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CHILDREN'S ONCOLOGY GROUP

ASCT1221

A Randomized Phase II Study Comparing Two Different Conditioning Regimens Prior to Allogeneic Hematopoietic Cell Transplantation (HCT) for Children with Juvenile Myelomonocytic Leukemia (JMML)

A Groupwide Phase II Study

STUDY CHAIR

[redacted]

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STUDY COMMITTEE

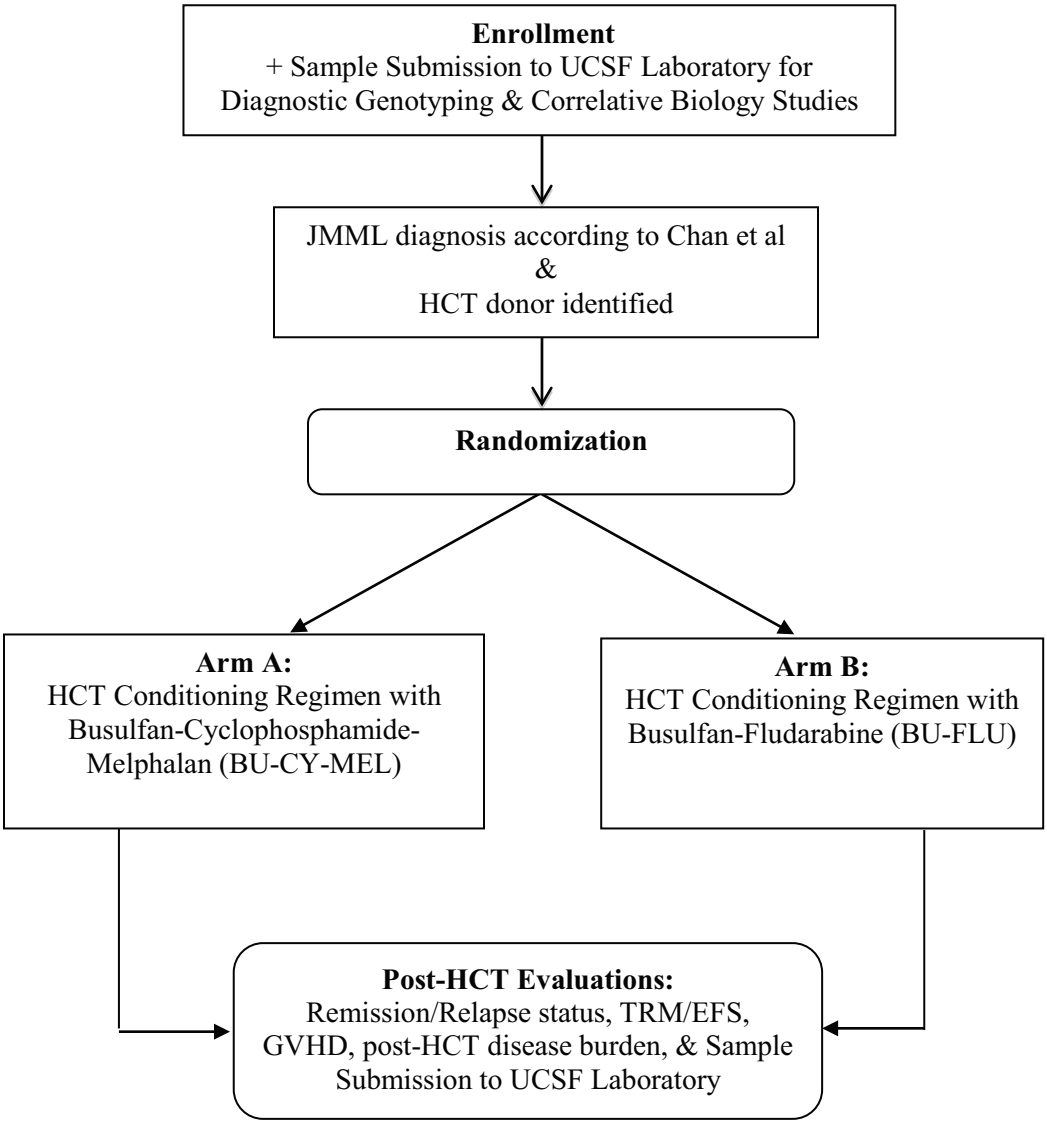
AGENT	NSC#	IND#
Antithymocyte Globulin	720095	Exempt
Busulfan	750	Exempt
Cyclophosphamide	26271	Exempt
Fludarabine	312887	Exempt
Melphalan	008806	Exempt
Mesna	113891	Exempt
Mycophenolate mofetil	724229	Exempt
Tacrolimus	717865	Exempt

SEE SECTION 14-16 FOR SPECIMEN SHIPPING ADDRESSES

ABSTRACT

ASCT1221 is a randomized Phase II trial designed to identify which myeloablative hematopoietic cell transplant (HCT) preparative regimen has a lower relapse and treatment related mortality (TRM) rate in children with juvenile myelomonocytic leukemia (JMML). Patients with JMML according to Chan et al (including diagnostic genotyping from a CLIA/CAP-certified central laboratory) will be randomized to one of two myeloablative conditioning regimens within strata defined by donor type and *PTPN11* mutation status. The objective of the study is to identify the better conditioning regimen. It is hypothesized that the regimens will have equivalent relapse rates but may differ in TRM, a feature that has been exploited in the study design. Our hypothesis is that patients randomized to receive a busulfan-fludarabine (BU-FLU) conditioning regimen will have less TRM and comparable event-free survival (EFS) when compared to a busulfan-cyclophosphamide-melphalan (BU-CY-MEL) conditioning regimen. Correlative biologic studies will determine the feasibility of assessing post-transplant disease burden by donor chimerism measurements and mutant allele burden. We will also validate gene expression classifiers using RNA-based or methylation-based arrays in patients with JMML that may predict relapse, and will comprehensively assess genetic and biochemical alterations amongst patients with JMML who are treated on this transplant protocol.

EXPERIMENTAL DESIGN SCHEMA



1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Objectives

1.1.1 To compare – in a randomized fashion – the Day 100 TRM incidence for two myeloablative conditioning regimens, busulfan-fludarabine (BU-FLU) and busulfan-cyclophosphamide-melphalan (BU-CY-MEL), prior to HCT for children with JMML, in order to determine the preferred regimen for future trials.

1.1.2 To compare – in a randomized fashion – the 18-month event-free survival (EFS) following two different myeloablative conditioning regimens (BU-FLU vs. BU-CY-MEL) prior to HCT for children with JMML, in order to determine the preferred regimen for future trials.

1.2 Secondary Objectives

1.2.1 To determine the 18-month relapse incidence (RI) following two different myeloablative conditioning regimens (BU-FLU vs. BU-CY-MEL) prior to HCT for children with JMML.

1.2.2 To determine the graft failure rates following two different myeloablative conditioning regimens (BU-FLU vs. BU-CY-MEL) prior to HCT for children with JMML.

1.3 Exploratory Objectives

1.3.1 To determine the rates of severe toxicities (Grade 3/4) at Day 100 post-HCT between the two myeloablative conditioning regimens (BU-FLU vs. BU-CY-MEL).

1.3.2 To determine the rates of acute and chronic (at 18 months post-HCT) graft-versus-host disease (GVHD) following HCT using two different conditioning regimens (BU-FLU vs. BU-CY-MEL) in children with JMML.

1.3.3 To create a JMML-specific pre-HCT index to allow better risk-stratification of future patients.

1.3.4 To determine the feasibility of assessing post-transplant disease burden by donor chimerism measurements and allele-specific PCR in mononuclear and sorted cell subsets.

1.3.5 To validate gene expression and methylation classifiers predictive of relapse in patients with JMML.

1.3.6 To comprehensively assess genetic and biochemical alterations amongst patients with JMML who are treated on this transplant protocol.

2.0 BACKGROUND

2.1 Rationale for Selected Approach and Trial Design

JMML is an uncommon disease occurring exclusively in young children who frequently present with a high disease burden and severe clinical symptoms including massive hepatosplenomegaly, pulmonary infiltrates, fevers, infections, and rash. Median survival of 1-2 years is commonly reported without HCT.[1,2] With few exceptions, long-term EFS has only been achieved following HCT, with the main cause of treatment failure being relapse.[3,4] Though not intuitive, there is little evidence that reduction of disease burden using chemotherapy prior to the preparative transplant regimen has an effect on post-transplant outcomes.[4] These patients are at high risk for transplant morbidity and mortality with conventional myeloablative regimens, which have been dose escalated to maximum tolerability in an attempt to prevent the high relapse risk. Reported rates of TRM range from 13-33% depending upon stem cell source utilized.[4-7] The median age at transplant is 2.5 years (0.3 – 15 years),[4] and thus, these very young children are also at increased risk of late effects when maximally intensive conditioning regimens are utilized.

Studies have demonstrated that immunotherapy-based interventions, either rapid withdrawal of immunosuppression[8,9] or administration of donor lymphocyte infusion (DLI),[10-12] can result in temporary and rarely long-lasting disappearance of residual/relapsing JMML cells following HCT, particularly when introduced in the setting of a low disease burden early in relapse. In addition, the use of *ex vivo* T-cell depletion has been shown to result in increased rates of relapse.[13] Therefore, we hypothesize that the primary mechanism by which HCT results in long-term disease-free survival in some patients with JMML is due to the provision of a source of alloreactive graft-versus leukemia (GVL) activity. As such, we further hypothesize that the myeloablative preparative regimen prior to HCT plays little role per se in the long-term disease-control of patients with JMML, provided that it allows donor cell engraftment and adequate transfer of alloreactive cells. Thus, in this trial, we will test if a BU/FLU conditioning regimen proves to have less TRM compared to BU-CY-MEL with equivalent EFS. If BU/FLU is chosen as the preferred regimen to carry forward, we believe that the reduced toxicity of this regimen will facilitate future trials incorporating novel pre- or post-HCT targeted agents to optimize the outcomes for these children. During the course of this trial, we will capture the molecular genotypes of patients and we will also validate approaches to measuring minimal residual disease, which we hypothesize will provide more sensitive new metrics that will facilitate assessing response to therapeutic interventions for JMML.

The study design is a modified randomized phase II trial. Patients will be assigned to BU-FLU or BU-CY-MEL myeloablative conditioning regimen using a randomized block strategy within strata defined by donor type and *PTPN11* mutation status. The outcome of the trial will be determined after all eligible patients have 18 months of follow-up. If the difference in the estimated 18-month EFS is more than 5%, then the regimen with the higher EFS will be declared the superior treatment. If the difference in EFS is less than 5%, then the regimen with the lowest 100 day TRM risk will be declared the superior treatment. The randomized phase II approach is appropriate since both arms can be considered experimental and requires a much smaller sample size to complete than a phase III trial. As discussed in Section 9.2, the modification of the design described above exploits the *a priori* belief that relapse probabilities will not influence the choice of regimen, therefore increasing the probability that the trial will pick the better regimen.

2.2 Trial Impact

Because JMML is a relatively rare malignancy unique to children, advances in the care of these children can only be accomplished in the setting of a cooperative group trial. This was demonstrated in Europe by the European Working Group on Childhood Myelodysplastic Syndromes and Bone Marrow Transplant Working Group (EWOG-MDS/EBMT). This consortium has used a maximally intense conditioning regimen, BU-CY-MEL in a limited institution setting.[4] However, it is unclear if the toxicity of the preparative regimen plays a significant role in the ultimate control of the disease, or if less toxic regimens will still accomplish the goal of providing a sustained production of alloreactive cells with less treatment-related morbidity and mortality. Since the current BU-CY-MEL conditioning regimen cannot be dose-escalated further, we hypothesize that superior results will be accomplished by maximizing the alloreactivity of the transplant process. In addition, we hypothesize that improving outcome will ultimately require therapies to block important cell growth pathways, either pre- or post-HCT with the use of targeted agents. Both of these goals may be significantly easier to accomplish if the patients have less morbidity and mortality from their HCT. By testing the approach of utilizing a fully myeloablative but lower toxicity conditioning regimen needed to achieve engraftment and thereby harnessing post-transplant GVL responses, we hope to implement a novel paradigm of how to approach HCT for JMML. Such a strategy is already being implemented in acute myeloid leukemia (AML); however the disease burden in JMML is generally higher at the start of the conditioning regimen and it remains untested if this will impede the use of lower doses of chemotherapy using BU-FLU.

2.3 HCT for JMML

2.3.1 "Standard of Care" HCT for JMML

There is no currently agreed upon "standard-of-care" preparative regimen in use for patients with JMML. Aricò *et al.* reviewed the outcome of 91 children with JMML treated with HCT in 16 different reports, with an OS of 41%.[1] The EWOG-MDS/EBMT reported the largest cohort to date (n = 100) in which the 5-year EFS was 52% (95% CI, 42-62%) and utilized a myeloablative preparative regimen with high doses of BU-CY-MEL.[4] Importantly, the European triple-alkylator-based approach was done in a limited-institution fashion. Thus, there is the potential that this regimen might be infeasible in a group-wide context. Because of the limited-center design of that trial, we cannot estimate a meaningful toxicity baseline for this regimen when implemented across the 50+ COG-accredited centers. Cyclophosphamide significantly contributes to the development of sinusoidal obstruction syndrome post-HCT,[14] and thus BU-FLU regimens have been noted to have a lower incidence of this toxicity.[15] The EWOG-MDS/EBMT also reported the results of 42 children with JMML undergoing umbilical cord blood transplant (UCBT).[6] The cumulative 5-year incidence of TRM, relapse, and EFS were 33%, 22%, and 45%, respectively. The results of AAML0122, which utilized a TBI-based conditioning regimen, were no better, with a 5-year EFS of 41% (95% CI, 30-52%) and a 2-year RI of 40% (95% CI, 29-51%) (Robert Gerbing, unpublished data). The lack of apparent benefit with a TBI-based conditioning, in conjunction with the increased risk of late effects due to TBI, led to the abandonment of a TBI-based approach for this trial. Further escalation of the conditioning regimen in JMML appears unlikely to produce significant improvement in EFS without unacceptable rates of TRM and

we hypothesize that novel strategies will ultimately be needed in order to reduce the high rates of relapse seen in these patients.

The COG protocol AAML0122 was closed in October 2006 after the European approach was published in January 2005.[4] A request to the CIBMTR in April 2011 revealed that from 2005 to 2009, the majority of children in North America with JMML (n = 75) were transplanted with busulfan and cyclophosphamide (63%). The COG approach, based on TBI and cyclophosphamide, was utilized in 16%, and the European approach of BU/CY/MEL was used in only 9% of cases, with TBI + Other (1%), BU + FLU +/- MEL (3%), MEL + Other (5%), and Unknown (4%) representing the remaining 12% of cases (Mary Horowitz, Scientific Director, personal communication). Thus, despite the fact that the European approach has been published since 2005, the majority of US transplant physicians have not adopted this approach for JMML, but rather utilized a preparative regimen considered standard for myeloid malignancies.

Busulfan can be administered on a variety of schedules following HCT. Classically, busulfan has been given every 6 hours for 16 doses. More recently, many centers have moved to a once daily for 4 doses regimen,[16] and this approach has been adopted for AAML1031. It has also been shown that every 12 hour dosing is comparable to every 6 hour dosing.[17] Since no head-to-head comparison has ever been performed, all three options will be acceptable practices on this trial.

