for 2 days. TBI, total dose 12 Gy, will be delivered in 8 fractions of 150 cGy each, two fractions (fxn) per day (typically separated by 6 hours). The dose rate will be <24 cGy/min. Partial pulmonary shielding will be used. Males with lymphoid lineage leukemia will receive an additional 200 cGy x 2 (400 cGy) testicular boost concurrent with TBI. Patients who receive a bone marrow product from MSD will not receive rATG. They will have a rest day on Day -1.

Table 1: TBI/Cy preparative regimen

DAY	MEDICATION	DOSE	DOSE #
-7	TBI	150 cGy x 2 fxn BID (<24 cGy/min)	1 & 2 of 8
-6	TBI	150 cGy x 2 fxn BID (<24 cGy/min)	3 & 4 of 8
-5	TBI	150 cGy x 2 fxn BID (<24 cGy/min)	5 & 6 of 8
-4	TBI	150 cGy x 2 fxn BID (<24 cGy/min)	7 & 8 of 8
-3	Cyclophosphamide	60 mg/kg/day IV	1 of 2
	Rabbit ATG*	1 mg/kg/day IV	1 of 3
-2	Cyclophosphamide	60 mg/kg/day IV	2 of 2
	Rabbit ATG*	3 mg/kg/day IV	2 of 3
-1	Rabbit ATG*	3 mg/kg/day IV	3 of 3
0	Bone Marrow infusion		1 of 1

*Only for MUD HCT

Thiotepa, Busulfan and Fludarabine (TBF) preparative regimen

The TBF preparative regimen is summarized in **Table 2**. The dose of thiotepa will be 5 mg/kg/day for 2 days. This will be followed by busulfan given at a dose of 3.2 mg/kg/day and fludarabine 50 mg/m^2/day for 3 days. Patients who receive a bone marrow product from a MSD will not receive rATG. They will have a rest day on Day -1.

Table 2: TBF preparative regimen

DAY	MEDICATION	DOSE	DOSE #
-6	Thiotepa	5 mg/kg/day IV	1 of 2
-5	Thiotepa	5 mg/kg/day IV	2 of 2
-4	Busulfan	3.2 mg/kg/day IV	1 of 3
	Fludarabine	50 mg/m^2/day IV	1 of 3
-3	Busulfan*	3.2 mg/kg/day IV	2 of 3
	Fludarabine	50 mg/m^2/day IV	2 of 3
	Rabbit ATG**	1 mg/kg/day IV	1 of 3
-2	Busulfan*	3.2 mg/kg/day IV	3 of 3
	Fludarabine	50 mg/m^2/day IV	3 of 3
	Rabbit ATG**	3 mg/kg/day IV	2 of 3
-1	Rabbit ATG**	3 mg/kg/day IV	3 of 3
0	Bone Marrow infusion		1 of 1

*Dosing of intravenous busulfan may be based on targeted levels (cumulative AUC goal of 75 mg*h/L ± 5).
**Only for MUD HCT

Graft-versus-Host Disease Prophylaxis

The regimen for GVHD prophylaxis will include the combination of CsA, methotrexate and ruxolitinib. rATG will be included for patients receiving a MUD HCT.

Cyclosporine will be initiated on day -1 at 3 mg/kg continuous intravenous infusion and adjusted to maintain a goal steady state concentration within 250-350 ng/mL. Once stable CsA concentrations are achieved on continuous infusion, CsA may be transitioned to intermittent administration every 12 hours (either IV or PO) to maintain a goal trough concentration within 175-250 ng/mL. For patients without GVHD, CsA will be tapered beginning day +42 and discontinued by day +60. Patients who have evidence of grade II-IV acute GVHD before day +60 will not be eligible for early CsA wean. Most likely, these patients may also require additional immunosuppressive agents and steroids as outlined in our SOP 20.01 “Acute GVHD”.

Methotrexate will be given at a dose of 10 mg/m^2/dose intravenously for four doses given on days +1, +3, +6, and +11. Leucovorin rescue will be administered starting approximately 12 hours after each dose of methotrexate per institution standard.

Ruxolitinib will be administered beginning day +40 at 5 mg twice daily. For patients without GVHD, ruxolitinib will be tapered beginning at approximately day +100 (+/- 1 week) over 2 weeks. The ruxolitinib dose will be reduced by 50% to 5 mg daily for 2 weeks, prior to discontinuation.

If at any point prior to initiation of ruxolitinib or during ruxolitinib treatment, whether still on CsA or not, the patient develops evidence of grade II-IV GVHD; the patient will be considered off therapy (but remain on study) and receive immunosuppressive therapy as outlined by SOP 20.01 "Acute GVHD".

Cellular Infusion Procedures and Monitoring

For the proper infusion procedures and monitoring of the HPC product please refer to BMTCT 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". Please note that all relevant SOPs can be found on the BMTCT Clinical Transplant Program intranet page: https://home.stjude.org/bmt/Pages/policies-transplant-program.aspx

Importantly, during the cellular infusions, monitoring of vital signs, breath sounds, heart rate, pulse oximetry, and I/O will be done per the established transplant nursing procedure, as well as appropriate Department of BMTCT SOPs, then documented on the Cellular Product Infusion Record. If a reaction is suspected at any time during the infusion, the nurse will 1. Stop the infusion, 2. Notify the Attending Transplant Physician immediately, 3. 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.

General Treatment Related Comments

- The bone marrow infusion may be delayed by approximately 24 hours in order to accommodate collection with the donor and/or HAL as well as the research participant clinical condition.
- The term "day" or "daily" used in tables is an approximate term meaning that these medications noted will be administered approximately every 24 hours. The drug administration timing may be modified by approximately +/- 4 hours or as clinically indicated such as to accommodate surgical procedures, radiation therapy, administration of other necessary medications, blood product delivery, or procedures (such as a needed CT scan). The term "day" or "daily" refers to a general 24-hour period.
- Dosing for the medications busulfan, cyclophosphamide, fludarabine and thiotepa may be modified for research recipients based upon actual body weight or adjusted ideal body weight when clinically indicated. Dose rounding at St. Jude will be allowed according to institutional policy (See St. Jude Policy # 20.109, Institutional Policy and Procedure Manual).
- Criteria for medication calculations based on body weight/body surface area and other medication related information can be found in the St. Jude Formulary http://www.crlonline.com/crlsql/servlet/crlonlineor the St. Jude Dept of Pharmaceutical Sciences intranet website https://home.stjude.org/pharmaceutical-

services/Pages/default.aspx. Medication doses may be rounded to the nearest integer or to the nearest appropriate quantity when clinically or pharmaceutically indicated as per the MD and PharmD.

Quality Assurance of Cellular Products

Quality assurance for cell products is overseen by the TPQ Quality Assurance division, which authorizes release of all products. Only trained cell processors will process the cell products. A labeling and product tracking system is in place to ensure that the correct cells are infused into the research participant.

Assays of cell numbers and immunophenotyping will be performed both before cell processing and at critical stages of the process. These values will be recorded according to SOP of the HAL. All products will be tested for viability and sterility (culture and Gram stain). Culture results are not available before infusion of cell products. If the gram stain is positive, the research participant/parent and/or guardian will be informed of this event and of the risks of proceeding prior to infusion. Positive results will be investigated as per the variance procedures of the HAL. The IRB and FDA will be notified, if at any time after infusion, cell product was determined to be contaminated.

5.2 Supportive care

Antimicrobial prophylaxis: All patients are required to start anti-pneumocystis jiroveci pneumonia (PJP), anti-fungal and anti-viral prophylaxis post-HCT as per BMTCT SOP. Acyclovir prophylaxis will be given to HSV and VZV seropositive patients. Surveillance for Epstein Barr virus (EBV), adenovirus (ADV), and cytomegalovirus (CMV) and pre-emptive treatment for viral reactivation will be done as per BMTCT SOP.

Initiation of azole prophylaxis: Micafungin prophylaxis may be replaced with azoles after neutrophil engraftment for better coverage against *Aspergillus spp.* Azoles will be used after day +8 in patients with prior history of proven or probable invasive fungal infections.

Use of growth factors: Use of GCSF is not recommended except after day +21 when a single dose not exceeding 2.5 mcg/kg may be given after rounding off if the absolute phagocytic count (APC) is >500 cells/mm^3, and ANC is <500 cells/mm^3 to mobilize cells into the mature compartments.

Hemorrhagic cystitis prophylaxis: The medication cyclophosphamide is known to increase the risk of hemorrhagic cystitis. Mesna (60 mg/kg; divided into 5 doses) will be administered approximately 15 minutes prior to each dose of cyclophosphamide and approximately 3, 6, 9, and 12 hours after each dose of cyclophosphamide. Mesna dose and administration schedule may vary based on physician or PharmD recommendation.

Seizure prophylaxis: The medication busulfan is known to increase the risk of seizures. Levetiracetam 10 mg/kg/dose every 12 hours (max 1000 mg/dose) starting 24 hours prior to the first dose of busulfan and continuing until at least 48 hours after the last dose will be prescribed as seizure prophylaxis for the transplant recipients receiving busulfan.

Pre-medication for rATG: Reactions to rATG administration are well documented and, in the most severe cases, include anaphylaxis. Pre-medications, including an antihistamine, a corticosteroid, and an antipyretic, will be given to prevent such severe reactions. NOTE: Patients receiving rATG on the TBF regimen will NOT be able to receive acetaminophen as a pre-medication due to interference with busulfan clearance.

5.3 Ruxolitinib dose modification

Concomitant CYP3A4 inhibitors/inducers: reduce ruxolitinib dose by 50% when administered with strong CYP3A4 inhibitors (e.g., voriconazole, posaconazole, etc.); use of CYP3A4 inducers should be avoided if possible.

Hematologic toxicity:

ANC <500 cells/mm^3 after day +40: reduce ruxolitinib dose by 50%*.

Hemoglobin less than 7gm% after day + 40 reduce ruxolitinib dose by 50%.

Platelet count less than 20,000 after day +40: reduce ruxolitinib dose by 50%.

Renal dysfunction: CrCl 15-59 mL/min: reduce ruxolitinib dose by 50%; Avoid use if CrCl < 15 mL/min and not on dialysis

Liver dysfunction: total bilirubin >3 x ULN: reduce ruxolitinib dose by 50% until recovery.

*A reduction in ruxolitinib dose by 50% is 5mg once daily.