The approach to GVHD prophylaxis for this trial was guided by an attempt to establish a uniform regimen for all patients despite differing stem cell sources. Standard GVHD prophylaxis regimens combine a calcineurin inhibitor (tacrolimus or cyclosporine) with short-course methotrexate. Cyclosporine and tacrolimus are relatively comparable medications, though tacrolimus has been used more often in COG trials (e.g. ASCT0431 and AAML1031). However, traditionally, methotrexate use has been avoided in recipients of UCB grafts, due to concerns of prolonging count recovery. For many years corticosteroids were utilized instead, however, recently mycophenolate mofetil (MMF) has become the most common second agent. It is a non-competitive selective inhibitor of inosine monophosphate dehydrogenase, and lacks major mucosal, hepatic, or renal toxicity compared to other prophylactic aGVHD immunosuppressant drugs. Because of this, MMF was utilized in the COG / Clinical Trials Network shared trial 0501 studying single vs. double UCB HCT at a dose of 15 mg/kg every 8 hours, a dose found to be important in children.[18] It has also been demonstrated that MMF can be successfully utilized in patients receiving non-UCB transplants.[19,20]

2.3.2 Use of Cyclophosphamide vs. Fludarabine

One study demonstrated that 15.75 Gy of TBI is superior to 12 Gy of TBI for preventing relapse in patients with AML, albeit with an increased risk of TRM, so that EFS and OS were identical.[21] Outside of this trial, we are unaware of any studies that demonstrate that – within the range of myeloablative preparative regimens, and with a comparable serotherapy regimen – there is any difference in relapse incidence (RI) for more toxic cyclophosphamide-based regimens versus less toxic fludarabine-based regimens. In fact, there are several retrospective studies that suggest that the RI for patients with AML/MDS is comparable

between the two regimens and potentially favors BU-FLU. Andersson *et al.* demonstrated a 3-year RI of 43% for the BU-CY group vs. 37% for the BU-FLU group,[22] while Chae *et al.* demonstrated a 2-year RI of 40% for the BU-CY group vs. 23% for the BU-FLU group.[15] In children with AML undergoing matched related donor HCT, unpublished CIBMTR data demonstrates a one year OS of 73% in 40 children conditioned with myeloablative doses of BU-FLU, compared to 63% in 200 children conditioned with BU-CY (per AAML1031).

Furthermore, as noted above, the introduction of the highly immunosuppressive fludarabine into myeloablative transplant preparative regimens for AML has demonstrated lower rates of TRM and equivalent relapse rates than cyclophosphamide-containing regimens.[23,24] The Japanese Childhood MDS Study Group reported a pilot trial in 10 children with JMML that demonstrated that fludarabine could be safely substituted for cyclophosphamide.[25] In addition, in a non-randomized cohort of 27 children with JMML in Japan, the 4-year EFS following HCT with a wide variety of conditioning regimens was similar for patients who received melphalan (67%; 95% CI, 42-85%) versus those who did not (58%; 95% CI 32-81%, $P = .71$).[5]

Given that Locatelli *et al.* demonstrated that pre-transplant chemotherapy intensity has not impacted outcome post-transplant in JMML,[4] it is reasonable to question whether highly toxic, dose-maximized myeloablative regimens, including BU-CY-MEL, are needed. However, many patients with JMML coming to transplant will be naïve to chemotherapy and thus may be at higher risk of graft failure if there is insufficient immunosuppression. Pilot studies in other patient groups with similar issues (chronic myeloid leukemia and non-malignant conditions) have demonstrated that approaches including busulfan, fludarabine, and anti-thymocyte globulin (ATG)[26] or alemtuzumab[27,28]-based regimens can successfully achieve engraftment in the majority of chemotherapy-naïve patients (89-91%), with low rates of Day 100 TRM (0-9%).

Taken together, since there is no data to suggest that the toxicity of the conditioning regimen in JMML is critical for long-term disease control, we therefore propose to determine which fully myeloablative[29] regimen (BU-FLU vs. BU-CY-MEL, plus/minus rabbit ATG) produces the least amount of TRM, while achieving stable donor cell engraftment and equivalent rates of relapse.

2.3.3 Serotherapy

Serotherapy (ATG) will only be utilized for patients undergoing unrelated adult donor and umbilical cord blood transplants, since a prospective trial showed no difference in 2-year RI in patients undergoing unrelated donor transplant who did (28.9%) or did not (23.6%) receive ATG ($P = .55$), but did decrease rates of both acute and chronic GVHD.[30] This was confirmed in another recent prospective trial.[31] Furthermore, Locatelli *et al.* reported no difference in 5-year RI in patients with JMML undergoing unrelated donor HCT who did (36%) or did not (33%) receive serotherapy (P = NS), though there was a trend towards less non-relapse mortality in the serotherapy group.[4] This practice is in uniformity with the conditioning regimen on the COG AAML1031 trial, in which only unrelated donors receive ATG. This is also similar to the European approach in which the use of serotherapy was optional, but was primarily given only to unrelated donors.[4]

The timing of serotherapy will vary between patients receiving UCB grafts vs. non-UCB grafts. Traditional dosing of ATG with non-UCB grafts occurs on the days immediately prior to HCT. However, there is concern that this proximal dosing of ATG causes excessive *in vivo* T-cell depletion in recipients of UCBTs, which typically have at least a 10-fold lower T cell amounts than non-UCB stem cell sources. In turn, this excessive T cell depletion has been linked to a higher incidence of mixed chimerism or frank graft rejection. One approach to solving this quandary has been to administer the ATG at an earlier time period before UCBT, and preliminary data suggests that this can produce excellent results (Boelens JJ, et al. unpublished data). In a European study of pediatric patients with either malignancy (n = 40) or inborn errors (n = 63), Bu-Flu recipients from 2009-11 were compared to a historical population of Bu-Cy+/-Mel recipients from 2005-09, with ATG administered to UCB recipients at Day -9 to -6. The majority of patients in the study (63%) received UCB, with a slight preponderance of UCB donor transplants in the Bu-Flu group (40/55 vs. 25/48; $P = 0.051$). Despite this imbalance, the incidence of graft failure was identical between the 2 groups (4%+/-3% in Bu-Flu vs. 5%+/-3% in Bu-Cy+/-Mel; p = 0.89), and this effect was retained in the minimally-pretreated inborn errors group (0%+/-6% in Bu-Flu vs. 7%+/-6% in Bu-Cy+/-Mel; $P = 0.34$), a group more similar to minimally-pretreated JMML patients than a typically heavily-pretreated patient with AML. We therefore have adopted this "early ATG" approach for patients getting UCB donor transplants on this trial in order to try to avoid excessive rates of graft failure.

2.4 Clinical Implications of Genetic Mutations in JMML

2.4.1 Diagnostic and Risk-Stratification Implications

Establishing the diagnosis of JMML can be difficult for many clinicians and is currently made by meeting a combination of clinical and laboratory parameters, according to expert modifications of the World Health Organization (WHO) criteria (see Table 1 in Section 4.3.1).[32,33] Taken together, 85% of patients with *de novo* JMML harbor a genetic lesion in molecules that encode proteins predicted to activate the Ras/MAPK pathway. Thirty-five percent have *PTPN11* mutations, 25% have *NRAS/KRAS* mutations, 10-15% have clinical NF1, and another 10-15% of patients have been recently described to harbor mutations in *CBL*.[34-36] Animal models demonstrate that *Nf1, Kras*, *Ptpn11*, and *Cbl* mutant mice all develop fatal myeloproliferative disorders that model JMML.[37-42] These genetic criteria have been recently added to the WHO criteria to reflect these advances in unraveling the molecular genetics of this disorder, with the caveat that a minority (15%) of patients will not have a lesion in one of the aforementioned genes, nor will they have clinical neurofibromatosis. Funding through the Biomarker, Imaging, and Quality of Life Studies Funding Program has been obtained for this protocol for the assessment of genetic alterations in *PTPN11*, *NRAS*, *KRAS*, and *CBL* through the UCSF CLIA/CAP approved laboratory environment. At the time of enrollment onto ASCT1221, samples will be submitted to UCSF for diagnostic genotyping, as well as research studies. Pyrosequencing with confirmation using an orthogonal platform (Sanger sequencing) will be performed on blood and/or bone marrow with further assessment of either buccal or fibroblasts to determine germline origin.

Considerable controversy remains about the prognostic significance of individual genetic lesions. The largest series of transplanted JMML patients did not document the presence of *NRAS/KRAS*, *PTPN11*, or *CBL* (first described in 2009) mutations in their cohort.[4] Only patients with clinical evidence of NF1 were reported, and though it did not reach statistical significance, there was a difference in outcomes in those patients with clinical NF1 (EFS 36%, RI 50%) compared to those without (EFS 55%, RI 34%).[4] A small Japanese study reported that a *PTPN11* mutation was the only unfavorable factor predicting EFS after HCT (30% in the *PTPN11* mutants vs. 69%; $P = .018$).[43] Another recently published small Korean report also demonstrated a trend towards an unfavorable outcome for patients with *PTPN11* mutations (59% vs. 86% for those without *PTPN11*, $P = .22$).[44] A trend towards worse EFS for *PTPN11* mutated patients, versus better EFS for *RAS* mutated patients was also noted in a European study (31% vs. 71%, respectively, vs. 44% for those with no mutation).[45] Finally, it is known that rare patients have been reported to spontaneously improve or resolve their disease. For example, 5 out of 6 patients with recently documented *CBL* lesions spontaneously resolved while only 1 died of progressive disease.[46] Spontaneous resolution is not uniform among *CBL* patients, as at least one of the patients who received HCT died from relapsed disease—and this patient's affected relatives also died from JMML. In other case reports, rare patients with *RAS* mutations have spontaneously improved.[47]

Given the genetic heterogeneity of JMML, we feel this is a unique opportunity to capture the "genetic demographics" of patients enrolled on this study. While randomization provides probabilistic balance of genetic risks for the outcomes across the treatment groups, there is good evidence that *PTPN11* mutation status is strong predictor of relapse so block randomization by *PTPN11* status (by donor type) will be used to assure absolute balance on this factor.

2.4.2 Transplant vs. No-Transplant for Patients with Germline Syndromes who Develop JMML

Patients with germline mutations in *PTPN11* (Noonan Syndrome) develop a myeloproliferative disorder that strongly resembles JMML, though nearly all cases appear to spontaneously regress over the first 18 months of life.[48,49] As such, these patients will not be eligible for treatment on this protocol. Patients with germline mutations in *CBL* develop JMML were discussed in Section 2.4.1, and as noted, we believe that patients with a germline *CBL* mutation are eligible for HCT if their clinical course is aggressive. Likewise, we feel that patients with *NRAS* or *KRAS* mutations are also eligible for HCT. Patients with germline mutations in *HRAS* (Costello Syndrome) are at increased risk of certain malignancies, but JMML has not been reported, and as such, there is no *a priori* reason to think that this would spontaneously regress. Therefore, such a patient would be eligible for treatment on this protocol.

2.4.3 Correlative Biology Studies

While very rare patients have been reported to resolve spontaneously,[47] the data also support that the best survival outcomes are associated with swift transplant at an early age. Thus, clinicians are routinely faced with the clinical dilemma of watchful waiting versus moving to swift HCT, a paradoxical approach that is understandably anxiety provoking for patients and physicians. Improved risk stratification for patients with JMML is warranted, but as we have presented

earlier, genotype may or may not be associated with outcome. Recent reports indicate that gene expression or methylation profiles may predict which patients will do well with currently available therapies[45,50] versus those patients who may require additional interventions to optimize cure. Furthermore, following these patients on therapy and diagnosing relapse has been problematic and has largely relied on measuring falling donor chimerism after HCT. We would thus propose a series of correlative biology studies to address these management issues for newly diagnosed JMML patients who are enrolled on this clinical trial.

A recent publication by Bresolin *et al.* identified an AML-type gene expression signature in JMML patients that was associated with a high risk of relapse[45] - such assays will be performed in RNA extracted from CD34+ cells selected from patients enrolled onto this protocol. The RNA will be extracted upon receipt, stored, and analyzed for only those patients who proceed to the HCT so that outcome can be assessed. An additional report by Olk-Batz and colleagues indicated that hypermethylation of 4 genes was associated with an inferior outcome for patients and raised the possibility of therapeutic interventions using DNA methyl-transferase inhibitors. Through additional collaborations, we will be assessing genome-wide methylation profiling[50] to determine the prognostic significance of such signatures and associated novel therapies.

Given the fact that JMML is a difficult disease to follow during therapy, we would also propose validation of an allele-specific PCR-based reaction for subsets of patients with mutations in *PTPN11* and *RAS*. These measurements will be done on diagnostic specimens (prior to receiving significant cytoreductive or differentiating therapy), pre-HCT (this will control for individualized pre-HCT therapy) and post-HCT at defined time-points in bulk mononuclear cells and in sorted cell subsets. We have previously designed and optimized a fluorescently based allele-specific PCR reaction for 12 Ras pathway mutations and published these results with EWOG-MDS colleagues to validate the assay.[51] In the analysis, we were able to demonstrate the a) sensitivity and specificity of the assay in selectively detecting these point mutations, b) detection of molecular relapse well ahead of clinical relapse and frequently ahead of falling donor chimerism measurements (currently the standard to detect relapse after HCT), and c) concordance of simultaneously obtained peripheral blood and bone marrow measurements. As a result of this successful collaboration, we will also be conducting these studies for our EWOG-MDS colleagues who will be treating their patients on their clinical trial. In this manner, we will be able to assess different clinical interventions (EWOG-MDS versus COG) using measurements obtained at the same timepoints post-HCT and analyzed in the same lab. Given the very recent developments of using sensitive 454 sequencing methodologies to detect JMML-specific mutations, we will also compare our allele-specific PCR assays against 454 sequencing results. While we predict that the pyrosequencing strategies will not be as sensitive as the allele-specific PCR, we know that they will be applicable to more patients and thus may have broader use in this population.

Because JMML exhibits considerable cellular heterogeneity, it has been difficult to elucidate the biologic features of cells that contribute to cancer phenotypes *in vivo*, and of precursor populations that might carry genetic lesions predisposing cells to an oncogenic fate. Phosphoflow cytometry is capable of measuring

surface antigens to identify subsets of cells with simultaneous assessments of intracellular protein phosphorylation to provide a dynamic view of perturbed signaling cascades.[52] JMML is an ideal disease for assessing the biochemical signature of aberrant signaling induced through GM-CSF stimulation and presumably via the Ras pathway. We previously identified a reproducible phosphoflow signature after exposing JMML samples to low doses of GM-CSF that closely mimics the readout of a 2-3 week CFU-GM assay, and will capitalize on this technology to design additional studies focused on identifying new drugs that could be employed in this disease.[53]

Finally, recent data indicates that a subset of JMML patients exhibit autoimmune features at diagnosis, manifested by high IgG levels and positivity on direct antibody tests using Coombs methods. Some patients may further exhibit features consistent with autoimmune lymphoproliferative disorders.[54] We recognize that this protocol will facilitate the capture of these variables in this patient cohort and wish to measure IgG and Coombs at the time of enrollment in order to determine their effect on patient outcomes. We anticipate that the laboratory assays that we and others have developed will allow us to identify patients at risk for poor outcomes and ultimately hope to identify novel agents that can be used to treat patients with this aggressive myeloid malignancy.

3.0 STUDY ENROLLMENT PROCEDURES AND PATIENT ELIGIBILITY

3.1 Study Enrollment

3.1.1 Patient Registration

Prior to enrollment on this study, patients must be assigned a COG patient ID number. This number is obtained via the COG Registry system once authorization for the release of protected health information (PHI) has been obtained. The COG patient ID number is used to identify the patient in all future interactions with COG. If you have problems with the registration, please refer to the online help.

In order for an institution to maintain COG membership requirements, every newly diagnosed patient needs to be offered participation in ACCRN07, *Protocol for the Enrollment on the Official COG Registry, The Childhood Cancer Research Network (CCRN)*.

A Biopathology Center (BPC) number will be assigned as part of the registration process. Each patient will be assigned only one BPC number per COG Patient ID. For additional information about the labeling of specimens please refer to the Pathology and/or Biology Guidelines in this protocol.

3.1.2 IRB Approval

Local IRB/REB approval of this study must be obtained by a site prior to enrolling patients. Sites must submit IRB/REB approvals to the NCI's Cancer Trials Support Unit (CTSU) Regulatory Office and allow 3 business days for processing. The submission must include a fax coversheet (or optional CTSU IRB Transmittal Sheet) and the IRB approval document(s). The CTSU IRB Certification Form may be submitted in lieu of the signed IRB approval letter. All CTSU forms can be located on the CTSU web page (https://www.ctsu.org). Any other regulatory documents needed for access to the study enrollment screens will be listed for the study on the CTSU Member's Website under the RSS Tab.

IRB/REB approval documents may be faxed (1-215-569-0206),
E-mailed (CTSURegulatory@ctsu.coccg.org) or mailed to the CTSU Regulatory office.

When a site has a pending patient enrollment within the next 24 hours, this is considered a "Time of Need" registration. For Time of Need registrations, in addition to marking your submissions as 'URGENT' and faxing the regulatory documents, call the CTSU Regulatory Helpdesk at: 1-866-651-CTSU. For general (non-regulatory) questions call the CTSU General Helpdesk at: 1-888-823-5923.

3.1.3 Study Enrollment

Patients may be enrolled on the study once all eligibility requirements for the study have been met. Study enrollment is accomplished by going to the CTSU OPEN (Oncology Patient Enrollment Network) https://www.ctsu.org/OPEN_SYSTEM/. For questions, please contact the CTSU OPEN helpdesk at ctsucontact@westat.com or call 1-888-823-5923.

3.1.4 Timing

Patients must be enrolled before treatment begins. Guidelines for patient management pre-HCT are provided in Appendix IV and may be used per physician discretion.

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated in the eligibility section below.

Protocol therapy (HCT conditioning) must begin within 1 year of enrollment.

3.1.4.1 Cytogenetics

Specimens for cytogenetic analysis are required and must be obtained prior to therapy initiation (other than hydroxyurea, 6-mercaptopurine, cis-retinoic acid, or low-dose intravenous cytarabine [≤ 200 mg/m^2/cumulative dose]). Use of a COG-approved institutional cytogenetics laboratory is required (see Section 13.2 for specimen submission guidelines). A listing of these laboratories may be found on the COG website as well as methods of attaining COG approval for local cytogenetics laboratories. Results of cytogenetics are not required to be completed prior to enrollment; however cytogenetic reports and associated materials must be sent for central review within 2 weeks of enrollment (see Section 13.2 for central review submission requirements).

3.1.4.2 Genotyping

Submission of diagnostic specimens for genotyping analysis at the UCSF CLIA/CAP-approved laboratory is required at the time of enrollment. See Section 15 for specimen submission and shipping requirements.

3.1.4.3 Pathology

Submission of central pathology review specimens is required. Specimens used for institutional diagnosis must have been obtained prior to therapy initiation (other than hydroxyurea, 6-mercaptopurine, cis-retinoic acid, or low-dose intravenous cytarabine [≤ 200 mg/m^2/cumulative dose]). Specimens must be sent for central review within 2 weeks of enrollment. See Section 14 for specimen submission guidelines.

3.1.4.4 Initiation of HCT Donor Search

At the time of diagnosis, the HCT donor search is to be initiated according to institutional procedures. Stem cell donor requirements are described in Section 4.2.

3.1.4.5 Obtaining Informed Consent

The consent process for ASCT1221 is divided into 2 stages. The Part 1 Consent is provided to allow patient enrollment and submission of clinical genotyping specimens and correlative biology to UCSF (see Section 15). When the requirements to proceed to HCT (as described in Section 4.3) are met, the Part 2 Consent is provided to allow HCT conditioning regimen randomization.

3.1.5 Randomization

Randomization will occur via OPEN when:

(1) The diagnosis of JMML according to Chan et al[33] is confirmed (see Section 4.3.1)

(2) The HCT donor source has been identified (see Section 4.2)

(3) The patient has met all requirements to proceed to HCT (see Section 4.3).

Patients will be randomly assigned to either myeloablative allogeneic HCT with a busulfan-fludarabine conditioning regimen or a busulfan-cyclophosphamide-melphalan conditioning regimen. HCT donor source and *PTPN11* mutational status must be entered into the OPEN system for patient stratification. Stratification groups are defined as:

(1) Matched related donor, *PTPN11* mutation present
(2) Matched related donor, *PTPN11* mutation absent
(3) Closely matched unrelated donor, *PTPN11* mutation present
(4) Closely matched unrelated donor, *PTPN11* mutation absent
(5) Umbilical cord blood, *PTPN11* mutation present
(6) Umbilical cord blood, *PTPN11* mutation absent

HCT conditioning regimen must begin within 5 days of randomization.

3.2 Patient Eligibility Criteria

Important note: The eligibility criteria listed below are interpreted literally and cannot be waived (per COG policy posted 5/11/01). All clinical and laboratory data required for determining eligibility of a patient enrolled on this trial must be available in the patient's medical/research record which will serve as the source document for verification at the time of audit.

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated. See Section 4.3 and Section 7.1 for required studies to be obtained prior to starting protocol therapy.

INCLUSION CRITERIA

3.2.1 Age

Patients must be ≥ 3 months and ≤ 18 years at the time of study enrollment.

3.2.2 Diagnosis

a. Patients must have a strong clinical suspicion of JMML, based on a modified Category 1 of the revised diagnostic criteria, as described by Chan et al.[33] Specifically, eligible patients must have all of the following:

- Splenomegaly*
- Absolute monocyte count (AMC) > 1000/µL
- Blasts in PB/BM < 20%

*For the 7-10% of patients without splenomegaly, the diagnostic entry criteria must include all other features described above and at least 2 of the following criteria (based on a modified Category 3 of Chan et al)[33]:

- Circulating myeloid precursors
- WBC > 10,000/μL
- Increased fetal hemoglobin (HgbF) for age
- GM-CSF hypersensitivity

OR

b. Patients must have been previously diagnosed with JMML (per Section 4.3.1).

3.2.3 Prior Therapy
Patients must be previously untreated with HCT.

Please see Section 4.5 for the concomitant therapy restrictions for patients during treatment.

EXCLUSION CRITERIA

3.2.4 Mutation Status of *PTPN11* (Germline)
Patients with a known germline mutation of *PTPN11* (Noonan's Syndrome) are not eligible.

3.2.5 Neurofibromatosis Type 1 (NF1) Status
Patients with a known history of NF1 (Neurofibromatosis Type 1) and either

- a history of a tumor of the central nervous system (astrocytoma or optic glioma), or
- a malignant peripheral nerve sheath tumor with a complete remission of < 1 year are not eligible.

3.2.6 HIV Status
HIV positive patients are not eligible.

REGULATORY

3.2.7 All patients and/or their parents or legal guardians must sign a written informed consent.

3.2.8 All institutional, FDA, and NCI requirements for human studies must be met.

4.0 TREATMENT PROGRAM

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

All transplants performed on COG trials must occur at FACT-accredited SCT programs.

4.1 Overview of Treatment Plan

Patients on ASCT1221 will receive an allogeneic stem cell transplant utilizing one of 2 conditioning regimens:

- **Arm A**: a Busulfan, Cyclophosphamide and Melphalan (BU-CY-MEL) regimen,

or

- **Arm B**: a Busulfan and Fludarabine (BU-FLU) regimen.

Patients will be randomized to Arm A or Arm B and stratified equally by stem cell source and *PTPN11* mutational status as described in Section 3.1.5. Randomization will occur when:

(1) The diagnosis of JMML according to Chan et al[33] is confirmed (see Section 4.3.1)

(2) The HCT donor source has been identified (see Section 4.2)

(3) The patient has met all requirements to proceed to HCT (see Section 4.3).

Note: If the patient was enrolled at a non-COG transplant center, the patient must be transferred to a COG-approved transplant center prior to randomization.

For the duration of the study, specimens will be collected and sent to the UCSF CLIA-certified laboratory and Biopathology Center (BPC; if patient has consented to specimen banking) (see Section 7 and 15 for details).

See Appendix IV for pre-transplant therapy recommendations.

"Protocol Therapy" for the purposes of this study encompasses the time from the initiation of the conditioning regimen through the end of the GVHD prophylaxis (Day +98) for matched family member donors. Day +180 for unrelated and cord blood donors).

4.2 Hematopoietic Stem Cell Matching and Cell Dose Requirements

4.2.1 Bone Marrow and Peripheral Blood Stem Cell (PBSC) Requirements

Allele level typing of HLA A, B, C, and DRB1 is required for unrelated or related bone marrow and PBSC donors but not matched siblings (i.e. matched parent, partially mismatched sibling, etc). Allele level typing of DQB1 is recommended.

4.2.2 Matched Related Donor and Cord Blood Unit Requirements

Serological matching of Class I antigens is acceptable, but allele level typing is required for DRB1 for matched related donors and cord blood units. Allele level typing of Class I antigens and DQB1 is recommended.

4.2.3 Hierarchy of Stem Cell Choices

The hierarchy of stem cell choices is as follows:

1. Preferred: Genotypically HLA matched relatives. Syngeneic (identical twin) donors are not allowed.

2. If number 1 above not available, choices a and b below are equally acceptable:
   a) **Unrelated or "other" related (non-genotypically matched) donor:** High resolution typing of 8 alleles required. Consistent with NMDP guidelines, minimum standard is a 5/6 match (antigen level at class I (HLA A, B) and allele level class II (DRB1)), but 7/8 or 8/8 including matching at HLA C at the allele level is desired. Haploidentical relatives (> 1 HLA mismatch) are not allowed.

   b) **Cord blood:** Serologic Class I (A and B) and allele level Class II (DRB1) typing is required. Minimum requirement is a 4/6 antigen match (HLA A, B, and DRB1). Minimum pre-thaw cell dose of 5 x 10^7 nucleated cells/kg recipient body weight is required.

4.3 Requirements to Proceed to HCT

The following are requirements for a patient to proceed to randomization and assignment of HCT preparative regimen. HCT preparative regimen must be initiated within 5 days of randomization. If a patient fails to meet the requirements described below within one year of enrollment, the patient will be Off Protocol Therapy and ASCT1221 Follow up Evaluations will be collected.

4.3.1 Confirmation of JMML Diagnosis

Patients must have confirmed JMML, ascertained upon completion of genotype testing at UCSF and cytogenetic analysis, based on the revised diagnostic criteria, as described by Chan et al[33] (see table below).

Table 1: JMML Diagnostic Criteria (Chan et al[33])

Category 1	Category 2	Category 3
All of the following: • Splenomegaly* • Absolute monocyte count (AMC) > 1000/μL • Blasts in PB/BM < 20% • Absence of the t(9;22) *BCR/ABL* fusion gene	At least 1 of the following • Somatic mutation in *RAS* or *PTPN11* • Clinical diagnosis of NF1 or *NF1* gene mutation • Homozygous mutation in *CBL* • Monosomy 7	At least 2 of the following • Circulating myeloid precursors • WBC > 10,000/μL • Increased fetal hemoglobin (Hgb F) for age • Clonal cytogenetic abnormality excluding monosomy 7 • GM-CSF hypersensitivity

The diagnosis of JMML is made if a patient meets all of the Category 1 criteria and one of the Category 2 criteria without needing to meet the Category 3 criteria. If there are no Category 2 criteria met, then the Category 3 criteria must be met.

* For the 7-10% of patients without splenomegaly, the diagnostic criteria must include all other features in Category 1 AND one of the parameters in Category 2 OR no features in Category 2 but two features in Category 3.

4.3.2 Prior Therapy Restrictions

To proceed with HCT randomization and preparative regimen, patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy.

a. Myelosuppressive Chemotherapy and Targeted Agents: Must not have received myelosuppressive chemotherapy or targeted agents within 2 weeks of the start of HCT conditioning regimen (4 weeks if prior nitrosourea).

b. Biologic (anti-neoplastic agent): Must not have received a biologic agent within 7 days of the start of HCT conditioning regimen.

c. Radiation therapy (RT): Must not have received local palliative RT (small port) within 2 weeks of the start of HCT conditioning regimen; ≥ 6 months must have elapsed if prior craniospinal RT or if ≥ 50% radiation of pelvis; ≥ 6 weeks must have elapsed if other substantial BM radiation.

Please see Section 4.5 for the concomitant therapy restrictions for patients during treatment.

4.3.3 Organ Function Requirements

To proceed with HCT randomization and preparative regimen, patients must meet the following organ function requirements. See Section 7.1 for timing requirements for organ function studies.

4.3.3.1 Adequate Renal Function Defined As:

- Creatinine clearance or radioisotope GFR ≥ 70 mL/min/1.73 m^2 or
- A serum creatinine based on age/gender as follows:

Age	**Maximum Serum Creatinine (mg/dL)**	
	Male	**Female**
3 months to < 6 months	0.4	0.4
6 months to < 1 year	0.5	0.5
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR[55] utilizing child length and stature data published by the CDC.

4.3.3.2 Adequate Liver Function Defined As:

- Total bilirubin < 2.5 mg/dL unless the increase in bilirubin is attributable to Gilbert's Syndrome, and
- SGOT (AST) or SGPT (ALT) < 5 x upper limit of normal (ULN) for age.

4.3.3.3 Adequate Cardiac Function Defined As:
- Shortening fraction of ≥ 27% by echocardiogram, or
- Ejection fraction of ≥ 50% by radionuclide angiogram.

4.3.3.4 Adequate Pulmonary Function Defined As:
- FEV1, FVC, and DLCO corrected for Hgb ≥ 60% by pulmonary function tests (PFTs).
- For children who are unable to cooperate for PFTs, the criteria are: no evidence of dyspnea at rest, no exercise intolerance, and not requiring supplemental oxygen therapy.

4.3.3.5 Central Nervous System Function Defined As:
- Patients with seizure disorder may proceed to HCT if on non-enzyme inducing anticonvulsants (see Appendix IIIB) and well controlled.
- CNS toxicity ≤ Grade 2.

4.3.4 Performance Level

Patients must have a Lansky or Karnofsky performance status score ≥ 60. Use Karnofsky for patients > 16 years of age and Lansky for patients ≤ 16 years of age.
See https://members.childrensoncologygroup.org/prot/reference_materials.asp under Standard Sections for Protocols.