Additionally, we will include stopping rules for ruxolitinib administration for the following reasons:

- ANC remains <500 cells/μL 2 weeks after the dose has been reduced by 50%.
- Secondary graft failure due to other causes with ANC <500 cells/μL and bone marrow cellularity <5%
- Uncontrolled infection

6.0 DRUG/DEVICE/BIOLOGIC AGENT INFORMATION

6.1 Medications

Anti-thymocyte globulin (rabbit) (Thymoglobulin®, rabbit ATG)	
Source & Pharmacology	Anti-thymocyte globulin is a purified, pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed on human T-lymphocytes. The mechanism of action by which polyclonal anti-lymphocyte preparations suppress immune responses is not fully understood. Possible mechanisms by which anti-thymocyte globulin may induce immunosuppression in vivo include: T-cell clearance from the circulation and modulation of T-cell activation, homing, and cytotoxic activities. Anti-thymocyte globulin (rabbit) includes antibodies against T-cell markers such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, HLA Class 1

	heavy chains, and β2 microglobulin. T-cell depletion is usually observed within a day from initiating anti-thymocyte globulin therapy.
Formulation and Stability	Anti-thymocyte globulin (refrigerated) is available as sterile, lyophilized powder to be reconstituted with sterile diluent (both lyophilized powder and diluent should be at room temperature before reconstitution). Reconstituted solutions provide a final concentration of 5 mg/mL x 5 mL. The product must be further diluted in normal saline prior to administration. Infusion solutions may be administered over 2 to 6 hours depending on dose and should be prepared immediately prior to administration.
Supplier	Commercially available
Toxicity Information	Frequently reported events include fever, chills, leukopenia, pain, headache, abdominal pain, diarrhea, hypertension, nausea, thrombocytopenia, peripheral edema, dyspnea, asthenia, hyperkalemia, tachycardia. The most serious toxicity is that of anaphylaxis. The full dose must be administered over at least 4 hours and the patient pretreated with antihistamine, corticosteroid, and antipyretic. Supportive medical resources must be readily available for patient management. Anaphylaxis precludes further administration of the drug.
Dosage and Route of Administration	1 mg/kg test dose followed by 2 additional consecutive days of 3 mg/kg, (7 mg/kg total dose); intravenous.

Busulfan (Myleran®, Busulfex)	
Source & Pharmacology	Busulfan is a polyfunctional alkylating agent. It interferes with the normal function of DNA by alkylating intracellular nucleophiles and cross linking DNA strands. It is cell cycle phase non-specific. It is well absorbed orally and is metabolized by the liver. Drugs that induce hepatic metabolism (e.g., phenytoin) increase clearance and those that inhibit hepatic metabolism (e.g., itraconazole) may decrease clearance. The plasma half life is ≈ 2.5 hours in adults, but children may have higher clearances.
Formulation and Stability	Busulfan is available as a 2 mg tablet and as a solution for intravenous administration.
Supplier	Commercially available.
Toxicity Information	Acute dose limiting toxicity is myelosuppression including leukopenia, thrombocytopenia, and anemia. This effect is delayed with a nadir of 14-21 days. Some patients may develop bone marrow fibrosis. Nausea and vomiting are generally mild. Other GI symptoms include diarrhea, anorexia and associated weight loss. Seizures are associated with high doses. Other side effects include liver dysfunction, skin hyperpigmentation, skin rash, gynecomastia, sterility, cataracts, and alopecia. Secondary cancers have occurred. "Busulfan lung", manifested

	by diffuse interstitial pulmonary fibrosis, persistent cough, fever, rales, and dyspnea may occur, most commonly after high doses or prolonged therapy.
Dosage and Route of Administration	Based on targeted levels (cumulative AUC of 75 mg*h/L ± 5), initial dose at 3.2 mg/kg/day; intravenous.

Cyclophosphamide (Cytoxan)	
Source & Pharmacology	Cyclophosphamide is a nitrogen mustard derivative. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. It is cell cycle phase non-specific. Cyclophosphamide is well absorbed from the GI tract with a bioavailability of >75%. It is a prodrug that requires activation. It is metabolized by mixed function oxidases in the liver to 4-hydroxycyclo-phosphamide, which is in equilibrium with aldophosfamide. Aldofosfamide spontaneously splits into nitrogen mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxy-cyclophosphamide may be enzymatically metabolized to 4-keto-cyclophosphamide and aldophosfamide may be enzymatically metabolized to carboxyphosphamide that is generally considered inactive. Cyclophosphamide and its metabolites are excreted mainly in the urine. Dose adjustments should be made in patients with a creatinine clearance of <25 mL/min.
Formulation and Stability	Cyclophosphamide is available in vials containing 100, 200, 500, 1000, and 2000 mg of lyophilized drug and 75 mg mannitol per 100 mg of cyclophosphamide. Both forms of the drug can be stored at room temperature. The vials are reconstituted with 5, 10, 25, 50, or 100 mL of sterile water for injection, respectively, to yield a final concentration of 20 mg/mL. Reconstituted solutions may be further diluted in either 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically stable for 24 hours at room temperature and 6 days if refrigerated, but contain no preservative, so it is recommended that they be used within 24 hours of preparation.
Supplier	Commercially available
Toxicity Information	Dose limiting toxicities of cyclophosphamide include myelosuppression and cardiac toxicity. Cardiac toxicity is typically manifested as congestive heart failure, cardiac necrosis, or hemorrhagic myocarditis and can be fatal. Hemorrhagic cystitis may occur and necessitates withholding therapy. The incidence of hemorrhagic cystitis is related to cyclophosphamide dose and duration of therapy. Forced fluid intake and/or the administration of mesna decreases the incidence and severity of hemorrhagic cystitis. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on dosage), diarrhea, anorexia, alopecia, immunosuppression, and sterility. Pulmonary fibrosis, SIADH, anaphylaxis, and secondary neoplasms have been reported rarely.

Dosage and Route of Administration	60 mg/kg for 2 consecutive days, (120 mg/kg total dose); intravenous.

Cyclosporine (Gengraf)	
Source & Pharmacology	Cyclosporine (cyclosporin A) is a cyclosporin immunosuppressive agent produced as a metabolite of the fungus species *Aphanocladium album* or *Beauveria nivea*. The drug is a nonpolar, cyclic polypeptide antibiotic consisting of 11 amino acids. Cyclosporine is one of several biologically active antibiotics (cyclosporins) produced by these fungi; cyclosporin A and C are the major metabolites. Cyclosporine occurs as a white or essentially white, finely crystalline powder. The drug is relatively insoluble in water, having an aqueous solubility of 0.04 mg/mL at 25°C, and is generally soluble in lipids and organic solvents, having a solubility of more than 80 mg/mL in alcohol at 25°C. The potency of cyclosporine is determined on the anhydrous basis; each mcg of cyclosporine is defined as the activity (potency) contained in 1.0173 mcg of the FDA's cyclosporine master standard.
Formulation and Stability	Cyclosporine concentrate for injection occurs as a clear, faintly brownish-yellow solution. Cyclosporine concentrate for injection is a sterile solution of the drug in polyoxyl 35 castor oil (Cremophor® EL, polyethoxylated castor oil) with 32.9% (v/v) alcohol. At the time of manufacture, the air in the ampuls of cyclosporine concentrate for injection is replaced with nitrogen. The concentrate for injection contains no more than 42 USP endotoxin units per mL. Cyclosporine also is commercially available as a modified, nonaqueous liquid formulation of the drug that immediately forms an emulsion in aqueous fluids; the formulation is available as an oral solution for emulsion and as oral 25- and 100-mg liquid-filled soft gelatin capsules containing the oral solution for emulsion. When exposed to an aqueous environment, the oral solution for emulsion forms a homogenous transparent emulsion with a droplet size smaller than 100 nm in diameter; as a result, the formulation has been referred to as an oral solution for microemulsion. In this formulation, the molecular structure of cyclosporine is unaltered, and aqueous dilution results in formation of an emulsion without reprecipitation of the drug. Cyclosporine is dispersed in a mixture of propylene glycol (hydrophilic solvent) and corn oil monoglycerides, diglycerides, and triglycerides (lipophilic solvent); when dispersed, polyoxyl 40 hydrogenated castor oil serves as a surfactant, and d,l-α-tocopherol is present as an antioxidant. Cyclosporine oral solution and liquid-filled capsules also contains dehydrated alcohol in a maximum concentration of 11.9% (v/v).
Supplier	Commercially available.
Toxicity Information	The principal adverse reactions of cyclosporine therapy are renal dysfunction, tremor, hirsutism, hypertension, and gum hyperplasia.

	Hypertension, which is usually mild to moderate, may occur in approximately 50% of patients following renal transplantation and in most cardiac transplant patients. The pathologic changes resembled those seen in the hemolytic-uremic syndrome and included thrombosis of the renal microvasculature, with platelet-fibrin thrombi occluding glomerular capillaries and afferent arterioles, microangiopathic hemolytic anemia, thrombocytopenia, and decreased renal function. Similar findings have been observed when other immunosuppressives have been employed post-transplantation. Hypomagnesemia has been reported in some, but not all, patients exhibiting convulsions while on cyclosporine therapy. Although magnesium-depletion studies in normal subjects suggest that hypomagnesemia is associated with neurologic disorders, multiple factors, including hypertension, high dose methylprednisolone, hypocholesterolemia, and nephrotoxicity associated with high plasma concentrations of cyclosporine appear to be related to the neurological manifestations of cyclosporine toxicity.
Dosage and Route of Administration	3 mg/kg; dose will be adjusted to maintain a steady concentration between 250-350 ng/mL or trough concentration between 175-250 ng/mL when transitioned to intermittent dosing.

Fludarabine (Fludara)	
Source & Pharmacology	Fludarabine phosphate is a synthetic purine nucleoside analog. It acts by inhibiting DNA polymerase, ribonucleotide reductase, and DNA primase by competing with the physiologic substrate, deoxyadenosine triphosphate, resulting in inhibition of DNA synthesis. In addition, fludarabine can be incorporated into growing DNA chains as a false base, thus interfering with chain elongation and halting DNA synthesis. Fludarabine is rapidly dephosphorylated in the blood and transported intracellularly via a carrier-mediated process. It is then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate form. Approximately 23% of the dose is excreted as the active metabolite in the urine (with dosages of 18-25 mg/m^2/day for 5 days). Renal clearance appears to become more important at higher doses, with approximately 41-60% of the dose being excreted as the active metabolite in the urine with dosages of 80-260 mg/m^2.
Formulation and Stability	Fludarabine is supplied in single-dose vials containing 50 mg fludarabine as a white lyophilized powder and 50 mg of mannitol. The intact vials should be stored under refrigeration. Each vial can be reconstituted by adding 2 mL of sterile water for injection resulting in a final concentration of 25 mg/mL. Because the reconstituted solution contains no antimicrobial preservative, the manufacturer recommends that it should be used within 8 hours of preparation. The solution should be further diluted in 5% dextrose or 0.9% NaCl prior to administration.
Supplier	Commercially available.
Toxicity Information	The major dose-limiting toxicity of fludarabine is myelosuppression. Nausea and vomiting are usually mild. Side effects reported commonly

	include anorexia, fever and chills, alopecia, and rash. Neurotoxicity can be manifested by somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness, and coma and is more common at high doses. Neurotoxicity is usually delayed, occurring 21-60 days after the completion of a course of therapy and may be irreversible. Side effects reported less commonly include diarrhea, stomatitis, increased liver function tests, liver failure, chest pain, arrhythmias, and seizures. Pulmonary toxicity includes allergic pneumonitis characterized by cough, dyspnea, hypoxia, and pulmonary infiltrates. Drug induced pneumonitis is a delayed effect, occurring 3-28 days after the administration of the third or later course of therapy. Administration of corticosteroids usually results in resolution of these symptoms.
Dosage and Route of Administration	50 mg/m^2 daily for 3 consecutive days (150 mg/m^2 total dose) intravenous.