Performance status must be assessed within the 14 days prior to the start of the HCT conditioning regimen.

4.3.5 Infection Status

Patients with HIV or uncontrolled fungal, bacterial or viral infections are not permitted to proceed to HCT conditioning regimen.
- Patients must not have begun treatment for or have any documentation of the presence of a new fungal infection within 30 days of the start of HCT conditioning regimen.

4.3.6 Pregnancy and Breast Feeding

4.3.6.1 Female patients who are pregnant are not permitted to proceed to HCT since fetal toxicities and teratogenic effects have been noted for several of the study drugs.

4.3.6.2 Lactating females are not permitted to proceed to HCT unless they have agreed not to breastfeed their infants.

4.3.6.3 Female patients of childbearing potential are not permitted to proceed to HCT unless a negative pregnancy test result has been obtained.

4.3.6.4 Sexually active patients of reproductive potential are not permitted to proceed to HCT unless they have agreed to use an effective contraceptive method for the duration of their study participation.

4.4 Hematopoietic Stem Cell Infusion Guidelines

Stem cells for allogeneic HCT are infused no sooner than 24 hours after the last dose of chemotherapy (not including ATG), according to the institution's standard operating procedure.

4.4.1 Donor Bone Marrow or Peripheral Blood Infusion Guidelines

For related donors, bone marrow (or peripheral blood stem cells, if donor is unwilling to provide bone marrow) will be collected locally according to the institution's standard operating procedure.

For unrelated donors, bone marrow (or peripheral blood stem cells, if donor is unwilling to provide bone marrow) will be collected and transported from the NMDP Donor Center (or equivalent country-specific national donor program) according to standard operating procedure. A minimum of 2×10^8 nucleated bone marrow cells/kg (2×10^6 CD34+ cells/kg if peripheral blood stem cells) of the recipients ideal weight must be collected, but 4×10^8 nucleated bone marrow cells/kg (4×10^6 CD34+ cells/kg if peripheral blood stem cells) is desirable. **There will be no manipulation of the bone marrow (or peripheral blood stem cells) to deplete T cells prior to marrow infusion**. Bone marrow should be processed to deplete red blood cells if there is a major ABO mismatch (e.g., O type in recipient and A type in donor) to avoid an acute hemolytic transfusion reaction, per institutional standard operating practices. Bone marrow (or peripheral blood stem cells) will be infused according to the institution's standard operating procedure. Donor bone marrow (or peripheral blood stem cell) grafts should be assessed for quantity of total nucleated cells, CD3, and CD34 cells.

4.4.2 Donor Umbilical Cord Blood Infusion Guidelines

4.4.2.1 UCB Unit Thaw

The cord blood should be thawed, diluted with or without wash per validated institutional or supplying cord blood bank procedures with the exception that bedside thawing and direct infusion is not allowed. Bedside thaws are not recommended because of the inability to rescue the product if there is loss of integrity of the UCB bag on thaw at the bedside and because of the instability of the cells in 10% DMSO post thaw.

All transplant centers/cellular therapy laboratories must be familiar with thawing of cord blood units. They must have validated procedures and maintain competency in the thaw process. The cord blood unit must be thawed in a qualified laboratory by trained personnel. Generally the cryopreserved unit is removed from the protective cassette, placed in a ziplock bag and thawed rapidly in a 37°C waterbath. The ziplock bag allows for recovery of cells if the cryopreservation bag cracks or leaks during the thawing process, a rare but possible event. Once the contents of the bag reach a slushy consistency, the cells can be diluted in dextran/albumin, a hypertonic solution that buffers against the intracellular hypertonicity created by DMSO. Cell suspensions can subsequently be washed to remove DMSO, free hemoglobin and other cellular debris allowing for resuspension in a volume appropriate for the size of the patient to be transplanted.

4.4.2.2 UCB Infusion

Under no circumstances is the cord blood to be irradiated. There must be no in-line leukocyte filter used with product infusion nor any medications or fluids infused in the same line with the cord blood (i.e., no piggyback fluids). Vital signs should be monitored before beginning the infusion and periodically during administration. Infusion should begin within 2 hours of washing. The infusion should take no longer than 1 hour. Pre-medications and hydration prior to cord blood infusion will be administered per institutional procedure. Diphenhydramine, epinephrine, and hydrocortisone should be available at the bedside for emergency use if infusion reactions occur. Oxygen with nasal prongs for standby use should be present in the room.

4.5 Concomitant Therapy and Study-Specific Supportive Care Guidelines

The following supportive care guidelines are provided for institutional consideration. Investigator discretion should be used, and individual considerations made for specific patient situations and institutional practices. Please see the COG Supportive Care Guidelines at:
https://members.childrensoncologygroup.org/prot/reference_materials.asp under Standard Sections for Protocols.

4.5.1 Growth Factors

Filgrastim (G-CSF) is not generally recommended for BM or PBSC sources, but is usually administered following UCB transplantation. If a center opts to administer filgrastim, 5 mcg/kg daily is recommended (with a start time according to institutional practice). In patients with evidence of Monosomy 7 by cytogenetics, center should consider avoiding filgrastim (by analogy to patients with AML).[56]

4.5.2 Infectious Prophylaxis

Co-enrollment on COG Cancer Control and Supportive Care clinical trials evaluating anti-infectious prophylaxis strategies is permitted.

Trimethoprim-sulfamethoxazole (TMP-SMX) and Intravenous Immunoglobulin: see COG Supportive Care Guidelines.

Fluconazole: ≥ 6 mg/kg dose QD IV or PO for at least the first 28 days (prophylaxis against molds is acceptable, according to institutional standards).

Acyclovir: Standard doses for up to one year after transplant. Acyclovir or valacyclovir will be used in patients who have been exposed to HSV, VZV or CMV or whose donors have been infected by CMV, as per institutional guidelines.

Weekly CMV antigenemia or PCR screening from Day +14 through at least Day +100 and pre-emptive treatment with ganciclovir.

Also see the Parenteral Chemotherapy Administration Guidelines (CAG) on the COG website at:
https://members.childrensoncologygroup.org/_files/disc/Pharmacy/ChemoAdminGuidelines.pdf for special precautions and suggestions for patient monitoring during the infusion. As applicable, see the CAG for suggestions on hydration, or hydrate according to institutional guidelines.

4.5.3 Sinusoidal Obstruction Syndrome/Veno-Occlusive Disease
When availale centers may use defibrotide for either prophylaxis or treatment per institutional guidelines

4.6 Matched Family Donor HCT for Patients Assigned to Arm A (BU-CY-MEL)

The condition regimen described below is for patients with a matched family donor randomized to receive the Arm A conditioning regimen. Patients with a matched family donor randomized to Arm A will receive an 8-day HCT conditioning regimen that consists of Busulfan, Cyclophosphamide and Melphalan (BU-CY-MEL). Tacrolimus and mycophenolate mofetil will be administered for GVHD prophylaxis.

Doses should be adjusted for patients > 125% of Ideal Body Weight (IBW). Adjusted weight = 1.25*IBW. See Section 5.1.

Anti-seizure Prophylaxis:
Days: -8 through at least Day -4
The preferred regimen is lorazepam (0.02-0.05 mg/kg/dose PO or IV every 6 hours) given 30 minutes prior to each busulfan dose and then continuing for at least 24 hours after last busulfan dose. An alternative regimen is levetiracetam (10 mg/kg/dose PO or IV BID, max dose 1000 mg) beginning 12 hours prior to busulfan and continuing for at least 24 hours after last busulfan dose.

Busulfan (BUS): IV over 3 hours
Day: -8, once daily (1 dose)
Dose: Patients < 10 kg: 3.2 mg/**kg**/dose
Patients ≥ 10 kg but ≤ 4 years old: 4 mg/**kg**/dose
Patients > 4 years: 3.2 mg/**kg**/dose

Days: -7 through -5, once daily (3 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16)

First-dose busulfan pharmacokinetic studies will be performed in all patients. Daily dosing will be adjusted to achieve an overall exposure target area under the curve (AUC) of 3600-6000 (micromole/liter)*minute. Refer to Section 16 for additional information.

Acetaminophen should be held for 72 hours before and during busulfan administration, but may be given per individual institutional standard policies if clinically necessary.

Note: Refrain from using polycarbonate syringes or filter needles during busulfan preparation.

Alternate Busulfan Dosing:
Divided dosing (every 6 or 12 hours) is permitted per institutional practice.

Q6 Hour Dosing Schedule: IV over 2 hours
Day: -8, every 6 hours
Dose: Patients < 10 kg: 0.8 mg/**kg**/dose q 6 hours (x 4 doses)
Patients ≥ 10 kg but ≤ 4 years old: 1 mg/**kg**/dose q 6 hours (x 4 doses)
Patients > 4 years: 0.8 mg/**kg**/dose q 6 hours (x 4 doses)

Days: -7 through -5, every 6 hours (x 12 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16). Every 6 hour dosing should target an AUC of 900 to 1500 (micromole/liter)*minute per dose.

Q12 Hour Dosing Schedule: IV over 2 hours
Day: -8, every 12 hours
Dose: Patients < 10 kg: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)
Patients ≥ 10 kg but ≤ 4 years old: 2 mg/**kg**/dose q 12 hours (x 2 doses)
Patients > 4 years: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)
Days: -7 through -5, every 12 hours (x 6 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16). Every 12 hour dosing should target an AUC of 1800 to 3000 (micromole/liter)*minute per dose.

Cyclophosphamide (CPM): IV over 60 minutes
Days: -4 through -3 (2 doses)
Dose: 60 mg/**kg**/dose, once daily

Mesna: IV over 15 minutes per dose or continuous infusion
Days: -4 through -3
Dose: 60 mg/**kg**/DAY, given as per the recommendations below:

Mesna can be administered in 5 divided doses by **short infusion** over 15 to 30 minutes. The initial bolus dose of mesna may be administered 15 minutes before or at the same time as the cyclophosphamide dose; subsequent doses are given 3, 6, 9, and 12 hours after the start of cyclophosphamide. This total daily dose of mesna can also be administered as IV **continuous infusion**. The continuous infusion should be started 15-30 minutes before or at the same time as cyclophosphamide and finished no sooner than 12 hours after the end of the cyclophosphamide infusion.

Examples:
Short infusion: if the cyclophosphamide dose is 1,000 mg, then the total daily mesna dose is 1,000 mg; 200 mg of mesna will be given 15 minutes before or with the cyclophosphamide dose (Hour 0) and 4 boluses of 200 mg each will be given at Hours 3, 6, 9 and 12.

Continuous infusion: if the cyclophosphamide dose is 1,000 mg, then the total daily mesna dose is 1,000 mg; the 1,000 mg mesna continuous infusion will start 15-30 minutes before or at the same time as the cyclophosphamide and be completed no sooner than 12 hours after **the end** of the cyclophosphamide infusion. If the cyclophosphamide is administered over 2 hours and mesna is started 30 minutes before the cyclophosphamide infusion, the total mesna infusion will last at least 14 hours and 30 minutes.

Melphalan (MEL): IV over 15-30 minutes (at a rate not to exceed 10 mg/minute)
Days: -1 (1 dose)
Dose: < 10 kg: 4.67 mg/**kg**/dose
≥ 10 kg: 140 mg/**m²**/dose

Note: Melphalan infusion must be completed within 60 minutes of preparation.

Tacrolimus (TAC): IV or PO
Days: -1 onwards (see below)
Dose is dependent upon route of administration.

IV administration, Dose: 0.03 mg/**kg**/DAY
PO administration, Dose: 0.12 mg/**kg**/DAY

Tacrolimus levels should be maintained between 5-12 ng/mL. The use of intermittent IV dosing, continuous IV dosing, or PO formulations may be used when clinically appropriate per institutional protocols. If ≤ Grade I acute GVHD, tacrolimus taper will begin no later than Day +60 and will be complete by Day +98. For patients with > Grade I acute GVHD, the tacrolimus taper will be per institutional protocols.

Serum tacrolimus troughs and serum magnesium, potassium, and creatinine should be drawn at least twice per week while hospitalized, then as per good clinical practice thereafter unless a change in medication (e.g. use of concomitant CYP3A4 inhibitors, see Appendix IIIA) or renal function might result in an acute change in level. At that point, levels will be measured as clinically indicated. Levels sent when dosing by continuous infusion are not true trough concentrations, however, the same target range of drug levels will be used for both continuous IV and bolus PO routes of administration. When converting patients at a therapeutic tacrolimus level from IV to PO formulation, multiply total daily IV dose times 4 and administer in 2 divided oral doses per day, every 12 hours (e.g., 1 mg of IV tacrolimus per day equates to 4 mg of PO tacrolimus per day). The oral dose should be administered 8-12 hours after the end of the tacrolimus continuous infusion.[57]

The target serum trough level for tacrolimus is 5-12 ng/mL. Dose adjustments are based on clinical judgment of the treating physician after considering clinical toxicity, serum levels, GVHD, concomitant drug use and the rate of rise or decline of the serum level. Refer to Section 5.2 for guidance on dose adjustment based on serum trough levels.

Mycophenolate Mofetil (MMF): IV over 2 hours
Days: +1 onwards (see below)
Dose: 15 mg/kg/dose, every 8 hours

The FDA has determined that a REMS (Risk Evaluation and Mitigation Strategy) program is necessary to ensure the benefits of mycophenolate outweigh the risks of first trimester pregnancy loss and congenital malformations associated with mycophenolate use during pregnancy. Mycophenolate REMS is a program to tell doctors, nurses, pharmacists and patients about the risks of taking mycophenolate during pregnancy. Providers and patients (females of child-bearing potential) are required to enroll in the program. The program also contains important information about patient education and reporting details for pregnancy. Providers and patients are required to sign acknowledgement forms prior to initiating therapy. Please refer to the website for additional information (https://www.mycophenolaterems.com/).

See Section 2.3.1 for additional information regarding the GVHD prophylaxis approach. If ≤ Grade I acute GVHD, MMF will be discontinued by Day +30. For patients with > Grade I acute GVHD, the MMF taper will be per institutional protocols. Patients may transition to PO formulations at a 1:1 IV to PO conversion when clinically appropriate. Refer to Section 5.3 for dose adjustments based on serum drug levels.

See Section 5.0 for Dose Modifications based on Toxicities.

The therapy delivery maps (TDMs) for this HCT treatment plan are on the next (one) page.

4.6.1 HCT Regimen: **Matched Family Donor -Arm A**

Treatment for the HCT preparative regimen lasts 8 days. After the HCT, the GVHD prophylaxis treatment lasts about 3+ months.

_____ Patient COG ID number _____ DOB

Extensive treatment details are in Section 4.1-4.6. This Therapy Delivery Map is on 1 page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Anti-Seizure Prophylaxis: _____ *Indicate agent, i.e., lorazepam, levetiracetam*	*IV or PO*	_____ *(indicate dose) Preferred regimen: lorazepam (0.02-0.05 mg/kg/dose every 6 hours) given 30 minutes prior to each busulfan dose. Continue until ≥ 24 hours after last busulfan dose.*	*-8 to -4*		a. History (incl. cardiac), PE w/ VS, NF1 status, pregnancy, PS b. BMA c. CBC, differential, HgbF, spleen & liver measure., concomitant tx d. Cytogenetics & FISH (not req. for all pts, see Section 7) e. GVHD, donor chimerism f. Complications g. Req. specimens: PB & BM (BM only if clinically indicated). See Section 7 for detailed list of evaluations. **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Busulfan (BUS)	IV over 3 hours ***For once daily dosing only.*** *See Section 4.6 for alternative dosing schedules.*	Age & wt-based (1st dose): < 10 **kg**: 3.2 mg/kg/dose ≥ 10 **kg** but ≤ 4 yr: 4 mg/kg/dose > 4 yr: 3.2 mg/**kg**/dose	-8	Submit BUS PK samples following 1st dose (Section 16). *See Section 4.6 for alternative dosing schedules.* **Note:** Refrain from using polycarbonate syringes or filter needles during busulfan preparation.	
		Dose adjusted per targeted AUC, once daily (3 doses)	-7 to -5		
Cyclophosphamide (CPM)	IV over 60 minutes	60 mg/**kg**/dose, once daily (2 doses)	-4 to -3		
MESNA	IV over 15 minutes per dose **or** continuous infusion	60 mg/**kg**/DAY	-4 to -3	See Section 4.6 for administration guidelines.	
Melphalan (MEL)	IV over 15-30 minutes at a rate not to exceed 10 mg/min	Weight-based dosing: < 10 kg: 4.67 mg/**kg**/dose ≥ 10 kg: 140 mg/**m²**/dose (1 dose)	-1	Melphalan infusion must be completed within 60 minutes of preparation.	
Tacrolimus (TAC)	IV or PO See Section 4.6	IV Dose: 0.03 mg/**kg**/DAY PO Dose: 0.12 mg/**kg**/DAY	-1 to +98	Maintain 5-12 ng/mL. If ≤ Grade I aGVHD, begin taper by Day +60. Discontinue by Day +98. See Section 4.6 for additional monitoring.	

****HEMATOPOIETIC CELL INFUSION: DAY 0 (see Section 4.4)****

Mycophenolate Mofetil (MMF)	IV over 2 hours. Convert to oral when tolerated.	15 mg/**kg**/dose, every 8 hours	+1 to +30	If ≤ Grade I aGVHD, discontinue by Day +30.	

Ht_____cm Wt_____kg BSA_____m²

Date Due	Date Given	Day	*Anti-seizure prophylaxis*	BUS ____mg	CPM ____mg	MESNA ___mg	MEL ___mg	TAC ___mg	MMF __mg __mg __mg	Studies	Comments
				Enter calculated dose above and actual dose administered below							
		-8	__mg __mg __mg __mg	____mg						(a, b, c, d, g) [1]	
		-7	__mg __mg __mg __mg	____mg							
		-6	__mg __mg __mg __mg	____mg							
		-5	__mg __mg __mg __mg	____mg							
		-4	__mg __mg __mg __mg		____mg	___mg					
		-3			____mg	___mg					
		-2									
		-1					____mg	____mg			
		0						↓			
		+1							__mg __mg __mg		
		+2							↓		
		+3									
		…									
		+30								b, c, d, e, f, g	
		…									
		+60								c, e, f, g	
		…									
		+90								b, c, d, e, f, g	
		…									
		+98						↓			
		…									
		+120								c, e, g	
		…									
		+180		See Section 7.2 for evaluations after Day +180.						c, e, g	

[1]Prior to HCT conditioning.

SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 4.5 FOR SUPPORTIVE CARE.

4.7 Unrelated Donor HCT for Patients Assigned to Arm A (BU-CY-MEL+ rATG)

The condition regimen described below is for patients with an unrelated donor randomized to receive the Arm A conditioning regimen. Patients with an unrelated donor and randomized to Arm A will receive an 8-day HCT conditioning regimen that consists of Busulfan, Cyclophosphamide and Melphalan (BU-CY-MEL), as well as rabbit Anti-Thymocyte Globulin (rATG). Tacrolimus and mycophenolate mofetil will be administered for GVHD prophylaxis.

Doses should be adjusted for patients >125% of Ideal Body Weight (IBW). Adjusted weight = 1.25*IBW. See Section 5.1

Anti-seizure Prophylaxis:
Days: -8 through at least Day -4
The preferred regimen is lorazepam (0.02-0.05 mg/kg/dose PO or IV every 6 hours) given 30 minutes prior to each busulfan dose and then continuing for at least 24 hours after last busulfan dose. An alternative regimen is levetiracetam (10 mg/kg/dose PO or IV BID, max dose 1000 mg) beginning 12 hours prior to busulfan and continuing for at least 24 hours after last busulfan dose.

Busulfan (BUS): IV over 3 hours
Day: -8, once daily (1 dose)
Dose: Patients < 10 kg: 3.2 mg/**kg**/dose
Patients ≥ 10 kg but ≤ 4 years old: 4 mg/**kg**/dose
Patients > 4 years: 3.2 mg/**kg**/dose

Days: -7 through -5, once daily (3 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16)

First-dose busulfan pharmacokinetic studies will be performed in all patients. Daily dosing will be adjusted to achieve an overall exposure target area under the curve (AUC) of 3600-6000 (micromole/liter)*minute. Refer to Section 16 for additional information.

Acetaminophen should be held for 72 hours before and during busulfan administration, but may be given per individual institutional standard policies if clinically necessary.

Note: Refrain from using polycarbonate syringes or filter needles during busulfan preparation.

Alternate Busulfan Dosing:
Divided dosing (every 6 or 12 hours) is permitted per institutional practice.

Q6 Hour Dosing Schedule: IV over 2 hours
Day: -8, every 6 hours
Dose: Patients < 10 kg: 0.8 mg/**kg**/dose q 6 hours (x 4 doses)
Patients ≥ 10 kg but ≤ 4 years old: 1 mg/**kg**/dose q 6 hours (x 4 doses)
Patients > 4 years: 0.8 mg/**kg**/dose q 6 hours (x 4 doses)

Days: -7 through -5, every 6 hours (x 12 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16). Every 6 hour dosing should target an AUC of 900 to 1500 (micromole/liter)*minute per dose.

Q12 Hour Dosing Schedule: IV over 2 hours
Day: -8, every 12 hours
Dose: Patients < 10 kg: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)
Patients ≥ 10 kg but ≤ 4 years old: 2 mg/**kg**/dose q 12 hours (x 2 doses)
Patients > 4 years: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)
Days: -7 through -5, every 12 hours (x 6 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16). Every 12 hour dosing should target an AUC of 1800 to 3000 (micromole/liter)*minute per dose.

Cyclophosphamide (CPM): IV over 60 minutes
Days: -4 through -3 (2 doses)
Dose: 60 mg/**kg**/dose, once daily

Mesna: IV over 15 minutes per dose or continuous infusion
Days: -4 through -3
Dose: 60 mg/**kg**/DAY, given as per the recommendations below:

Mesna can be administered in 5 divided doses by **short infusion** over 15 to 30 minutes. The initial bolus dose of mesna may be administered 15 minutes before or at the same time as the cyclophosphamide dose; subsequent doses are given 3, 6, 9, and 12 hours after the start of cyclophosphamide. This total daily dose of mesna can also be administered as IV **continuous infusion**. The continuous infusion should be started 15-30 minutes before or at the same time as cyclophosphamide and finished no sooner than 12 hours after the end of the cyclophosphamide infusion.

Examples:
Short infusion: if the cyclophosphamide dose is 1,000 mg, then the total daily mesna dose is 1,000 mg; 200 mg of mesna will be given 15 minutes before or with the cyclophosphamide dose (Hour 0) and 4 boluses of 200 mg each will be given at Hours 3, 6, 9 and 12.

Continuous infusion: if the cyclophosphamide dose is 1,000 mg, then the total daily mesna dose is 1,000 mg; the 1,000 mg mesna continuous infusion will start 15-30 minutes before or at the same time as the cyclophosphamide and be completed no sooner than 12 hours after **the end** of the cyclophosphamide infusion. If the cyclophosphamide is administered over 2 hours and mesna is started 30 minutes before the cyclophosphamide infusion, the total mesna infusion will last at least 14 hours and 30 minutes.

Rabbit Anti-thymocyte globulin (rATG): IV over 4-6* hours
Days: -4 through -1 (4 doses)
Dose: 2 mg/**kg**/dose, once daily

*The first dose should be infused over at least 6 hours through a high-flow vein. Subsequent doses should be administered over at least 4 hours. Administer through an in-line 0.22 micron filter.

Premedications should be considered prior to rATG per institutional protocols. This may include diphenhydramine 1 mg/kg/dose (max 50 mg), or a comparable anti-histamine; acetaminophen 10-15 mg/kg/dose (max 1000 mg); and methylprednisolone 1 mg/kg/dose. Premedication may be repeated or increased as needed to control allergic reactions, chills, or fever. Patients who are unable to tolerate the rabbit product may receive equine ATG 25 mg/kg/day x 4 days with similar premedication.

Melphalan (MEL): IV over 15-30 minutes at a rate not to exceed 10 mg/minute
Days: -1 (1 dose)
Dose: < 10 kg: 4.67 mg/**kg**/dose
≥ 10 kg: 140 mg/**m²**/dose

Note: Melphalan infusion must be completed within 60 minutes of preparation.

Tacrolimus (TAC): IV or PO
Days: -1 onwards (see below)
Dose is dependent upon route of administration.
IV administration, Dose: 0.03 mg/**kg**/DAY
PO administration, Dose: 0.12 mg/**kg**/DAY

Tacrolimus levels should be maintained between 8-12 ng/mL. The use of intermittent IV dosing, continuous IV dosing, or PO formulations may be used when clinically appropriate per institutional protocols. If ≤ Grade I acute GVHD, tacrolimus taper will begin no later than Day +100 and will be complete by Day +180. For patients with > Grade I acute GVHD, the tacrolimus taper will be per institutional protocols.

Serum tacrolimus troughs and serum magnesium, potassium, and creatinine should be drawn at least twice per week while hospitalized, then as per good clinical practice thereafter unless a change in medication (e.g. use of concomitant CYP3A4 inhibitors, see Appendix IIIA) or renal function might result in an acute change in level. At that point, levels will be measured as clinically indicated. Levels sent when dosing by continuous infusion are not true trough concentrations, however, the same target range of drug levels will be used for both continuous IV and bolus PO routes of administration. When converting patients at a therapeutic tacrolimus level from IV to PO formulation, multiply total daily IV dose times 4 and administer in 2 divided oral doses per day, every 12 hours (e.g., 1 mg of IV tacrolimus per day equates to 4 mg of PO tacrolimus per day). The oral dose should be administered 8-12 hours after the end of the tacrolimus continuous infusion.[57]

The target serum trough level for tacrolimus is 8-12 ng/mL for mismatched or unrelated donors. Dose adjustments are based on clinical judgment of the treating physician after considering clinical toxicity, serum levels, GVHD, concomitant drug use and the rate of rise or decline of the serum level. Refer to Section 5.2 for guidance on dose adjustment based on serum trough levels.

Mycophenolate Mofetil (MMF): IV over 2 hours
Days: +1 onwards (see below)
Dose: 15 mg/**kg**/dose, every 8 hours

The FDA has determined that a REMS (Risk Evaluation and Mitigation Strategy) program is necessary to ensure the benefits of mycophenolate outweigh the risks of first trimester pregnancy loss and congenital malformations associated with mycophenolate use during pregnancy. Mycophenolate REMS is a program to tell doctors, nurses, pharmacists and patients about the risks of taking mycophenolate during pregnancy. Providers and patients (females of child-bearing potential) are required to enroll in the program. The program also contains important information about patient education and reporting details for pregnancy. Providers and patients are required to sign acknowledgement forms prior to initiating therapy. Please refer to the website for additional information (https://www.mycophenolaterems.com/).

See Section 2.3.1 for additional information regarding the GVHD prophylaxis approach. If ≤ Grade I acute GVHD, MMF will be discontinued by Day +45. For patients with > Grade I acute GVHD, the MMF taper will be per institutional protocols. Patients may transition to PO formulations at a 1:1 IV to PO conversion when clinically appropriate. Refer to Section 5.3 for dose adjustments based on serum drug levels.

See Section 5.0 for Dose Modifications based on Toxicities. The therapy delivery maps (TDMs) for this HCT treatment plan are on the next (one) page.

4.7.1 HCT Regimen - **Unrelated Donor -Arm A**

Treatment for the HCT preparative regimen lasts 8 days. After the HCT, the GVHD prophylaxis treatment lasts about 6 months.

Patient COG ID number _____ DOB _____

Extensive treatment details are in Section 4.1-4.5 and 4.7. This Therapy Delivery Map is on 1 page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Anti-Seizure Prophylaxis: _____ *Indicate agent, i.e., lorazepam, levetiracetam*	*IV or PO*	_____ *(indicate dose) Preferred regimen: lorazepam (0.02-0.05 mg/kg/dose every 6 hours) given 30 minutes prior to each busulfan dose. Continue until ≥ 24 hours after last busulfan dose.*	*-8 to -4*		a. History (incl. cardiac), PE w/ VS, NF1 status, pregnancy, PS b. BMA c. CBC, differential, HgbF, spleen & liver measure., concomitant tx d. Cytogenetics & FISH (not req. for all pts, see Sect. 7) e. GVHD, donor chimerism f. Complications g. Req. specimens: PB & BM (BM only if clinically indicated). See Section 7 for detailed list of evaluations. **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Busulfan (BUS)	IV over 3 hours ***For once daily dosing only.*** *See Section 4.7 for alternative dosing schedules.*	Age & wt-based (1st dose): < 10 kg: 3.2 mg/**kg**/dose ≥ 10 kg but ≤ 4 yr: 4 mg/**kg**/dose > 4 yr: 3.2 mg/**kg**/dose	-8	Submit BUS PK samples following 1st dose (Section 16). *See Section 4.7 for alternative dosing schedules.* **Note:** Refrain from using polycarbonate syringes or filter needles during busulfan preparation.	
		Dose adjusted per targeted AUC, once daily (3 doses)	-7 to -5		
Cyclophosphamide (CPM)	IV over 60 minutes	60 mg/**kg**/dose, once daily (2 doses)	-4 to -3		
MESNA	IV over 15 minutes per dose **or** continuous infusion	60 mg/**kg**/DAY	-4 to -3	See Section 4.7 for administration guidelines.	
Rabbit ATG (rATG)	IV over 4-6* hours	2 mg/**kg**/dose, once daily (4 doses)	-4 to -1	***1st dose**: infuse over ≥ 6 hours through a high-flow vein. **Subsequent doses:** infuse over ≥ 4 hours. Administer through an in-line 0.22 micron filter. Premedications should be considered. Equine ATG may be substituted if rabbit product is not tolerated. See Section 4.7 for details.	
Melphalan (MEL)	IV over 15-30 minutes at a rate not to exceed 10 mg/min	Weight based dosing: < 10 kg: 4.67 mg/**kg**/dose ≥ 10 kg: 140 mg/**m²**/dose (1 dose)	-1	Note: Melphalan infusion must be completed within 60 minutes of preparation.	
Tacrolimus (TAC)	IV or PO See Section 4.7.	IV Dose: 0.03 mg/**kg**/DAY PO Dose: 0.12 mg/**kg**/DAY	-1 to +180	Maintain 8-12 ng/mL. If ≤ Grade I aGVHD begin taper by Day +100. Discontinue by Day +180. See Section 4.7 for additional monitoring.	