Mesna (Mesnex)	
Source & Pharmacology	Mesna is a synthetic sulfhydryl (thiol) compound. Mesna contains free sulfhydryl groups that interact chemically with urotoxic metabolites of oxaza-phosphorine derivatives such as cyclophosphamide and ifosfamide. Oral bioavailability is 50%. Upon injection into the blood, mesna is oxidized to mesna disulfide, a totally inert compound. Following glomerular filtration, mesna disulfide is rapidly reduced in the renal tubules back to mesna, the active form of the drug. Mesna and mesna disulfide are excreted primarily via the urine.
Formulation and Stability	Mesna is available in 2 mL, 4 mL and 100 mL amps containing 100 mg/mL of mesna solution. The intact vials can be stored at room temperature. Mesna may be further diluted in 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically and chemically stable for at least 24 hours under refrigeration.
Supplier	Commercially available
Toxicity Information	Mesna is generally well tolerated. Nausea and vomiting, headache, diarrhea, rash, transient hypotension, and allergic reactions have been reported. Patients may complain of a bitter taste in their mouth during administration. Mesna may cause false positive urine dipstick readings for ketones.
Dosage and Route of Administration	Intravenous. Mesna is dosed based on the cyclophosphamide dose and generally administered in fractionated doses at approximately 20% of the total cyclophosphamide dose. It is generally given prior to and again at 3, 6, 9 and 12 hours following each dose of cyclophosphamide.

Methotrexate	
Source & Pharmacology	A folate analogue which reversibly inhibits dihydrofolate reductase, the enzyme that reduces folic acid to tetrahydrofolic acid. Inhibition of tetrahydrofolate formation limits the availability of one carbon fragments necessary for the synthesis of purines and the conversion of deoxyuridylate to thymidylate in the synthesis of DNA and cell reproduction. The polyglutamated metabolites of MTX also contribute to the cytotoxic effect of MTX on DNA repair and/or strand breaks. MTX cytotoxicity is highly dependent on the absolute drug concentration and the duration of drug exposure. MTX is actively transported across cell membranes. At serum methotrexate concentrations exceeding 0.1 μmol/mL, passive diffusion becomes a major means of intracellular transport of MTX. The drug is widely distributed throughout the body with the highest concentration in the kidney, liver, spleen, gallbladder, and skin. Plasma concentrations following high dose IV MTX decline in a biphasic manner with an initial half-life of 1.5-3.5 hours and a terminal half-life of 8-15 hours. About 50% is bound to protein. After oral administration, approximately 60% of a 30 mg/m^2 dose is rapidly absorbed from the GI tract, with peak blood levels at 1 hour. At doses >30 mg/m^2 absorption decreases significantly. Even at low doses, absorption may be very erratic, varying between 23% and 95%. The elimination of MTX from the CSF after an intrathecal dose is characterized by a biphasic curve with half-lives of 4.5 and 14 hours. After intrathecal administration of 12 mg/m^2, the lumbar concentration of MTX is ~100 times higher than in plasma (ventricular concentration is ~ 10% of lumbar concentration). MTX is excreted primarily by the kidneys via glomerular filtration and active secretion into the proximal tubules. Renal clearance usually equals or exceeds creatinine clearance. Small amounts are excreted in the feces. There is significant entero-hepatic circulation of MTX. The distribution of MTX into third-space fluid collections, such as pleural effusions and ascitic fluid, can substantially alter MTX pharmacokinetics. The slow release of accumulated MTX from these third spaces over time prolongs the terminal half-life of the drug, leading to potentially increased clinical toxicity.
Formulation and Stability	Methotrexate for Injection is available as a lyophilized powder for injection in 1000 mg vials. The powder for injection contains approximately 7 mEq sodium in the 1000 mg vial. Methotrexate for Injection is also available as a 25 mg/mL solution in 2, 4, 8, 10, and 40 mL preservative free vials and 2 and 10 mL vials with preservative. The 2, 4, 8, 10, and 40 mL solutions contain approximately 0.43, 0.86, 1.72, 2.15, and 8.6 mEq sodium per vial, respectively. The preserved vials contain 0.9% benzyl alcohol as a preservative. Sterile methotrexate powder or solution is stable at 20º-25ºC (68º-77ºF); excursions permitted to 15º-30ºC (59º- 86 Fº). Protect from light.
Supplier	Commercially available

Toxicity Information	The dose limiting toxicities of methotrexate are generally bone marrow suppression, ulcerative stomatitis, severe diarrhea, or acute nephrotoxicity. Toxicities reported frequently include nausea and vomiting, diarrhea, anorexia, alopecia, hepatic toxicity, and alopecia. Less common side effects include blurred vision, photosensitivity, anaphylaxis, headache, pneumonitis, skin depigmentation or hyperpigmentation, rash, vasculitis, and encephalopathy. During high-dose methotrexate therapy, most patients experience a transient decrease in GFR, but renal failure can occur, particularly if the patient does not receive urinary alkalinization and aggressive hydration before, during, and after receiving high dose methotrexate. Leucovorin rescue should be initiated within 48 hours of starting high-dose methotrexate and adjusted based on MTX levels to prevent bone marrow toxicity and mucositis. Leucovorin may also be necessary after IT administration, especially if IT methotrexate therapy is given to patients with renal dysfunction. Patients with Down Syndrome have a tendency to have delayed methotrexate clearance and a greater risk of toxicity, despite increased leucovorin rescue.
Dosage and Route of Administration	10 mg/m^2/dose for four doses given on days +1, +3, +6, and +11; intravenous. Doses may be omitted based on clinical status of patient (e.g., liver dysfunction, mucositis). Each case should be discussed with PI.

Ruxolitinib (Jakafi®)	
Source & Pharmacology	Ruxolitinib (INCB018424 phosphate, INC424, ruxolitinib phosphate) represents a novel, potent, and selective inhibitor of JAK1 (Janus kinase 1) (inhibition concentration 50% [IC50]=3.3 ± 1.2 nM) and JAK2 (IC50=2.8 ± 1.2 nM) with modest to marked selectivity against TYK2 (tyrosine kinase 2) (IC50=19 ± 3.2 nM) and JAK3 (IC50=428 ± 243 nM), respectively.
Formulation and Stability	Ruxolitinib (Jakafi®) is commercially available in the US in 5, 10, 15, 20, and 25 mg strength tablets. The tablet contains the active ingredient and may include the following commonly used excipients: microcrystalline cellulose, lactose, stearic acid, magnesium stearate, colloidal silicone dioxide, sodium starch glycolate, povidone, and hydroxylpropyl cellulose. All excipients are of US and EuPh compendial grade. The 5 mg (free base equivalent) and 25 mg (free base equivalent) tablets are packaged in HDPE bottles. Ruxolitinib has been shown to be stable for up to 6 months at 40°C and up to 24 months when stored at 25°C. Ruxolitinib may be taken either with food or without food. Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.
Supplier	Commercially available
Toxicity Information	The following adverse events were reported as common (occurring in at least 1%) or very common (occurring in at least 10%) side effects in

	patients who were treated with ruxolitinib for myelofibrosis (MF) or polycythemia vera: **Very common (at least 10%):** Anemia, thrombocytopenia, bruising, neutropenia, raised ALT and AST, hypercholesterolemia, hypertriglyceridemia, dizziness, headache, urinary tract infections, and weight gain, peripheral edema, hypertension, hypokalemia. **Common (more than 1% but less than 10%):** Flatulence, constipation, herpes zoster, hypertension. Non-melanoma skin cancers (NMSCs), including basal cell, squamous cell, and a rare and aggressive type of skin cancer called Merkel Cell Carcinoma has been reported in patients who took ruxolitinib. It is unknown whether this was due to ruxolitinib treatment, as many of these patients had been either diagnosed with non-melanoma skin cancer in the past or had previously been treated with hydroxyurea which is associated with multiple types of skin cancers. **Uncommon: (occurring in less than 1% of patients):** These events were uncommon but have occurred in patients with MF during ruxolitinib treatment and are potentially serious. • Tuberculosis (TB) has occurred in a small number of patients (less than 1%) with MF who were treated with ruxolitinib, but it is not known whether this was due to MF, ruxolitinib, or other factors that are known to increase the risk of tuberculosis (such as diabetes, bronchitis, asthma, smoking, emphysema, or steroid use. • About one week following interruption or discontinuation of ruxolitinib, some patients with MF experienced a return of symptoms (such as fatigue, bone pain, fever, itching, night sweats, weight loss, or an enlarged spleen). There have been cases of MF patients stopping ruxolitinib during another ongoing illness who became more severely ill, but it was not clear whether stopping ruxolitinib therapy contributed to the patients' conditions worsening. • A rare disease called progressive multifocal leukoencephalopathy (PML) has been reported during ruxolitinib treatment for MF. PML comes from a viral infection that causes brain damage and can be fatal. It is unknown whether this was due to ruxolitinib treatment since PML has occurred in patients with blood cancers, including MF, who were not treated with ruxolitinib. •The effect of ruxolitinib on viral replication in patients with chronic hepatitis B virus is unknown.
Dosage and Route of Administration	Oral. See Treatment and Dose Modifications sections of protocol. Ruxolitinib tablets should be administered orally BID, approximately every 12 hours, continuously. Ruxolitinib may be taken with or without food. If a patient vomits within 30 minutes following a dose of ruxolitinib,

	the dose should be repeated. If dose is vomited after more than 30 minutes following a dose of ruxolitinib, the dose will not be repeated. For patients unable to ingest tablets, ruxolitinib can be administered through a nasogastric tube (8 French or greater) as follows: • Suspend one tablet in approximately 40 mL of water with stirring for approximately 10 minutes • Within 6 hours after the tablet has dispersed, the suspension can be administered through a nasogastric tube using an appropriate syringe The tube should be rinsed with approximately 75 mL of water. The effect of tube feeding preparations on ruxolitinib exposure during administration through a nasogastric tube has not been evaluated.

Thiotepa (Triplex® by Immunex) (TESPA, TSPA)	
Source & Pharmacology	Thiotepa is a cell-cycle nonspecific polyfunctional alkylating agent. It reacts with DNA phosphate groups to produce cross-linking of DNA strands leading to inhibition of DNA, RNA and protein synthesis. Thiotepa is extensively metabolized in the liver to metabolites that retain activity, primarily triethylene-phosphoramide (TEPA). The main route of elimination is via the urine, mainly as metabolites; the elimination half-life of the thiotepa is 2.5 hours, and that of TEPA is 17.6 hours.
Formulation and Stability	Thiotepa is supplied in single-use vials containing 15 mg of lyophilized thiotepa, 80 mg NaCl and 50 mg $NaHCO_3$. The intact vials should be stored under refrigeration and protected from light. Each vial should be reconstituted with 1.5 mL of sterile water for injection to yield a concentration of 10 mg/mL. Further dilution with sterile water for injection to a concentration of 1 mg/mL yields an isotonic solution; if larger volumes are desired for intracavitary, intravenous infusion, or perfusion therapy, this solution may then be diluted with 5% dextrose or 0.9% NaCl containing solutions. The 10 mg/mL reconstituted solution is chemically stable when stored in the refrigerator for up to 5 days, however, it is recommended that solutions be prepared just prior to administration since they do not contain a preservative. Reconstituted solutions should be clear to slightly opaque: the solutions may be filtered through a 0.22 micron filter to eliminate haze.
Supplier	Commercially available; manufactured by Immunex
Toxicity Information	Dose limiting toxicity is myelosuppression. The leukocyte nadir may occur at any time from 10 to 30 days. Other toxicities include pain at the injection site, nausea and vomiting, anorexia, mucositis, dizziness, headache, amenorrhea, interference with spermatogenesis, and depigmentation with topical use. Allergic reactions, including skin rash and hives, have been reported rarely. Rare cases of apnea, hemorrhagic cystitis, and renal failure have occurred. Thiotepa is mutagenic, carcinogenic, and teratogenic in animals. Pregnancy category D.

Dosage and Route of Administration	5 mg/kg daily for 2 consecutive days, (10 mg/kg total dose); intravenous.

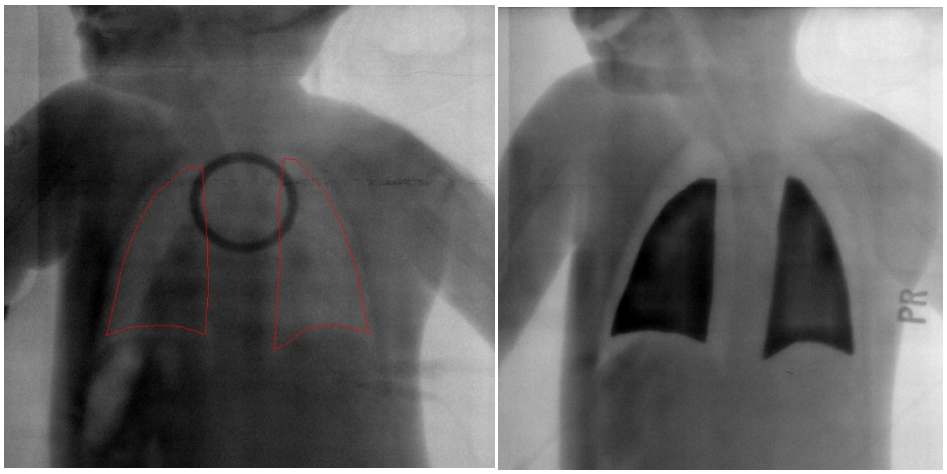
6.2 Total Body Irradiation (TBI)

Total body Irradiation (TBI) will be used in this clinical trial as a part of a conditioning regimen for patients with lymphoid malignancies.

6.2.1 Treatment Planning

Planning will be based on measurements obtained in the treatment room at the time of the "in room simulation". The following will be recorded:

- Patient thickness at umbilicus
- Distance from the source to the patient surface
- Distance from beam central axis to umbilicus
- Testicular treatment cutout size (may also be obtained during treatment) and testicular thickness or depth
- Gantry and couch coordinates
- PA film for design of pulmonary shields (shown below and with verification image)



CT

6.2.2 Target Volume

The target for this treatment is the whole body including the head.

Partial transmission lung shields are used from the posterior direction to bring the lung dose to approximately a mean dose of 1000 cGy.

Electron cutots for testicular irradiation (semi-custom) will be designed for males.

6.2.3 Target Dose

1200 cGy total dose delivered 150 cGy per treatment fraction delivered BID over 4 days with 6 MV photons.

Dose rate should be < 10 cGy/min in patients treated at extended SSD (450-500 cm) in the TBI couch and will be less than 15 cGy/min in young children and infants treated at extended SSD (200-220 cm) on the floor.

Intra-fraction interval should be 6 hours.

Custom posteriorly placed (PA only) partial transmission lung shields (blocks) will be used to reduce the average dose to the lung to approximately 1000 cGy total dose.

An additional 400 cGy supplemental testicular radiation delivered in 2 fractions of 200 cGy delivered for males with lymphoid lineage leukemia.

6.2.4 Treatment Technique

Immobilization and Position

TBI rotational couch: Patient lays prone and supine in a clock bag on the TBI rotational couch with alpha cradle on the posterior and anterior of patient. Head turned to left, arms down by sides, fingers spread out, and legs relaxed and straight. TBI rotational couch rotated to face beam.

TBI drop-off board on floor (patients < 100 cm tall): Patient lays prone and supine on TBI drop-off board, with arms away from body and by sides with fingers spread, legs frogged, head turned to left. Baby blanket roll under feet for prone position.

Beam Configuration – Open fields are delivered to the patient AP/PA by changing the orientation of the patient relative to the treatment beam.

Testicular treatment (males with lymphoid lineage leukemia) will be delivered with an en face electron field using standardized cutouts and an appropriate beam energy to cover the testes by 90% dose.

6.2.5 Dose Calculation

The monitor units per treatment field are manually calculated for each patient using the following equation.

$$MU\ per\ field = \frac{\frac{Dose}{fraction}\ per\ field\ (cGy)}{\left(1\frac{cGy}{MU}\right)\left(S_c(40x40cm^2)\right)\left(S_p(30x30cm^2)\right)\left(TMR\left(30x30, d_{\frac{1}{2}}\right)\right)\left(OAR\left(d_{\frac{1}{2}}\right)\right)\left(\frac{101.5}{SSD + d_{\frac{1}{2}}}\right)^2}$$

Where $d_{1/2}$ = the mid-plane depth = separation/2, MU = monitor unit, TMR = tissue maximum ratio, SSD = source to surface distance, OAR = off-axis ratio, S_c = collimator scatter factor, S_p = patient scatter factor.

The dose rate required on the linear accelerator is manually calculated to achieve the prescribed dose rate.

6.2.6 Supplemental site of irradiation

Patients with CNS involvement from leukemia, persistent choloroma, gross testicular involvement or other sites of disease may require supplemental irradiation. Dosing and timing should be selected in consultation between the radiation oncologist and bone marrow transplant physician.

7.0 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS

7.1 Schedule of evaluations

All evaluations for these participants will be carried out as outlined in **Appendix E**, and guided by the Standard Operating Procedures (SOPs) of the St. Jude Children's Research Hospital, Department of BMTCT, for recipients of allogeneic stem cell transplantation. Copies of these SOPs and ongoing updates can be found at the following site: Please note that all relevant SOPs can be found on the BMT&CT Clinical Transplant Program intranet page:
https://home.stjude.org/bmt/Pages/policies-transplant-program.aspx

Furthermore, to accommodate the research studies, flexibility in the date is allowed without a deviation from protocol. The degree of flexibility in the timing is also provided in Appendix E. Other studies, or additional routine studies may be obtained as needed for good clinical care.

7.2 Evaluation for chimerism and engraftment

Evaluation for chimerism and engraftment will be performed on bone marrow or peripheral blood samples according to the timelines noted in **Appendix E**. Bone marrow chimerism studies will be conducted on or about the following time points: day +21, day +100, and year one post-transplant. However, for research participants who have less than 100% donor chimerism at or about day +21 post-transplant, a repeat bone marrow study (to include chimerism) may be performed as indicated.

The time to neutrophil engraftment will be recorded. Neutrophil engraftment will be defined as the first of 3 consecutive days of an absolute neutrophil count (ANC) greater than or equal to 500/mm^3.

If there is an initial decrease in donor chimerism to less than 90% at any time on peripheral blood studies, a bone marrow examination will subsequently be performed within approximately two weeks to confirm this initial decline. In addition, chimerism analysis may be performed in subsets of lymphocytes, granulocytes, and monocytes for research participants with increasing host chimerism until the research participant attains 100% donor chimerism. Chimerism studies will be performed in the St. Jude Department of Pathology using standard techniques.

7.3 Evaluation for immune reconstitution

Performed according to the schedule outlined in **Appendix E** until the immune parameters recover to normal level or donor pattern:

7.3.1 Lymphocyte subsets study: Flow cytometry enumeration.

7.3.2 Quantitative immunoglobulins: IgG, IgM, and IgA levels.

7.3.3 CNI60 Lymphocyte Research Lab, CNI60 Phenotype Research Lab and CNI60 pSTAT 3/5 Assay Research Lab: Correlative studies will be performed in the Immune Monitoring and Flow Cytometry cores of the BMTCT Department and through the Center of Translational Immunology and Immunotherapy ($CeTI^2$), which is a joint endeavour between the BMTCT and Immunology Departments. Studies are focused on characterizing the reconstituted immune cells, and may include flow cytometry, functional assays, and cytokine/chemokine, T cell receptor (TCR) diversity or TREC analyses. In addition, sequencing studies (genetic test) will be performed to

i) evaluate and track immune cells, e.g. single cell RNA sequencing (sc RNA-seq), single cell ATAC-sequencing (sc ATAC-seq),

ii) analyze the clonal structure and specificity of immune cells, e.g. sc TCR-seq, single nucleotide polymorphism (SNP) analysis,

iii) analyze the immune cell methylome, e.g. whole genome bisulfite sequencing (WGBS), sc ATAC-seq.

All of these assays may not be performed on every specimen as a limited number of cells will be available.