__HEMATOPOIETIC CELL INFUSION: DAY 0 (see Section 4.4)__

Mycophenolate mofetil (MMF)	IV over 2 hours. Convert to oral when tolerated.	15 mg/**kg**/dose, every 8 hours	+1 to +45	If ≤ Grade I aGVHD, discontinue by Day +45.	

Ht_____cm Wt_____kg BSA_____m²

Date Due	Date Given	Day	*Anti-seizure prophylaxis*	BUS ___mg	CPM ___mg	MESNA ___mg	rATG ___mg	MEL ___mg	TAC ___mg	MMF __mg __mg __mg	Studies	Comments
			Enter calculated dose above and actual dose administered below									
		-8	__mg __mg __mg __mg	____mg							(a, b, c, d, g) [1]	
		-7	__mg __mg __mg __mg	____mg								
		-6	__mg __mg __mg __mg	____mg								
		-5	__mg __mg __mg __mg	____mg								
		-4	__mg __mg __mg __mg		____mg	____mg	____mg					
		-3			____mg	____mg	____mg					
		-2					____mg					
		-1					____mg	____mg	____mg			
		0							↓			
		+1								__mg __mg __mg		
		+2								↓		
		+3										
		+30									b, c, d, e, f, g	
		+45										
		+60									c, e, f, g	
		+90									b, c, d, e, f, g	
		+120									c, e, g	
		+180									c, e, g	
			See Section 7.2 for evaluations after Day +180.									

[1]Prior to HCT conditioning. **SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 4.5 FOR SUPPORTIVE CARE.**

4.8 UCB Donor HCT for Patients Assigned to Arm A (BU-CY-MEL+ rATG)

The condition regimen described below is for patients with an UCB donor randomized to receive the Arm A conditioning regimen. Patients with an UCB donor and randomized to Arm A will receive an 8-day HCT conditioning regimen that consists of Busulfan, Cyclophosphamide and Melphalan (BU-CY-MEL), as well as rabbit Anti-Thymocyte Globulin (rATG). Tacrolimus and mycophenolate mofetil will be administered for GVHD prophylaxis.

Doses should be adjusted for patients >125% of Ideal Body Weight (IBW). Adjusted weight = 1.25*IBW. See Section 5.1

Anti-seizure Prophylaxis:
Days: -8 through at least Day -4
The preferred regimen is lorazepam (0.02-0.05 mg/kg/dose PO or IV every 6 hours) given 30 minutes prior to each busulfan dose and then continuing for at least 24 hours after last busulfan dose. An alternative regimen is levetiracetam (10 mg/kg/dose PO or IV BID, max dose 1000 mg) beginning 12 hours prior to busulfan and continuing for at least 24 hours after last busulfan dose.

Busulfan (BUS): IV over 3 hours
Day: -8, once daily (1 dose)
Dose: Patients < 10 kg: 3.2 mg/**kg**/dose
Patients ≥ 10 kg but ≤ 4 years old: 4 mg/**kg**/dose
Patients > 4 years: 3.2 mg/**kg**/dose

Days: -7 through -5, once daily (3 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16)

First-dose busulfan pharmacokinetic studies will be performed in all patients. Daily dosing will be adjusted to achieve an overall exposure target area under the curve (AUC) of 3600-6000 (micromole/liter)*minute. Refer to Section 16 for additional information.

Acetaminophen should be held for 72 hours before and during busulfan administration, but may be given per individual institutional standard policies if clinically necessary.

Note: Refrain from using polycarbonate syringes or filter needles during busulfan preparation.

Alternate Busulfan Dosing:
Divided dosing (every 12 hours) is permitted per institutional practice. Every 6 hour dosing is NOT permitted with UCB donors due to conflicts with ATG timing.

Q12 Hour Dosing Schedule: IV over 2 hours
Day: -8, every 12 hours
Dose: Patients < 10 kg: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)
Patients ≥ 10 kg but ≤ 4 years old: 2 mg/**kg**/dose q 12 hours (x 2 doses)
Patients > 4 years: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)
Days: -7 through -5, every 12 hours (x 6 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16). Every 12 hour dosing should target an AUC of 1800 to 3000 (micromole/liter)*minute per dose.

Cyclophosphamide (CPM): IV over 60 minutes
Days: -4 through -3 (2 doses)
Dose: 60 mg/**kg**/dose, once daily

Mesna: IV over 15 minutes per dose or continuous infusion
Days: -4 through -3
Dose: 60 mg/**kg**/DAY, given as per the recommendations below:

Mesna can be administered in 5 divided doses by **short infusion** over 15 to 30 minutes. The initial bolus dose of mesna may be administered 15 minutes before or at the same time as the cyclophosphamide dose; subsequent doses are given 3, 6, 9, and 12 hours after the start of cyclophosphamide. This total daily dose of mesna can also be administered as IV **continuous infusion**. The continuous infusion should be started 15-30 minutes before or at the same time as cyclophosphamide and finished no sooner than 12 hours after the end of the cyclophosphamide infusion.

Examples:
Short infusion: if the cyclophosphamide dose is 1,000 mg, then the total daily mesna dose is 1,000 mg; 200 mg of mesna will be given 15 minutes before or with the cyclophosphamide dose (Hour 0) and 4 boluses of 200 mg each will be given at Hours 3, 6, 9 and 12.

Continuous infusion: if the cyclophosphamide dose is 1,000 mg, then the total daily mesna dose is 1,000 mg; the 1,000 mg mesna continuous infusion will start 15-30 minutes before or at the same time as the cyclophosphamide and be completed no sooner than 12 hours after **the end** of the cyclophosphamide infusion. If the cyclophosphamide is administered over 2 hours and mesna is started 30 minutes before the cyclophosphamide infusion, the total mesna infusion will last at least 14 hours and 30 minutes.

Rabbit Anti-thymocyte globulin (rATG): IV over 4-6* hours
Days: -8 through -5 (4 doses)
Dose: 2 mg/**kg**/dose, once daily

*The first dose should be infused over at least 6 hours through a high-flow vein. Subsequent doses should be administered over at least 4 hours. Administer through an in-line 0.22 micron filter.

Premedications should be considered prior to rATG per institutional protocols. This may include diphenhydramine 1 mg/kg/dose (max 50 mg), or a comparable anti-histamine; acetaminophen 10-15 mg/kg/dose (max 1000 mg); and methylprednisolone 1 mg/kg/dose. Premedication may be repeated or increased as needed to control allergic reactions, chills, or fever. Patients who are unable to tolerate the rabbit product may receive equine ATG 25 mg/kg/day x 4 days with similar premedication.

Melphalan (MEL): IV over 15-30 minutes at a rate not to exceed 10 mg/minute
Days: -1 (1 dose)
Dose: < 10 kg: 4.67 mg/**kg**/dose
≥ 10 kg: 140 mg/$\mathbf{m^2}$/dose

Note: Melphalan infusion must be completed within 60 minutes of preparation.

Tacrolimus (TAC): IV or PO
Days: -1 onwards (see below)
Dose is dependent upon route of administration.
IV administration, Dose: 0.03 mg/**kg**/DAY
PO administration, Dose: 0.12 mg/**kg**/DAY
Tacrolimus levels should be maintained between 8-12 ng/mL. The use of intermittent IV dosing, continuous IV dosing, or PO fomulatios may be used when clinically appropriate per institutional protocols. If ≤ Grade I acute GVHD, tacrolimus taper will begin no later than Day +100 and will be complete by Day +180. For patients with > Grade I acute GVHD, the tacrolimus taper will be per institutional protocols.

Serum tacrolimus troughs and serum magnesium, potassium, and creatinine should be drawn at least twice per week while hospitalized, then as per good clinical practice thereafter unless a change in medication (e.g. use of concomitant CYP3A4 inhibitors, see Appendix IIIA) or renal function might result in an acute change in level. At that point, levels will be measured as clinically indicated. Levels sent when dosing by continuous infusion are not true trough concentrations, however, the same target range of drug levels will be used for both continuous IV and bolus PO routes of administration. When converting patients at a therapeutic tacrolimus level from IV to PO formulation, multiply total daily IV dose times 4 and administer in 2 divided oral doses per day, every 12 hours (e.g., 1 mg of IV tacrolimus per day equates to 4 mg of PO tacrolimus per day). The oral dose should be administered 8-12 hours after the end of the tacrolimus continuous infusion.[57]

The target serum trough level for tacrolimus is 8-12 ng/mL for mismatched or unrelated donors. Dose adjustments are based on clinical judgment of the treating physician after considering clinical toxicity, serum levels, GVHD, concomitant drug use and the rate of rise or decline of the serum level. Refer to Section 5.2 for guidance on dose adjustment based on serum trough levels.

Mycophenolate Mofetil (MMF): IV over 2 hours
Days: +1 onwards (see below)
Dose: 15 mg/**kg**/dose, every 8 hours
The FDA has determined that a REMS (Risk Evaluation and Mitigation Strategy) program is necessary to ensure the benefits of mycophenolate outweigh the risks of first trimester pregnancy loss and congenital malformations associated with mycophenolate use during pregnancy. Mycophenolate REMS is a program to tell doctors, nurses, pharmacists and patients about the risks of taking mycophenolate during pregnancy. Providers and patients (females of child-bearing potential) are required to enroll in the program. The program also contains important information about patient education and reporting details for pregnancy. Providers and patients are required to sign acknowledgement forms prior to initiating therapy. Please refer to the website for additional information (https://www.mycophenolaterems.com/).

See Section 2.3.1 for additional information regarding the GVHD prophylaxis approach. If ≤ Grade I acute GVHD, MMF will be discontinued by Day +45. For patients with > Grade I acute GVHD, the MMF taper will be per institutional protocols. Patients may transition to PO formulations at a 1:1 IV to PO conversion when clinically appropriate. Refer to Section 5.3 for dose adjustments based on serum drug levels.

See Section 5.0 for Dose Modifications based on Toxicities. The therapy delivery maps (TDMs) for this HCT treatment plan are on the next (one) page.

4.8.1 HCT Regimen - **UCB Donor -Arm A**

Treatment for the HCT preparative regimen lasts 8 days. After the HCT, the GVHD prophylaxis treatment lasts about 6 months.

Patient COG ID number _____ DOB _____

Extensive treatment details are in Section 4.1-4.5 and 4.8. This Therapy Delivery Map is on 1 page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Anti-Seizure Prophylaxis: _____ *Indicate agent, i.e., lorazepam, levetiracetam*	*IV or PO*	_____ *(indicate dose) Preferred regimen: lorazepam (0.02-0.05 mg/kg/dose every 6 hours) given 30 minutes prior to each busulfan dose. Continue until ≥ 24 hours after last busulfan dose.*	*-8 to -4*		a. History (incl. cardiac), PE w/ VS, NF1 status, pregnancy, PS b. BMA c. CBC, differential, HgbF, spleen & liver measure., concomitant tx d. Cytogenetics & FISH (not req. for all pts, see Sect. 7) e. GVHD, donor chimerism f. Complications g. Req. specimens: PB & BM (BM only if clinically indicated). See Section 7 for detailed list of evaluations. **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Busulfan (BUS)	IV over 3 hours ***For once daily dosing only.*** *See Section 4.8 for alternative dosing schedules.*	Age & wt-based (1st dose): < 10 kg: 3.2 mg/**kg**/dose ≥ 10 kg but ≤ 4 yr: 4 mg/**kg**/dose > 4 yr: 3.2 mg/**kg**/dose	-8	Submit BUS PK samples following 1st dose (Section 16). *See Section 4.8 for alternative dosing schedule.* **Note:** Refrain from using polycarbonate syringes or filter needles during busulfan preparation.	
		Dose adjusted per targeted AUC, once daily (3 doses)	-7 to -5		
Cyclophosphamide (CPM)	IV over 60 minutes	60 mg/**kg**/dose, once daily (2 doses)	-4 to -3		
MESNA	IV over 15 minutes per dose **or** continuous infusion	60 mg/**kg**/DAY	-4 to -3	See Section 4.8 for administration guidelines.	
Rabbit ATG (rATG)	IV over 4-6* hours	2 mg/**kg**/dose, once daily (4 doses)	-8 to -5	***1st dose**: infuse over ≥ 6 hours through a high-flow vein. **Subsequent doses:** infuse over ≥ 4 hours. Administer through an in-line 0.22 micron filter. Premedications should be considered Equine ATG may be substitutued if rabbit product is not tolerated. See Section 4.8 for details.	
Melphalan (MEL)	IV over 15-30 minutes at a rate not to exceed 10 mg/min	Weight based dosing: < 10 kg: 4.67 mg/**kg**/dose ≥ 10 kg: 140 mg/**m²**/dose (1 dose)	-1	Note: Melphalan infusion must be completed within 60 minutes of preparation.	
Tacrolimus (TAC)	IV or PO See Section 4.8.	IV Dose: 0.03 mg/**kg**/DAY PO Dose: 0.12 mg/**kg**/DAY	-1 to +180	Maintain 8-12 ng/mL. If ≤ Grade I aGVHD begin taper by Day +100. Discontinue by Day +180. See Section 4.8 for additional monitoring.	

__HEMATOPOIETIC CELL INFUSION: DAY 0 (see Section 4.4)__

Mycophenolate mofetil (MMF)	IV over 2 hours. Convert to oral when tolerated.	15 mg/**kg**/dose, every 8 hours	+1 to +45	If ≤ Grade I aGVHD, discontinue by Day +45.	

Ht_____cm Wt_____kg BSA_____m²

Date Due	Date Given	Day	*Anti-seizure prophylaxis*	BUS mg	CPM mg	MESNA mg	rATG mg	MEL mg	TAC mg	MMF mg mg mg	Studies	Comments
			Enter calculated dose above and actual dose administered below									
		-8	mg mg mg mg	mg			mg				(a, b, c, d, g)[1]	
		-7	mg mg mg mg	mg			mg					
		-6	mg mg mg mg	mg			mg					
		-5	mg mg mg mg	mg			mg					
		-4	mg mg mg mg		mg	mg						
		-3			mg	mg						
		-2										
		-1						mg	mg			
		0							↓			
		+1								mg mg mg		
		+2								↓		
		+3										
		+30									b, c, d, e, f, g	
		+45										
		+60									c, e, f, g	
		+90									b, c, d, e, f, g	
		+120									c, e, g	
		+180									c, e, g	
			See Section 7.2 for evaluations after Day +180.									

[1]Prior to HCT conditioning. **SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 4.5 FOR SUPPORTIVE CARE.**

4.9 Matched Family Donor HCT for Patients Assigned to Arm B (BU-FLU)

The condition regimen described below is for patients with a matched family donor randomized to receive the Arm B conditioning regimen. Patients randomized to Arm B will receive an 8-day HCT conditioning regimen that consists of Busulfan and Fludarabine (BU-FLU). Tacrolimus and mycophenolate mofetil will be administered for GVHD prophylaxis.

Doses should be adjusted for patients > 125% of Ideal Body Weight (IBW). Adjusted weight = 1.25*IBW. See Section 5.1.