7.4 Minimal residual disease evaluation

Minimal residual disease (MRD) assays in BM by immunologic and molecular methods will be performed for those research participants who have had this test performed during prior therapy for their disease at St. Jude only or those for who samples of diseased marrow were available to identify a leukemic marker for MRD testing. MRD assays may be performed more frequently in participants with increasing host chimerism. Tests will be performed in the appropriate St. Jude laboratories.

7.5 Evaluations for ruxolitinib pharmacokinetics testing

Ruxolitnib PK testing will be completed in the PK Shared Resource at St. Jude. PK testing on ruxolitinib will be obtained on HCT recipients to evaluate drug exposure and correlate the exposure with transplant-related outcomes. 2 mL of peripheral blood will be drawn into a heparinized (dark green top) tube at the following timepoints: pre-dose and 0.5, 1, 2, 4, and 8 hours post-dose on day +40 and +47 and pre-dose and 2 hours post-dose on days +54 and +61[47] (total samples = 16). NOTE: Do NOT give the next dose until the 8-hour sample is collected. Sample collection date may be adjusted ±2 days as needed (such as to accommodate blood volume concerns, line access issues, etc.). Ideally, ruxolitinib PK samples should be obtained on the same day and same time as pSTAT3/5 activity if needing to adjust collection days. Additionally, leftover PK samples from days +54 and +61 will be utilized for pSTAT 3/5 testing (no additional blood draws will be required from the patient). The pharmacokinetics of ruxolitinib will be evaluated using nonlinear mixed-effects modeling approaches.

Detailed instructions for collection, handling and shipping of PK samples can be found in Appendix G: CNI60 Data Collection, Specimen Transmittal Forms.

7.6 Evaluations for rATG pharmacokinetics testing

rATG PK testing will be completed in the PK Shared Resource at St. Jude. PK testing on rabbit ATG will be obtained on HCT recipients to evaluate drug exposure and correlate the exposure with transplant-related outcomes. We will evaluate whether covariates such as weight and lymphocyte count are related to rATG exposure. 2 mL of peripheral blood will be drawn into a heparinized (dark green top) tube at the following timepoints: pre- and post- rATG infusions on day -3, -2, and -1 and on days 0, +3, +7, +14, +21 (total samples = 11).[51, 58] Sample collection date may be adjusted ±2 days as needed (such as to accommodate blood volume concerns, line access issues, etc.).

Detailed instructions for collection, handling and shipping of PK samples can be found in Appendix G: CNI60 Data Collection, Specimen Transmittal Forms.

7.7 Evaluations for fludarabine pharmacokinetics testing

Fludarabine PK testing will be completed in the PK Shared Resource at St. Jude. PK testing on fludarabine will be obtained on HCT recipients to evaluate drug exposure and correlate the exposure with transplant-related outcomes. We will evaluate whether covariates such as weight and renal function are related to fludarabine exposure. 2 mL of peripheral blood will be drawn into a heparinized (dark green top) tube at the following timepoints: 0.5, 2, 7, 24 hours after fludarabine infusion on day -4[4] (total samples = 4).[59] Sample collection date may be adjusted ±2 days as needed (such as to accommodate blood volume concerns, line access issues, etc.). These coincide with the peak concentration (at the end of infusion) along with samples either before or after the inflection in the pharmacokinetic curve. The pharmacokinetics of fludarabine will be evaluated using nonlinear mixed-effects modeling approaches.

Detailed instructions for collection, handling and shipping of PK samples can be found in Appendix G: CNI60 Data Collection, Specimen Transmittal Forms.

7.8 Evaluation for ex-vivo target inhibition

Patient's plasma/whole blood will be used to determine if circulating levels of ruxolitinib inhibit pSTAT 3 and/or 5 *ex vivo*. Samples will be obtained on day +40 and day +47 before and after dosing with ruxolitinib. Leftover PK samples from days +54 and +61 will be utilized for pSTAT 3/5 testing (no additional blood draws will be required from the patient). This will allow us to assess the level of inhibition of JAK-STAT pathway by ruxolitinib at these time points and allow us to trend over a 3 week period.

7.9 Long-term follow-up evaluations

In general, recipients of allogeneic HCT at St. Jude are seen at least annually until 10 years post-transplant in the Department of BMTCT outpatient clinic or in the after completion of therapy (ACT) clinic. For the purpose of this study, research participants will be followed to year 1 post-transplantation..

8.0 EVALUATION CRITERIA

8.1 Response criteria and evaluations

8.1.1 Hematologic Recovery Criteria

- *Neutrophil recovery* will be defined as the first of 3 consecutive tests performed on different days of an ANC $\geq$500/mm^3 with evidence of donor cell engraftment.
- *Platelet recovery* will be defined as the first of 3 consecutive tests performed on different days of a platelet count $\geq$20,000/mm^3 with no platelet transfusions in the preceding 7 days.

8.1.2 Graft Failure Criteria

- *Primary graft failure* will be defined as an ANC never meeting or exceeding 500/mm^3 for 3 consecutive tests performed on different days and no evidence of donor chimerism (<5%) by day +30 post-HCT.
- *Secondary graft failure* will be defined as a decline in ANC to <500/mm^3 with a decline in donor chimerism to <5% in research participants with prior engraftment.

8.1.3 Relapse criteria

Relapse is defined as excess of 5% blasts in bone marrow by morphology confirmed by flow cytometry analysis.

8.2 Toxicity evaluation criteria

Adverse event (AE) monitoring for on-study research participants will be assessed using the NCI Common terminology Criteria for Adverse Events Version 5.0. The standard procedures for adverse event collection and monitoring are noted in **Appendix D**. An exception will be made with evaluating, staging and grading of GVHD, which will be evaluated using consensus criteria which are provided for both acute and chronic GVHD in Appendices B and C respectively.

9.0 OFF STUDY AND OFF THERAPY CRITERIA

9.1 Off-study criteria

Recipient research participants will remain on-study until one of the following occurs:

- Death.
- Lost to follow-up, unable to be contacted and/or effectively monitored by the Principal Investigator (PI) and/or designees.
- Noncompliance, participant misses so many appointments that the data cannot be used in the study.

- Study evaluations are complete. One year after HPC infusion (i.e. has completed the year +1 post-primary transplant evaluation)
- Discretion of the Study PI, including but not limited to:
    - PI decides continuing in the study would be harmful.
    - A treatment is needed that is not allowed on this study.
    - Donor unable to provide the cell product required for recipient to undergo the study procedure.
    - Participant's condition gets worse or a change in health status which renders the study interventions medically unsafe or not in the participant's best interest.
    - New information is learned that a better treatment is available, or that the study is not in the participant's best interest.

9.2 Off-therapy criteria

Recipient research participants will remain on-study, but considered off-therapy, if one of the following occurs:

- Requires conventional chemotherapy for confirmed (generally >5%) disease relapse: epigenetic or targeted therapy, immune therapy, and low dose lymphodepleting chemotherapy with DLI, even if molecularly detectable disease is present, would not trigger this off-therapy criterion.
- Experiences graft failure/rejection, requiring non-protocol therapy.
- Positive pregnancy test post cellular product infusion.
- Development of unacceptable toxicity during treatment.
- Grade II-IV GVHD prior to day +100 post-HCT.

10.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

10.1 Adverse events (AEs)

Adverse events will be monitored from the start of transplant conditioning throughout the first year post-transplant. Participants will be instructed to report all AEs during the study and will be assessed for the occurrence of AEs throughout the study by the assigned personnel within the Department of Bone Marrow Transplantation and Cellular Therapy Clinical Research Office (BMTCT CRO).

10.2 Definitions

Adverse event (AE): Any untoward medical occurrence associated in a study participant after the start of transplant conditioning and until the participant completes study interventions or meets any of the defined off-study criteria. Adverse Events will be graded by the NCI CTC AE version 5.0; and will be documented and reported per guidance defined in **Appendix D**.

Serious adverse event (SAE) 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.

Unanticipated problem (UP) 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.

10.3 Handling of adverse events (AEs) and deaths

Recording **of Adverse Events and Serious Adverse Events:** All NCI Grade III-V adverse events from the start of conditioning and throughout the first year post HCT, regardless of their relationship to the treatment given will be collected in the study database. GVHD events will be recorded on an ongoing basis regardless of stage or grade using the criteria defined in Appendix B and C, and will not be graded according to NCI criteria.

Reporting **Adverse Events and Serious Adverse Events**: The St. Jude PI, upon awareness of an event, will determine the seriousness of AEs and ensure that all related or possibly related AND unexpected SAEs, and all UPs are entered into the electronic submission system (iRIS) within 10 days. All recorded AEs, serious or not, will be recorded in a log, spreadsheet, or report and submitted to the St. Jude IRB at the time of continuing review. The St. Jude Regulatory Affairs Office, upon receipt, reports SAEs on study to the FDA within mandated regulatory timelines per 21 CRF 312 or 812.

Reporting **of unanticipated problems**: The St. Jude PI will refer to St. Jude Human Research Protection Program (HRPP) Policy 01.720 for specifics on the reporting of unanticipated problems to the St. Jude IRB. The St. Jude IRB reports UPs to BIMO as per 21 CFR 56. The UP link follows: https://home.stjude.org/hrpp/Policies/01-720.pdf#search=unanticipated.

***Reporting* of deaths:** When an unexpected death that is related or possibly related to the study treatment occurs, the PI should report it to the Director of Human Subject's Protection immediately, by phone: (901) 595-4359, cell: (901) 336-2894, fax: (901) 595-4361, or e-mail: hsp-1@stjude.org. A reportable event entry into iRIS should follow within 48 hours of notification of the event.

10.4 Reporting to the CIBMTR

The Transplant Program at St. Jude is required by the federal government to report transplant information to the Center for International Blood and Marrow Transplant Research (CIBMTR). The CIBMTR is a research partnership of the International Bone Marrow Transplant Registry, the National Marrow Donor Program (NMDP), and the Foundation for the Accreditation of Cellular Therapy (FACT). This organization is responsible for the collection and maintenance of a standardized data warehouse registry of autologous and all allogeneic (related and unrelated donor) transplants performed in the United States.

The Office of General Counsel, U.S. Department of Health and Human Services, had deemed the CIBMTR not a covered entity under the Privacy Rule (45 CFR 164.512), 45 CFR Parts 160 and 164, and the Health Insurance Portability and Accountability Act (HIPPA) of 1996. For this reason, the submission and disclosure of certain protected health information (PHI), including that required for CIBMTR, is allowable without the individual's authorization (i.e. consent is waived) when such disclosure is made to public health authorities authorized by law for the purpose of preventing or controlling disease, injury, or disability.

Data resulting from this transplant procedure will be sent for general registry purposes to comply with the federal government requirements. This information for both donor and recipient is submitted using a unique participant identification number. The information submitted for haploidentical recipients is less extensive than recipients of other donor products. For this reason, variables submitted may include but are not limited to the transplant recipient's date of birth, country/state of current residence, diagnosis, basic lympho-hematopoietic reconstitution (e.g. date of ANC and platelet engraftment), post-HCT disease status, and basic AEs (e.g. GVHD- yes or no), survival status, date/cause of death.

11.0 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

11.1 Data collection

Data from the participant's source record will be entered directly into a secure study-specific database (Trial Master). This will be completed by the assigned personnel within the BMTCT CRO and will be supervised by the PI. Instructions for data entry are outlined in the database.

The PI and designees will be responsible for the review of data for accuracy and completeness. Protocol-specific data will be recorded in the electronic database within 2 to 4 weeks of availability within the source record. All questions will be directed to

the PI or designee and will be reviewed at regularly scheduled working meetings (monthly).

Regular summaries of toxicity and protocol events will be generated for the PI and the Department of Biostatistics to review.

11.2 Study Monitoring

This study is considered moderate risk for monitoring purposes. Protocol and regulatory compliance, including essential regulatory documentation, will be assessed as well as the accuracy and completeness of data points related to the primary study objective semi- annually. If the study design has strata, accrual will be tracked continuously. The first two enrollees and then 10 % of participants will be monitored semi-annually.

The PI and study team are responsible for protocol and regulatory compliance, and for data accuracy and completeness. The study team will meet at appropriate intervals to review case histories or quality summaries on participants and retain copies of the minutes which are signed by the PI.

Clinical Trials Operations (CTO) will verify informed consent documentation and eligibility status for 50% of trial participants within 5 days of enrollment. Additionally, a quality review will be performed by CTO personnel on 100% of St. Jude participants' informed consent forms to assure completeness. The Clinical Research Monitor (CRM) will verify the informed consent and eligibility processes of all non-St. Jude participants during routine monitoring intervals (every 6 months).

Overall study conduct, compliance with primary objectives, age of majority consenting, safety assessments and reporting, and the timeliness and accuracy of database entries are monitored routinely. Study documents routinely monitored on selected participants include medical records, database entries, study worksheets, and case report forms. Study documents are monitored for participant status, demographics, staging, subgroup assignment, treatments, investigational drug accountability, evaluations, responses, participant protocol status, off-study and off-therapy criteria, and for all other specifics as detailed in a separate study-specific monitoring plan. The frequency and intensity of monitoring will adapt over time, based on study progress, site compliance, accrual, participants on active treatment, rate of adverse events, and monitoring findings thus allowing refocusing on critical study risks based on findings. Once all participants selected for monitoring have completed treatment and are in long term follow-up, the monitoring frequency may be changed to annual monitoring until a new participant enrolls. Once a site is closed to accrual and participants selected for monitoring have completed treatment, the monitoring will be conducted annually.

The recording and reporting of Adverse Events, Serious Adverse Events (SAEs), and Unanticipated Problems (UPs) to include type, grade, attribution, duration, timeliness and appropriateness will be reviewed by the Monitor/ CRM. The CRM will generate a follow-up letter which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC).

Continuing reviews by the Institutional Review Board (IRB) and Scientific Review Committee (CT-SRC) will occur at least annually. In addition, unanticipated problems are reviewed in a timely manner by the IRB.

11.3 Confidentiality

Unique study numbers will be used in place of an identifier such as a medical record number. These numbers will be used to identify any data that is released to persons or agencies outside of the study team. 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 password protected file that is accessible only to study team members.

The medical records of study participants may be reviewed by the St. Jude IRB, FDA, clinical research monitors, auditors, etc.

11.4 Genomic Data Sharing

Genomic and epigenomic data may be shared through the St. Jude Cloud, the Database for Genotypes and Phenotypes (dbGAP) and the Gene Expression Omnibus (GEO), which are both run by the NIH, and the Sequence Read Archive (SRA). Prior to submitting data, data will be stripped of identifiers such as name, date of birth, medical record number, and any other information that could be used to identify participants and will be fully de-identified by standards consistent with the Common Rule and HIPAA. The genotype data will be made publicly available no later than six months after completion of sequencing and analysis for all patients on the study, or the date of initial publication, whichever comes first.

12.0 STATISTICAL CONSIDERATIONS

This is a phase II study with development of saGVHD within 100 days post-HCT as the primary endpoint.

12.1 Historical St. Jude data relating to the primary endpoint

We have been accruing 15-20 patients each year for the past 30 years on the BEAL1 clinical practice plan for treatment of patients with hematological malignancies with MSD or MUD HCT using the same eligibility criteria. This experience indicates that it is logistically feasible to enroll 30 evaluable patients in 4 years.

A retrospective study of 255 patients with ALL and AML who underwent HCT at St. Jude between January 2000 through December 2015 on BEAL1 practice plan with a bone marrow graft was evaluated for OS. NRM and relapse rates. The treatment plan consisted of conditioning with TBI/Cy for patients with ALL and Bu/Cy for patients with AML. GVHD prophylaxis was with a calcineurin inhibitor and either mycophenolate mofetil or methotrexate. Patients who had an unrelated donor in addition received rATG.

The mean age at HCT was 9.89 years, approximately 60% were 12 years of age or older, approximately 38% were diagnosed with ALL and 43% with AML, 72% of

patients were in remission prior to HCT, 38% received a MSD graft, and 62% a MUD graft. Approximately **92**% of patients engrafted neutrophils at 28 days, 33% had acute GVHD, **19**% had saGVHD, and 4% had chronic GVHD. The OS was 65%, 28% patients relapsed, 24% patients died due to relapse, and 11% from NRM.

12.2 Primary objective

To estimate the incidence of severe acute GVHD (saGVHD) using a prophylaxis regimen with no calcineurin inhibitors after day +60 post first allogeneic Human Leukocyte antigen (HLA)-matched sibling or unrelated donor HCT for hematological malignancies.

We regard any patient experiencing any competing event (except death of any non-GVHD-cause within 100 days) that renders the patient nevaluable for GVHD, as unevaluable for the primary endpoint and GVHD monitoring (Table 3 below); such patients will be replaced. Death of any cause within 100 days will be considered as a failure for both the primary endpoint and monitoring.

We regard any patient experiencing toxicity from CNI requiring replacement of CNI with another GVHD prophylaxis agent other than ruxolitinib as inevaluable for the primary endpoint, such patients will be replaced. However, patients switched to ruxolitinib before Day +60 will be considered evaluable.

GVHD will be defined using Children's Oncology Group (COG) Committee consensus guidelines (Appendix B). Proportion (probability) of sa GVHD defined as Gr II-IV GVHD events within 100 days post-HCT will be estimated by sample proportions and exact 95% binomial-based confidence interval. Patients who are alive but drop out within 100 days due to events unrelated to saGVHD are considered as unevaluable and will be replaced. Notably from our experience, the probability of dropout during this period is below 1%. Historically, the saGVHD proportion is 0.19; if the proposed regimen reduces the saGVHD proportion to 0.10 (0.05) then with n=30 evaluable patients the estimation accuracy as represented by the half-length of the 95% confidence interval is expected to be 0.11 (0.07).

12.3 Secondary objective

12.3.1 *Estimate the cumulative incidence of relapse, NRM, chronic GVHD, and OS in study participants at one year post-transplant*.

Cumulative incidence (probability) function of relapse, NRM, chronic GVHD will be estimated by the Kalbfleisch-Prentice method. For each endpoint (relapse, NRM, chronic GVHD) deaths and drop outs not related to the endpoint are treated as competing risks. For OS, the survival probability function will be estimated by Kaplan-Meier estimate with standard error estimated according to Peto' method.[60] Subset analyses in patients with lymphoid and myeloid malignancies respectively will also be conducted.

12.4 Exploratory objectives

12.4.1 *To evaluate the PK/PD profiles of ruxolitinib, fludarabine, and rATG.*

PK.PD parameters estimated for each individual patient will be summarized across the study cohort using descriptive statistics including mean, standard deviation, and the five number summary (minimum, 3 quartiles, and maximum). Subset analyses in patients with lymphoid and myeloid malignancies respectively will also be conducted.

12.4.2 *To assess immune reconstitution in study participants within the first year psot-HCT*

Biomarkers of immune reconstitution will be summarized using descriptive statistics including mean, standard-deviation, and the five number summary (minimum, 3 quartiles, and maximum) or sample proportions, as appropriate for the data type. Subset analyses in patients with lymphoid and myeloid malignancies respectively will also be conducted.

12.5 Safety Monitoring

To ensure that the proposed therapy does not significantly increase saGVHD that the historical control rate of 19%, the following safety stoppint rule will be followed after the evaluation of 5, 15, 25 and 30 patients. A trial will be suspended if we observe the incidence of saGVHD of $\geq$ 2/5, 4/15, 6/25 or 7/30.

The stopping rule is obtained based on Bayesian toxicity monitoring developed by Lee J, Kuo Y-K, Liu D and Chen N at MD Anderson: https://biostatistics.mdanderson.org/shinyapps/BTOX/

The parameter specifications are: the maximum acceptable saGVHD rate of 19%, a Beta(0.5,0.5) prior distribution, and the probability threshold of 0.7 (i.e., the trial is suspended if the posterior probability of saGVHD rate being greater than 19% is at least 0.7).

Toxicity Stopping Boundaries		
Table SB1: Toxicity Stopping Boundaries		
# Patients (inclusive)	**# saGVHD's considered excessive**	**Actions**
5	≥2	Early stopping
15	≥4	Early stopping
25	≥6	Early stopping
30	≥7	Reach to Nmax

This stopping rule has reasonable operating characteristics as illustrated in the table and the figures below.