Anti-seizure Prophylaxis:
Days: -8 through at least Day -4
The preferred regimen is lorazepam (0.02-0.05 mg/kg/dose PO or IV every 6 hours) given 30 minutes prior to each busulfan dose and then continuing for at least 24 hours after last busulfan dose. An alternative regimen is levetiracetam (10 mg/kg/dose PO or IV BID, max dose 1000 mg) beginning 12 hours prior to busulfan and continuing for at least 24 hours after last busulfan dose.

Busulfan (BUS): IV over 3 hours
Day: -8, once daily (1 dose)
Dose: Patients < 10 kg: 3.2 mg/**kg**/dose
Patients ≥ 10 kg but ≤ 4 years old: 4 mg/**kg**/dose
Patients > 4 years: 3.2 mg/**kg**/dose

Days: -7 through -5, once daily (3 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16)

First-dose busulfan pharmacokinetic studies will be performed in all patients. Daily dosing will be adjusted to achieve an overall exposure target area under the curve (AUC) of 3600-6000 (micromole/liter)*minute. Refer to Section 16 for additional information.

Acetaminophen should be held for 72 hours before and during busulfan administration, but may be given per individual institutional standard policies if clinically necessary.

Note: Refrain from using polycarbonate syringes or filter needles during busulfan preparation.

Alternate Busulfan Dosing:
Divided dosing (every 6 or 12 hours) is permitted per institutional practice.

Q6 Hour Dosing Schedule: IV over 2 hours
Day: -8, every 6 hours
Dose: Patients < 10 kg: 0.8 mg/**kg**/dose q 6 hours (x 4 doses)
Patients ≥ 10 kg but ≤ 4 years old: 1 mg/**kg**/dose q 6 hours (x 4 doses)
Patients > 4 years: 0.8 mg/**kg**/dose q 6 hours (x 4 doses)

Days: -7 through -5, every 6 hours (x 12 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16). Every 6 hour dosing should target an AUC of 900 to 1500 (micromole/liter)*minute per dose.

Q12 Hour Dosing Schedule: IV over 2 hours
Day: -8, every 12 hours
Dose: Patients < 10 kg: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)
Patients ≥ 10 kg but ≤ 4 years old: 2 mg/**kg**/dose q 12 hours (x 2 doses)
Patients > 4 years: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)

Days: -7 through -5, every 12 hours (x 6 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16). Every 12 hour dosing should target an AUC of 1800 to 3000 (micromole/liter)*minute per dose.

Fludarabine (FLUD): IV over 30-60 minutes
Days: -5 through -2 (4 doses)
Dose: < 10 kg: 1.33 mg/**kg**/dose once daily
≥ 10 kg: 40 mg/**m²**/dose once daily

Tacrolimus (TAC): IV or PO
Days: -1 onwards (see below)
Dose is dependent upon route of administration.
IV administration, Dose: 0.03 mg/**kg**/DAY
PO administration, Dose: 0.12 mg/**kg**/DAY

Tacrolimus levels should be maintained between 5-12 ng/mL. The use of intermittent IV dosing, continuous IV dosing, or PO formulations may be used when clinically appropriate per institutional protocols. If ≤ Grade I acute GVHD, tacrolimus taper will begin no later than Day +60 and will be complete by Day +98. For patients with > Grade I acute GVHD, the tacrolimus taper will be per institutional protocols.

Serum tacrolimus troughs and serum magnesium, potassium, and creatinine should be drawn at least twice per week while hospitalized, then as per good clinical practice thereafter unless a change in medication (e.g. use of concomitant CYP3A4 inhibitors, see Appendix IIIA) or renal function might result in an acute change in level. At that point, levels will be measured as clinically indicated. Levels sent when dosing by continuous infusion are not true trough concentrations, however, the same target range of drug levels will be used for both continuous IV and bolus PO routes of administration. When converting patients at a therapeutic tacrolimus level from IV to PO formulation, multiply total daily IV dose times 4 and administer in 2 divided oral doses per day, every 12 hours (e.g., 1 mg of IV tacrolimus per day equates to 4 mg of PO tacrolimus per day). The oral dose should be administered 8-12 hours after the end of the tacrolimus continuous infusion.[57]

The target serum trough level for tacrolimus is 5-12 ng/mL. Dose adjustments are based on clinical judgment of the treating physician after considering clinical toxicity, serum levels, GVHD, concomitant drug use and the rate of rise or decline of the serum level. Refer to Section 5.2 for guidance on dose adjustment based on serum trough levels.

Mycophenolate Mofetil (MMF): IV over 2 hours
Days: +1 onwards (see below)
Dose: 15 mg/**kg**/dose, every 8 hours

The FDA has determined that a REMS (Risk Evaluation and Mitigation Strategy) program is necessary to ensure the benefits of mycophenolate outweigh the risks of first trimester pregnancy loss and congenital malformations associated with mycophenolate

use during pregnancy. Mycophenolate REMS is a program to tell doctors, nurses, pharmacists and patients about the risks of taking mycophenolate during pregnancy. Providers and patients (females of child-bearing potential) are required to enroll in the program. The program also contains important information about patient education and reporting details for pregnancy. Providers and patients are required to sign acknowledgement forms prior to initiating therapy. Please refer to the website for additional information (https://www.mycophenolaterems.com/).

See Section 2.3.1 for additional information regarding the GVHD prophylaxis approach. If ≤ Grade I acute GVHD, MMF will be discontinued by Day +30. For patients with > Grade I acute GVHD, the MMF taper will be per institutional protocols. Patients may transition to PO formulations at a 1:1 IV to PO conversion when clinically appropriate. Refer to Section 5.3 for dose adjustments based on serum drug levels.

See Section 5.0 for Dose Modifications based on Toxicities.
The therapy delivery maps (TDMs) for this HCT treatment plan are on the next (one) page.

4.9.1 HCT Regimen: **Matched Family Donor -Arm B**
Treatment for the HCT preparative regimen lasts 8 days.
After the HCT, the GVHD prophylaxis treatment lasts about 3+ months.

Patient COG ID number _____ DOB _____

Extensive treatment details are in Section 4.1-4.5 and 4.9. This Therapy Delivery Map is on 1 page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Anti-Seizure Prophylaxis _____ *Indicate agent, i.e., lorazepam, levetiracetam*	*IV or PO*	_____ *(indicate dose)* *Preferred regimen: lorazepam (0.02-0.05 mg/kg/dose every 6 hours) given 30 minutes prior to each busulfan dose. Continue until ≥ 24 hours after last busulfan dose.*	*-8 to -4*		a. History (incl. cardiac), PE w/ VS , NF1 status, pregnancy, PS b. BMA c. CBC, differential, HgbF, spleen & liver measure., concomitant tx d. Cytogenetics & FISH (not req. for all pts, see Sect. 7) e. GVHD, donor chimerism f. Complications g. Req. specimens: PB & BM (BM only if clinically indicated). See Section 7 for detailed list of evaluations. **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Busulfan (BUS)	IV over 3 hours ***For once daily dosing only.*** *See Section 4.9 for alternative dosing schedules.*	Age & wt-based (1 dose): < 10 kg: 3.2 mg/**kg**/dose ≥ 10 kg but ≤ 4 yr: 4 mg/**kg**/dose > 4 yr: 3.2 mg/**kg**/dose	-8	Submit BUS PK samples following 1st dose (Section 16). *See Section 4.9 for alternative dosing schedules.* **Note:** Refrain from using polycarbonate syringes or filter needles during busulfan preparation.	
		Dose adjusted per targeted AUC, once daily (3 doses)	-7 to -5		
Fludarabine (FLUD)	IV over 30-60 minutes	Weight-based dosing: < 10 kg: 1.33 mg/**kg**/dose ≥ 10 kg: 40 mg/**m²**/dose (4 doses)	-5 to -2		
Tacrolimus (TAC)	IV or PO See Section 4.9.	IV Dose: 0.03 mg/**kg**/DAY PO Dose: 0.12 mg/**kg**/DAY	-1 to +98	Maintain levels between 5-12 ng/mL. If ≤ Grade I acute GVHD, taper will begin no later than Day +60 and will be complete by Day +98. See Section 4.9 for additional monitoring.	

****HEMATOPOIETIC CELL INFUSION: DAY 0 (see Section 4.4)****

Mycophenolate mofetil (MMF)	IV over 2 hours. Convert to oral when tolerated.	15 mg/**kg**/dose, every 8 hours	+1 to +30	If ≤ Grade I acute GVHD, MMF will be discontinued by Day +30.	

Ht_____cm Wt_____kg BSA_____m²

Date Due	Date Given	Day	Anti-seizure prophylaxis ____mg	BUS ____mg	FLUD ____mg	TAC ____mg	MMF ____mg ____mg ____mg	Studies	Comments
			Enter calculated dose above and actual dose administered below						
		-8	__mg __mg __mg __mg	____mg				(a, b, c, d, g) [1]	
		-7	__mg __mg __mg __mg	____mg					
		-6	__mg __mg __mg __mg	____mg					
		-5	__mg __mg __mg __mg	____mg	_____mg				
		-4	__mg __mg __mg __mg		_____mg				
		-3			_____mg				
		-2			_____mg				
		-1				____mg			
		0				____mg			
		+1				____mg	____mg ____mg ____mg		
		+2							
		+3							
		...							
		+30						b, c, d, e, f, g	
		...							
		+60						c, e, f, g	
		...							
		+90						b, c, d, e, f, g	
		...							
		+98							
		...							
		+120						c, e, g	
		...							
		+180	See Section 7.2 for evaluations after Day +180					c, e, g	

[1]Prior to HCT conditioning. **SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 4.5 FOR SUPPORTIVE CARE.**

4.10 Unrelated Donor HCT for Patients Assigned to Arm B (BU-FLU + rATG)

The condition regimen described below is for patients with an unrelated donor randomized to receive the Arm B conditioning regimen. Patients randomized to Arm B will receive an 8-day HCT conditioning regimen that consists of Busulfan and Fludarabine (BU-FLU), as well as rabbit Anti-Thymocyte Globulin (rATG). Tacrolimus and mycophenolate mofetil will be administered for GVHD prophylaxis.

Doses should be adjusted for patients > 125% of Ideal Body Weight (IBW). Adjusted weight = 1.25*IBW. See Section 5.1.

Anti-seizure Prophylaxis:
Days: -8 through at least Day -4
The preferred regimen is lorazepam (0.02-0.05 mg/kg/dose PO or IV every 6 hours) given 30 minutes prior to each busulfan dose and then continuing for at least 24 hours after last busulfan dose. An alternative regimen is levetiracetam (10 mg/kg/dose PO or IV BID, max dose 1000 mg) beginning 12 hours prior to busulfan and continuing for at least 24 hours after last busulfan dose.

Busulfan (BUS): IV over 3 hours
Day: -8, once daily (1 dose)
Dose: Patients < 10 kg: 3.2 mg/**kg**/dose
Patients ≥ 10 kg but ≤ 4 years old: 4 mg/**kg**/dose
Patients > 4 years: 3.2 mg/**kg**/dose

Days: -7 through -5, once daily (3 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16)

First-dose busulfan pharmacokinetic studies will be performed in all patients. Daily dosing will be adjusted to achieve an overall exposure target area under the curve (AUC) of 3600-6000 (micromole/liter)*minute. Refer to Section 16 for additional information.

Acetaminophen should be held for 72 hours before and during busulfan administration, but may be given per individual institutional standard policies if clinically necessary.

Note: Refrain from using polycarbonate syringes or filter needles during busulfan preparation.

Alternate Busulfan Dosing:
Divided dosing (every 6 or 12 hours) is permitted per institutional practice.

Q6 Hour Dosing Schedule: IV over 2 hours
Day: -8, every 6 hours
Dose: Patients < 10 kg: 0.8 mg/**kg**/dose q 6 hours (x 4 doses)
Patients ≥ 10 kg but ≤ 4 years old: 1 mg/**kg**/dose q 6 hours (x 4 doses)
Patients > 4 years: 0.8 mg/**kg**/dose q 6 hours (x 4 doses)

Days: -7 through -5, every 6 hours (x 12 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16). Every 6 hour dosing should target an AUC of 900 to 1500 (micromole/liter)*minute per dose.

Q12 Hour Dosing Schedule: IV over 2 hours
Day: -8, every 12 hours
Dose: Patients < 10 kg: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)
Patients ≥ 10 kg but ≤ 4 years old: 2 mg/**kg**/dose q 12 hours (x 2 doses)
Patients > 4 years: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)

Days: -7 through -5, every 12 hours (x 6 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16). Every 12 hour dosing should target an AUC of 1800 to 3000 (micromole/liter)*minute per dose.

Fludarabine (FLUD): IV over 30-60 minutes
Days: -5 through -2 (4 doses)
Dose: < 10 kg: 1.33 mg/**kg**/dose once daily
≥ 10 kg: 40 mg/**m²**/dose once daily

Rabbit Anti-thymocyte globulin (rATG): IV over 4-6* hours
Days: -4 through -1 (4 doses)
Dose: 2 mg/**kg**/dose

*The first dose should be infused over at least 6 hours through a high-flow vein. Subsequent doses should be administered over at least 4 hours. Administer through an in-line 0.22 micron filter.

Premedications should be considered prior to rATG per institutional protocols. This may include diphenhydramine 1 mg/kg/dose (max 50 mg), or a comparable anti-histamine; acetaminophen 10-15 mg/kg/dose (max 1000 mg); and methylprednisolone 1 mg/kg/dose. Premedication may be repeated or increased as needed to control allergic reactions, chills, or fever. Patients who are unable to tolerate the rabbit product may receive equine ATG 25 mg/kg/day x 4 days with similar premedication.

Tacrolimus (TAC): IV or PO
Days: -1 onwards (see below)
Dose is dependent upon route of administration.
IV administration, Dose: 0.03 mg/**kg**/DAY
PO administration, Dose: 0.12 mg/**kg**/DAY

Tacrolimus levels should be maintained between 8-12 ng/mL. The use of intermittent IV dosing, continuous IV dosing, or PO formulations may be used when clinically appropriate per institutional protocols. If ≤ Grade I acute GVHD, tacrolimus taper will begin no later than Day +100 and will be complete by Day +180. For patients with > Grade I acute GVHD, the tacrolimus taper will be per institutional protocols.

Serum tacrolimus troughs and serum magnesium, potassium, and creatinine should be drawn at least twice per week while hospitalized, then as per good clinical practice thereafter unless a change in medication (e.g. use of concomitant CYP3A4 inhibitors, see Appendix IIIA) or renal function might result in an acute change in level. At that point, levels will be measured as clinically indicated. Levels sent when dosing by continuous infusion are not true trough concentrations, however, the same target range of drug levels will be used for both continuous IV and bolus PO routes of administration. When converting patients at a therapeutic tacrolimus level from IV to PO formulation, multiply total daily IV dose times 4 and administer in 2 divided oral doses per day, every 12 hours (e.g., 1 mg of IV tacrolimus per day equates to 4 mg of PO tacrolimus per day). The oral

dose should be administered 8-12 hours after the end of the tacrolimus continuous infusion.[57]

The target serum trough level for tacrolimus is 8-12 ng/mL for mismatched or unrelated donors. Dose adjustments are based on clinical judgment of the treating physician after considering clinical toxicity, serum levels, GVHD, concomitant drug use and the rate of rise or decline of the serum level. Refer to Section 5.2 for guidance on dose adjustment based on serum trough levels.