Operating characteristics (stopping probabilities) at each interim

Interim	Cum. (n)	Suspend if cum # saGVHD reaches	Postulated true saGVHD rate				
			0.28	**0.25**	**0.20**	**0.15**	**0.10**
1	5	≥2	0.43	0.37	0.26	0.16	0.08
2	15	≥4	0.27	0.24	0.17	0.09	0.03
3	25	≥6	0.12	0.12	0.09	0.05	0.01
4	30	≥7	0.04	0.04	0.04	0.02	0.00
		≤6 saGVHDs, continue to full accrual	0.14	0.23	0.44	0.68	0.87

Figure OC2: Cumulative Probability of Stopping by Patient Number

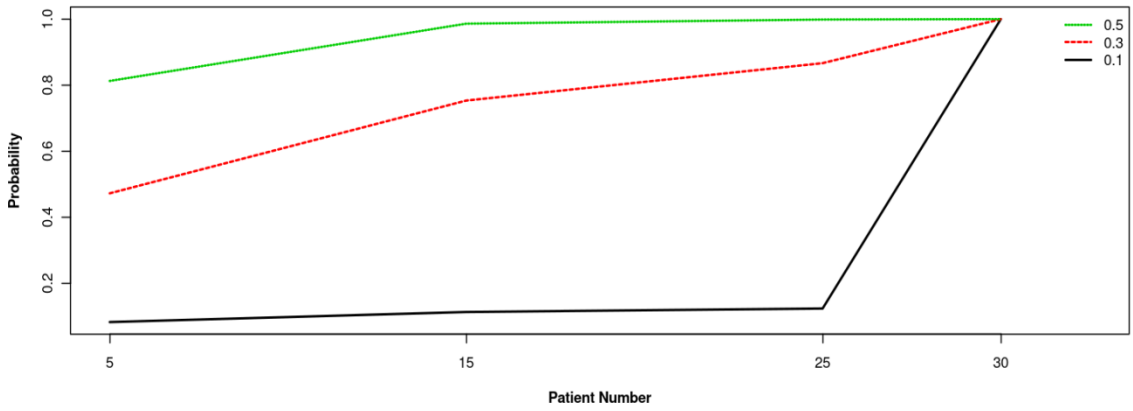
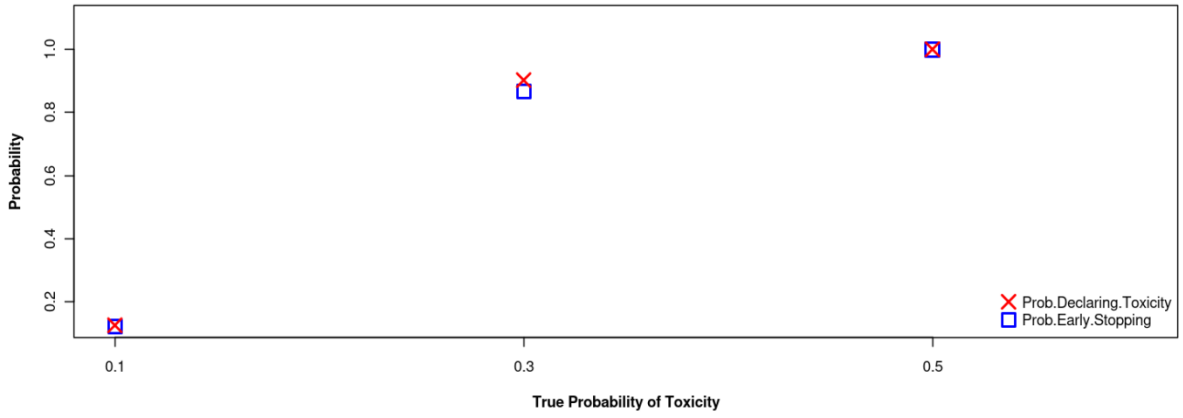


Figure OC3: Probability of Early Stopping and Declaring Toxicity by True Probability of Toxicity



12.6 Anticipated Completion Dates

Anticipated Primary Completion Date**:** 4 years and 6 months from start of the study.

Anticipated Study Completion Date: 5 years from the start of the study.

13.0 OBTAINING INFORMED CONSENT

13.1 Informed Consent Prior to Research Interventions

The PI or physician sub-investigator will conduct the signature authorization portion of the consent process. Authorization for the recipient procedure should be conducted in the presence of an independent witness, such as a nurse from the St. Jude Department of Nursing or the St. Jude Institutional Review Board Ombudsperson/Patient Advocate, as applicable and available to serve as a witness.

13.2 Consent at Age of Majority

The age of majority in the state of Tennessee is 18 years old. Research participants must be consented at the next clinic visit after their 18th birthday.

13.3 Consent When English is Not the Primary Language

When English is not the patient, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information 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 CTO websites. Please refer to HRPP Policy 01.724 Informed Consent Involving Participants with Limited English Proficiency for more details.

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APPENDIX A

KARNOFSKY PERFORMANCE STATUS SCALE ≥16 YEARS OLD	
Score	**General Description**
100	Normal. No complaints. No evidence of disease.
90	Able to carry on normal activity. Minor signs or symptoms of disease.
80	Normal activity with effort. Some signs or symptoms of disease.
70	Care of self. Unable to carry out normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled. Requires special care and assistance.
30	Severely disabled. Hospitalization is indicated although death is not imminent.
20	Hospitalization necessary, very sick, active support treatment necessary.
10	Moribund. Fatal processes progressing rapidly.
0	Dead.

LANSKY PERFORMANCE STATUS SCALE <16 YEARS OLD	
Score	**General Description**
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restriction of and less time spent in play activity
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed but lies around much of the day, no active play but able to participate in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	No play; does not get out of bed
0	Unresponsive

APPENDIX B

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 Thrombotic Thrombocytopenic Purpura (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 TTP). 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 <u>Assessment of Skin GVHD</u>

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 C

CRITERIA FOR GRADING CHRONIC GVHD GRADE

	Score 0	**Score 1**	**Score 2**	**Score 3**
Performance Score: _____ **KPS ECOG LPS**	☐Asymptomatic and fully active (ECOG 0: KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐Symptomatic limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐Maculopapular rash ☐Lichen planus-like features ☐Papuloquamous lesions or ichthyosis ☐Hyperpigmentation ☐Hypopigmentation ☐Keratosis pilaris ☐Erythema ☐Erythroderma ☐Poikiloderma ☐Sclerotic features ☐Pruritus ☐Hair involvement ☐Nail involvement **% BSA Involved** _____	☐ No symptoms	☐<18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐>50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	☐No symptoms	☐ Mild symptoms with disease signs but **NOT** limiting oral intake significantly	☐ Moderate symptoms with disease signs **WITH** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **WITH** major limitation of oral intake
EYES ☐**Mean tear test (mm):** ☐**>10** ☐**6-10** ☐**≥5** ☐**Not done**	☐No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops) **≤**3 x per day) **OR** asymptomatic signs of keratoconjunctivitissiccca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops ≥3x per day or punctual plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision cause by keratoconjunctivitissicca
GI Tract	☐No symptoms	☐ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss) 5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-3 x ULN	☐ Bilirubin or enzymes > 5 x ULN

APPENDIX C (continued)

CRITERIA FOR GRADING CHRONIC GVHD GRADE

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
Lungs† FEV1 _____ DLCO _____	☐ No symptoms ☐ FEV1 >80% **OR** LFS=2	☐ Mild symptoms (shortness of breath after climbing one flight of steps) ☐ FEV1 60-79% **OR** LFS 3-5	☐ Moderate symptoms (shortness of breath after walking on flat ground) ☐ FEV1 40-59% **OR** LFS 6-9	Severe symptoms (shortness of breath at rest; requiring 0_2 ☐ FEV1 ≥39% **OR** LFS 10-12
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐Contractures WITH significant decrease or ROM AND significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advance signs (stricture, labial agglutination or severe ulcerations **AND** severe pain with coitus or inability it insert vaginal speculum

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

Esophageal stricture or web ____ **Pericarial Effusion ____** **Pleural Effusion(s) ____**

Ascites (serositis) ____ **Nephrotic syndrome ____** **Peripheral Neuropathy ____**

Myasthenia Gravis ____ **Cardiomyopathy ____** **Eosinophilia > 500/µl ____**

Polymyositis ____ **Cardiac conduction defects ____** **Coronary artery involvement ____**

Platelets <100,000/µl ____ **Progressive Onset ____**

Other: Specify: _____

Organ scoring of chronic GVHD.

* AP may be elevated in growing children, and not reflective of liver dysfunction.

† Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptoms or PFT scores, the higher value should be used for final scoring. Scoring using the Lung Function Score (LFS**) i**s preferred, but if DLCO is not available, grading using FEV1 should be used. The LFS is a global assessment of lung function after the diagnosis of bronchiolitis obliterans has already been established. The percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows: >80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; <40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12. GVHD indicates graft versus host disease, ECOG, Eastern Cooperative Oncology Group, KPS, Karnofsky Performance Status; LPS, Lansky Performance Status; BSA, body surface area; ADL, activities of daily living; LFTs, liver function tests; AP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

APPENDIX C (continued)

CRITERIA FOR GRADING CHRONIC GVHD GRADE

GLOBAL GRADING OF CHRONIC GVHD[84]

Final Grade	Number of Organs/Sites*	Maximum Organ Score**	Lung Score
Mild	1 - 2	1	0
Moderate	3 or more	1	1
	At least 1	2	
Severe	At least 1	3	2 - 3

*Determined by adding the total number of organs receiving score > 0 using Figure 1, Appendix C.

**Defined as the maximum score given to any organ system amongst all organs scored using Figure 1, Appendix C.

APPENDIX D

CRITERIA FOR ADVERSE EVENT (AE) EVALUATION AND REPORTING

The St. Jude Department of BMTCT Clinical Research Office standard operating procedure for the documenting and reporting of adverse (SOP 10 Documenting and Reporting of Adverse Events https://home.stjude.org/bmt/Policies/10.pdf) will provide guidance on the evaluation, collection and reporting of adverse events for this clinical trial. The current version of this document, as well as ongoing updates, can be located at the following website: http://home.stjude.org/bmt/Pages/policies-research.aspx

APPENDIX E

Recommended routine clinical testing and evaluation schedule for Transplant Recipient

STANDARD OF CARE STUDIES	SAMPLE	VOLUME	PRE	MONTH 1	MONTH 2	MONTH 3	MONTH 6	MONTH 12
Pregnancy Test	PB	2 mL	X	As clinically indicated				
Physical Exam	N/A	N/A	X	Weekly			X	X
GVHD Assessment	N/A	N/A		Weekly				
CBC with diff.	PB	0.5-2 mL	X	Daily until engrafted, then weekly			X	X
Chemistry	PB	0.25-2 mL	X	Weekly			X	X
Lipid Panel	PB	1-2mL				X		
Viral surveillance (BMTPCR)	PB	4 mL	X	Weekly			As clinically indicated	
Chimerism	PB	1-2 mL		Weekly upon engraftment			X	X
	BM	2 mL		X		X		X
Disease Status Evaluation	N/A	N/A	X	X		X		X
MRD Bone Marrow	BM	3 mL	X	X		X		X
Lymphocyte Subset Study	PB	2.5-4 mL	X	X	X	X	X	X
Quantitative Immunoglobulins	PB	2 mL	X			X		X
Total CLINICAL blood sample volumes per time point			19 mL	17 mL	12 mL	17 mL	10 mL	19 mL

- The above-indicated follow-up regimen for these evaluations is guided by the SOPs of the Department of BMTCT, for recipients of allogeneic stem cell transplantation. As these evaluations are considered standard clinical care (non-research), variations in frequency (more or less frequent) of these evaluations can occur due to the participant's current clinical condition and will not be noted as protocol deviations.
- Disease status evaluations/BM testing results obtained prior to enrollment may be used for the baseline/pre-infusion assessments.
- Lymphocyte subset studies may be omitted without variance when the absolute lymphocyte count (ALC) is zero.
- In the event of graft failure/rejection, bone marrows, chimerisms and several applicable immune studies would no longer be clinically indicated and these studies may be held.

APPENDIX E (continued)

Immune reconstitution testing and evaluation schedule for Transplant Recipient

RESEARCH STUDIES	SAMPLE	VOLUME	CONTAINER	PRE	DAY +40	DAY +47	MONTH 3	MONTH 6	MONTH 12
CNI60 Lymphocyte Research Lab	PB	15 mL	Yellow ACD	X			X	X	X
CNI60 Phenotype Research Lab	PB	5 mL	Yellow ACD	X			X	X	X
CNI60 pSTAT 3/5 Assay Research Lab	PB	5 mL	Yellow ACD		0800 (pre Ruxolitinib) x1 1200 (4H post Ruxolitinib) x1	0800 (pre Ruxolitinib) x1 1200 (4H post Ruxolitinib) x1			
Total RESEARCH blood sample volumes per time point				20 mL	10 mL	10 mL	20 mL	20 mL	20 mL

- CNI60 Lymphocyte Research Lab, CNI60 Phenotype Research Lab and CNI60 pSTAT 3/5 Assay Research Lab results will be maintained in the Immune Monitoring Core database.
- For these research studies, the posted volumes are the minimum volumes required to perform the respective protocol evaluations, *unless otherwise specified*.

APPENDIX E (continued)

Proposed PK sampling schedule calendar for TBI/Cy regimen

Drug	Time	Sample	Volume	Day -3	Day -2	Day -1	Day 0	Day +3	Day +7	Day +14	Day +21	Day +40	Day +47	Day +54	Day +61
rATG (test dose)	0800-1000	Dark Green (sodium heparin)	2 mL (1 mL min)	0800 (pre) 1000 (post)											
rATG (full dose)	0800-1400				0800 (pre) 1400 (post)	0800 (pre) 1400 (post)	0800	0800	0800	0800	0800				
Ruxolitinib	0800/2000		2 mL (1 mL min)									0800 (pre) 0830 0900 1000 1200 1600	0800 (pre) 0830 0900 1000 1200 1600	0800 1000	0800 1000
Total PK Sample Volume/day		MSD (no rATG)										12 mL	12 mL	4 mL	4 mL
		MUD (rATG)		4 mL	4 mL	4 mL	2 mL	2 mL	2 mL	2 mL	2 mL	12 mL	12 mL	4 mL	4 mL

*Minimal volumes are posted in parentheses when applicable.

- Leftover PK samples from days +54 and +61 will be utilized for pSTAT 3/5 testing (no additional blood draws will be required from the patient).

APPENDIX E (continued)

Proposed PK sampling schedule calendar for TBF regimen

Drug	Time	Sample	Volume	Day -4	Day -3	Day -2	Day -1	Day 0	Day +3	Day +7	Day +14	Day +21	Day +40	Day +47	Day +54	Day +61
Busulfan	0200-0500	Dark Green (sodium heparin)	2 mL (1 mL min)	0500 (EOI) 0600 0800 1000	0500 (EOI) 0600 0800 1000											
Fludarabine	0530-0600		2 mL	0600 (EOI) 0800 1300	0530 (24 hr)											
rATG (test dose)	0800-1000		2 mL (1 mL min)		0800 (pre) 1000 (post)											
rATG (full dose)	0800-1400					0800 (pre) 1400 (post)	0800 (pre) 1400 (post)	0800	0800	0800	0800	0800				
Ruxolitinib	0800-2000		2 mL (1 mL min)										0800 (pre) 0830 0900 1000 1200 1600	0800 (pre) 0830 0900 1000 1200 1600	0800 1000	0800 1000
Total PK Sample Volume/day		Busulfan		8 mL	8 mL											
		MSD (no rATG)		6 mL	2 mL								12 mL	12 mL	4 mL	4 mL
		MUD (rATG)		6 mL	6 mL	4 mL	4 mL	2 mL	2 mL	2 mL	2 mL	2 mL	12 mL	12 mL	4 mL	4 mL

*Minimal volumes are posted in parentheses when applicable.

- Leftover PK samples from days +54 and +61 will be utilized for pSTAT 3/5 testing (no additional blood draws will be required from the patient).

APPENDIX E (continued)

Research Study Evaluation Target Windows

Several laboratory tests can only be processed on weekdays; therefore, if the scheduled evaluation falls on a weekend, or during a holiday period, an adjustment in the follow-up visit is expected and would not be noted as a protocol variation. Additionally, in order to accommodate such logistical constraints, evaluation/collection dates of all protocol assessments (*unless otherwise specified*), may be performed within a reasonable window of the intended date following the guidelines provided in the table below:

If the Planned Evaluation Time Point is:	Window
Weekly	±3 Days
Month 1	Week 2 to Week 6
Month 2	Week 7 to Week 11
Month 3	Week 12 to Month 4
Month 6	Month 5 to Month 7
Month 9	Month 8 to Month 10
Month 12	Month 10 to Month 14

APPENDIX F

The St. Jude Department of BMTCT Clinical SOPs for standard of care for all allogeneic stem cell infusion recipients and stem cell donors will provide guidance on the evaluation, ongoing clinical care and follow up for this clinical trial. The current versions of these SOPs, as well as ongoing updates, of these documents can be located at the following website: http://home.web.stjude.org /bone_marrow/clinicalHome.shtml.

APPENDIX G

Pharmacokinetic Data Collection & Specimen Transmittal Forms

CNI60: Ruxolitinib Pharmacokinetic Data Collection & Specimen Transmittal Form

Complete collection and dosing data at time of administration. Email completed forms to claire.mills@stjude.org and PKNurses@stjude.org. For questions, call PK Nurses at 901-595-2482.

Patient Name:		SJ MRN:
Hospital/Clinic:		DOB:
Contact Name:	Contact Phone:	Contact Email:

Subject BSA (m^2): _____ Ruxolitinib Dose (mg/m^2): _____ Ruxolitinib Actual Dose mg): _____

Collection and Processing of Peripheral Blood Samples:
Whole blood (2 mL) will be drawn into a green top sodium heparin tube. Centrifuge samples and place plasma only into two 2ml screw top tubes. Samples should be frozen at -80°C; overnight refrigeration prior to centrifugation is acceptable for late collections.

Ruxolitinib Day +40 (+2 days) PK: Date: _____ Ruxolitinib Dose Administration Time: _____		
Protocol Time Point	**Date Sample Collected**	**Time Sample Collected**
Pre		
30 min (+/- 15 min)		
1 hr (+/- 15 min)		
2 hr (+/- 15 min)		
4 hr (+/- 15 min)		
8 hr (+/- 15 min)		
Ruxolitinib Day +47 (+/- 2 days) PK: Date: _____ Dose Administration Time: _____		
Protocol Time Point	**Date Sample Collected**	**Time Sample Collected**
Pre		
30 min (+/- 15 min)		
1 hr (+/- 15 min)		
2 hr (+/- 15 min)		
4 hr (+/- 15 min)		
8 hr (+/- 15 min)		

Ruxolitinib Day +54 (+/- 2 days) PK: Date: _____ Ruxolitinib Dose Administration Time: _____		
Protocol Time Point	**Date Sample Collected**	**Time Sample Collected**
Pre		
2 hr (+/- 15 min)		
Ruxolitinib Day +61 (+/- 2 days) PK: Date: _____ Dose Administration Time: _____		
Protocol Time Point	**Date Sample Collected**	**Time Sample Collected**
Pre		
2 hr (+/- 15 min)		

Complete collection and dosing data at time of administration. Email completed forms to claire.mills@stjude.org and PKNurses@stjude.org. For questions, call PK Nurses at 901-595-2482.

Patient Name:		SJ MRN:
Hospital/Clinic:		DOB:
Contact Name:	Contact Phone:	Contact Email:

Subject BSA (m^2): _____

Collection and Processing of Peripheral Blood Samples:
Whole blood (2 mL) will be drawn into a green top sodium heparin tube. Centrifuge samples at 2000 rpm for 10 minutes at 4C° within 4 hours of collection. Place plasma only into two 2ml screw top tubes. Samples should be frozen at -80°C within 30 minutes of separation.

Dose Day	rATG Dose (mg/kg IV)	rATG Actual Dose (mg)	Infusion Start Time	Infusion Stop Time	Date Sample Collected	Time Sample Collected
Day -3					Pre:	Pre (- 15 min):
					EOI:	EOI (+ 15 min):
Day -2					Pre:	Pre (- 15 min):
					EOI:	EOI (+ 15 min):
Day -1					Pre:	Pre (- 15 min):
					EOI:	EOI (+ 15 min):
Day 0					Pre:	Pre (- 15 min):
					EOI:	EOI (+ 15 min):
Day +3 (+/- 2 days)					Pre:	Pre (- 15 min):
					EOI:	EOI (+ 15 min):
Day +7 (+/- 2 days)					Pre:	Pre (- 15 min):
					EOI:	EOI (+ 15 min):
Day +14 (+/- 2 days)					Pre:	Pre (- 15 min):
					EOI:	EOI (+ 15 min):
Day +21 (+/- 2 days)					Pre:	Pre (- 15 min):
					EOI:	EOI (+ 15 min):

Complete collection and dosing data at time of administration. Email completed forms to claire.mills@stjude.org and PKNurses@stjude.org. For questions, call PK Nurses at 901-595-2482.

Patient Name:		SJ MRN:
Hospital/Clinic:		DOB:
Contact Name:	Contact Phone:	Contact Email:

Subject BSA (m^2): _____ Fludarabine Dose (mg/m^2/day IV): _____ Fludarabine Actual Dose (mg): _____

Collection and Processing of Peripheral Blood Samples:
Whole blood (2 mL) will be drawn into a green top sodium heparin tube. Centrifuge samples and place plasma only into two 2ml screw top tubes. Samples should be frozen at -80°C within 30 minutes of collection; however, overnight refrigeration prior to centrifugation is acceptable for late collections.

Fludarabine Day -4 (-2 days) PK:

Date: _____

Fludarabine Infusion Start Time: _____ Fludarabine Infusion Stop Time: _____

Protocol Time Point	Date Sample Collected	Time Sample Collected
30 min (+/- 5 min):		
2 hr (+/- 15 min):		
7 hr (+/- 15 min):		
24 hr (+/- 15 min): ****PRIOR to start of infusion**		