Mycophenolate Mofetil (MMF): IV over 2 hours
Days: +1 onwards (see below)
Dose: 15 mg/**kg**/dose, every 8 hours

The FDA has determined that a REMS (Risk Evaluation and Mitigation Strategy) program is necessary to ensure the benefits of mycophenolate outweigh the risks of first trimester pregnancy loss and congenital malformations associated with mycophenolate use during pregnancy. Mycophenolate REMS is a program to tell doctors, nurses, pharmacists and patients about the risks of taking mycophenolate during pregnancy. Providers and patients (females of child-bearing potential) are required to enroll in the program. The program also contains important information about patient education and reporting details for pregnancy. Providers and patients are required to sign acknowledgement forms prior to initiating therapy. Please refer to the website for additional information (https://www.mycophenolaterems.com/).

See Section 2.3.1 for additional information regarding the GVHD prophylaxis approach. If ≤ Grade I acute GVHD, MMF will be discontinued by Day +45. For patients with > Grade I acute GVHD, the MMF taper will be per institutional protocols. Patients may transition to PO formulations at a 1:1 IV to PO conversion when clinically appropriate. Refer to Section 5.3 for dose adjustments based on serum drug levels.

See Section 5.0 for Dose Modifications based on Toxicities.
The therapy delivery maps (TDMs) for this HCT treatment plan are on the next (one) page.

4.10.1 HCT Regimen: **Unrelated Donor -Arm B**

Treatment for the HCT preparative regimen lasts 8 days.
After the HCT, the GVHD prophylaxis treatment lasts about 6 months.

_____ _____
Patient COG ID number DOB

Extensive treatment details are in Section 4.1-4.5 and 4.10. This Therapy Delivery Map is on 1 page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Anti-Seizure Prophylaxis: _____ *Indicate agent, i.e., lorazepam, levetiracetam*	*IV or PO*	_____ *(indicate dose)* *Preferred regimen: lorazepam (0.02-0.05 mg/kg/dose every 6 hours) given 30 minutes prior to each busulfan dose. Continue until ≥ 24 hours after last busulfan dose.*	*-8 to -4*		a. History (incl. cardiac), PE w/ VS, NF1 status, pregnancy, PS b. BMA c. CBC, differential, HgbF, spleen & liver measure., concomitant tx d. Cytogenetics & FISH (not req. for all pts, see Sect. 7) e. GVHD, donor chimerism f. Complications g. Req. specimens: PB & BM (BM only if clinically indicated). See Section 7 for detailed list of evaluations. **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Busulfan (BUS) ***For once daily dosing only.***	IV over 3 hours ***For once daily dosing only.*** *See Section 4.10 for alternative dosing schedules.*	Age & wt-based (1 dose): < 10 kg: 3.2 mg/**kg**/dose ≥ 10 kg but ≤ 4 yr: 4 mg/**kg**/dose > 4 yr: 3.2 mg/**kg**/dose	-8	Submit BUS PK samples following 1st dose (Section 16). *See Section 4.10 for alternative dosing schedules.*	
		Dose adjusted per targeted AUC, once daily (3 doses)	-7 to -5	**Note:** Refrain from using polycarbonate syringes or filter needles during busulfan preparation.	
Fludarabine (FLUD)	IV over 30-60 minutes	Weight-based dosing: ≥ 10 kg: 40 mg/**m²**/dose < 10 kg: 1.33 mg/**kg**/dose (4 doses)	-5 to -2		
Rabbit ATG (rATG)	IV over 4-6* hours	2 mg/**kg**/day (4 doses)	-4 to -1	***1st dose**: infuse over ≥ 6 hours through a high-flow vein. **Subsequent doses:** infuse over ≥ 4 hours. Administer through an in-line 0.22 micron filter. Premedications should be considered. Equine ATG may be substitutued if rabbit product is not tolerated. See Section 4.10 for details.	
Tacrolimus (TAC)	IVor PO See Section 4.10.	IV Dose: 0.03 mg/**kg**/DAY PO Dose: 0.12 mg/**kg**/DAY	-1 to +180	Maintain levels between 8-12 ng/mL. If ≤ Grade I aGVHD, begin taper by Day +100. Discontinue by Day +180. See Section 4.10 for additional monitoring.	

__HEMATOPOIETIC CELL INFUSION: DAY 0 (see Section 4.4)__

Mycophenolate mofetil (MMF)	IV over 2 hours. Convert to oral when tolerated.	15 mg/**kg**/dose, every 8 hours	+1 to +45	If ≤ Grade I aGVHD, discontinue by Day +45.	

			Ht ___ **cm**	**Wt** ___ **kg**	**BSA** ___ **m²**				
Date Due	Date Given	Day	Anti-seizure prophylaxis ___mg	BUS ___mg	FLUD ___mg	rATG ___mg	TAC ___mg	MMF ___mg ___mg ___mg	Studies	Comments
				Enter calculated dose above and actual dose administered below						
		-8	__mg __mg __mg __mg	___mg					(a, b, c, d, g)[1]	
		-7	__mg __mg __mg __mg	___mg						
		-6	__mg __mg __mg __mg	___mg						
		-5	__mg __mg __mg __mg	___mg	___mg					
		-4	__mg __mg __mg __mg		___mg	___mg				
		-3			___mg	___mg				
		-2			___mg	___mg				
		-1				___mg	___mg			
		0					___mg			
		+1					___mg	___mg ___mg ___mg		
		+2					↓	↓		
		+3								
		…								
		+30							b, c, d, e, f, g	
		…								
		+45								
		…								
		+60							c, e, f, g	
		…								
		+90							b, c, d, e, f, g	
		…								
		+120							c, e, g	
		…								
		+180							c, e, g	
			See Section 7.2 for evaluations after Day +180.							

[1]Prior to HCT conditioning. **SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 4.5 FOR SUPPORTIVE CARE.**

4.11 UCB Donor HCT for Patients Assigned to Arm B (BU-FLU + rATG)

The condition regimen described below is for patients with an UCB donor randomized to receive the Arm B conditioning regimen. Patients randomized to Arm B will receive an 8-day HCT conditioning regimen that consists of Busulfan and Fludarabine (BU-FLU), as well as rabbit Anti-Thymocyte Globulin (rATG). Tacrolimus and mycophenolate mofetil will be administered for GVHD prophylaxis.

Doses should be adjusted for patients > 125% of Ideal Body Weight (IBW). Adjusted weight = 1.25*IBW. See Section 5.1.

Anti-seizure Prophylaxis:
Days: -8 through at least Day -4
The preferred regimen is lorazepam (0.02-0.05 mg/kg/dose PO or IV every 6 hours) given 30 minutes prior to each busulfan dose and then continuing for at least 24 hours after last busulfan dose. An alternative regimen is levetiracetam (10 mg/kg/dose PO or IV BID, max dose 1000 mg) beginning 12 hours prior to busulfan and continuing for at least 24 hours after last busulfan dose.

Busulfan (BUS): IV over 3 hours
Day: -8, once daily (1 dose)
Dose: Patients < 10 kg: 3.2 mg/**kg**/dose
Patients ≥ 10 kg but ≤ 4 years old: 4 mg/**kg**/dose
Patients > 4 years: 3.2 mg/**kg**/dose

Days: -7 through -5, once daily (3 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16)

First-dose busulfan pharmacokinetic studies will be performed in all patients. Daily dosing will be adjusted to achieve an overall exposure target area under the curve (AUC) of 3600-6000 (micromole/liter)*minute. Refer to Section 16 for additional information.

Acetaminophen should be held for 72 hours before and during busulfan administration, but may be given per individual institutional standard policies if clinically necessary.

Note: Refrain from using polycarbonate syringes or filter needles during busulfan preparation.

Alternate Busulfan Dosing:
Divided dosing (every 12 hours) is permitted per institutional practice. Every 6 hour dosing is NOT permitted with UCB donors due to conflicts with ATG timing.

Q12 Hour Dosing Schedule: IV over 2 hours
Day: -8, every 12 hours
Dose: Patients < 10 kg: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)
Patients ≥ 10 kg but ≤ 4 years old: 2 mg/**kg**/dose q 12 hours (x 2 doses)
Patients > 4 years: 1.6 mg/**kg**/dose q 12 hours (x 2 doses)

Days: -7 through -5, every 12 hours (x 6 doses)
Dose: Targeted dose adjustment based on PK results (see Section 16). Every 12 hour dosing should target an AUC of 1800 to 3000 (micromole/liter)*minute per dose.

Fludarabine (FLUD): IV over 30-60 minutes
Days: -5 through -2 (4 doses)
Dose: < 10 kg: 1.33 mg/**kg**/dose once daily
≥ 10 kg: 40 mg/**m²**/dose once daily

Rabbit Anti-thymocyte globulin (rATG): IV over 4-6* hours
Days: -8 through -5 (4 doses)
Dose: 2 mg/**kg**/dose

*The first dose should be infused over at least 6 hours through a high-flow vein. Subsequent doses should be administered over at least 4 hours. Administer through an in-line 0.22 micron filter.

Premedications should be considered prior to rATG per institutional protocols. This may include diphenhydramine 1 mg/kg/dose (max 50 mg), or a comparable anti-histamine; acetaminophen 10-15 mg/kg/dose (max 1000 mg); and methylprednisolone 1 mg/kg/dose. Premedication may be repeated or increased as needed to control allergic reactions, chills, or fever. Patients who are unable to tolerate the rabbit product may receive equine ATG 25 mg/kg/day x 4 days with similar premedication.

Tacrolimus (TAC): IV or PO
Days: -1 onwards (see below)
Dose is dependent upon route of administration.
IV administration, Dose: 0.03 mg/**kg**/DAY
PO administration, Dose: 0.12 mg/**kg**/DAY

Tacrolimus levels should be maintained between 8-12 ng/mL. The use of intermittent IV dosing, continuous IV dosing, or PO formulations may be used when clinically appropriate per institutional protocols. If ≤ Grade I acute GVHD, tacrolimus taper will begin no later than Day +100 and will be complete by Day +180. For patients with > Grade I acute GVHD, the tacrolimus taper will be per institutional protocols.

Serum tacrolimus troughs and serum magnesium, potassium, and creatinine should be drawn at least twice per week while hospitalized, then as per good clinical practice thereafter unless a change in medication (e.g. use of concomitant CYP3A4 inhibitors, see Appendix IIIA) or renal function might result in an acute change in level. At that point, levels will be measured as clinically indicated. Levels sent when dosing by continuous infusion are not true trough concentrations, however, the same target range of drug levels will be used for both continuous IV and bolus PO routes of administration. When converting patients at a therapeutic tacrolimus level from IV to PO formulation, multiply total daily IV dose times 4 and administer in 2 divided oral doses per day, every 12 hours (e.g., 1 mg of IV tacrolimus per day equates to 4 mg of PO tacrolimus per day). The oral dose should be administered 8-12 hours after the end of the tacrolimus continuous infusion.[57]

The target serum trough level for tacrolimus is 8-12 ng/mL for mismatched or unrelated donors. Dose adjustments are based on clinical judgment of the treating physician after considering clinical toxicity, serum levels, GVHD, concomitant drug use and the rate of rise or decline of the serum level. Refer to Section 5.2 for guidance on dose adjustment based on serum trough levels.

Mycophenolate Mofetil (MMF): IV over 2 hours
Days: +1 onwards (see below)
Dose: 15 mg/**kg**/dose, every 8 hours

The FDA has determined that a REMS (Risk Evaluation and Mitigation Strategy) program is necessary to ensure the benefits of mycophenolate outweigh the risks of first trimester pregnancy loss and congenital malformations associated with mycophenolate use during pregnancy. Mycophenolate REMS is a program to tell doctors, nurses, pharmacists and patients about the risks of taking mycophenolate during pregnancy. Providers and patients (females of child-bearing potential) are required to enroll in the program. The program also contains important information about patient education and reporting details for pregnancy. Providers and patients are required to sign acknowledgement forms prior to initiating therapy. Please refer to the website for additional information (https://www.mycophenolaterems.com/).

See Section 2.3.1 for additional information regarding the GVHD prophylaxis approach. If ≤ Grade I acute GVHD, MMF will be discontinued by Day +45. For patients with > Grade I acute GVHD, the MMF taper will be per institutional protocols. Patients may transition to PO formulations at a 1:1 IV to PO conversion when clinically appropriate. Refer to Section 5.3 for dose adjustments based on serum drug levels.

See Section 5.0 for Dose Modifications based on Toxicities.
The therapy delivery maps (TDMs) for this HCT treatment plan are on the next (one) page.

4.11.1 HCT Regimen: **UCB Donor -Arm B**

Treatment for the HCT preparative regimen lasts 8 days.
After the HCT, the GVHD prophylaxis treatment lasts about 6 months.

_____ _____
Patient COG ID number DOB

Extensive treatment details are in Section 4.1-4.5 and 4.11. This Therapy Delivery Map is on 1 page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Anti-Seizure Prophylaxis: _____ *Indicate agent, i.e., lorazepam, levetiracetam*	*IV or PO*	_____ *(indicate dose) Preferred regimen: lorazepam (0.02-0.05 mg/kg/dose every 6 hours) given 30 minutes prior to each busulfan dose. Continue until ≥ 24 hours after last busulfan dose.*	*-8 to -4*		a. History (incl. cardiac), PE w/ VS, NF1 status, pregnancy, PS b. BMA c. CBC, differential, HgbF, spleen & liver measure., concomitant tx d. Cytogenetics & FISH (not req. for all pts, see Sect. 7) e. GVHD, donor chimerism f. Complications g. Req. specimens: PB & BM (BM only if clinically indicated). See Section 7 for detailed list of evaluations. **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Busulfan (BUS) ***For once daily dosing only.***	IV over 3 hours ***For once daily dosing only.*** *See Section 4.11 for alternative dosing schedule.*	Age & wt-based (1 dose): < 10 kg: 3.2 mg/**kg**/dose ≥ 10 kg but ≤ 4 yr: 4 mg/**kg**/dose > 4 yr: 3.2 mg/**kg**/dose	-8	Submit BUS PK samples following 1st dose (Section 16). *See Section 4.11 for alternative dosing schedule.* **Note:** Refrain from using polycarbonate syringes or filter needles during busulfan preparation.	
		Dose adjusted per targeted AUC, once daily (3 doses)	-7 to -5		
Fludarabine (FLUD)	IV over 30-60 minutes	Weight-based dosing: ≥ 10 kg: 40 mg/**m²**/dose < 10 kg: 1.33 mg/**kg**/dose (4 doses)	-5 to -2		
Rabbit ATG (rATG)	IV over 4-6* hours	2 mg/**kg**/day (4 doses)	-8 to -5	***1st dose**: infuse over ≥ 6 hours through a high-flow vein. **Subsequent doses:** infuse over ≥ 4 hours. Administer through an in-line 0.22 micron filter. See Section 4.11: premedications should be considered Equine ATG may be substitutued if rabbit product is not tolerated. See Section 4.11 for details.	
Tacrolimus (TAC)	IV or PO See Section 4.11	IV Dose: 0.03 mg/**kg**/DAY PO Dose: 0.12 mg/**kg**/DAY	-1 to +180	Maintain levels between 8-12 ng/mL. If ≤ Grade I aGVHD, begin taper by Day +100. Discontinue by Day +180. See Section 4.11 for add'l monitoring.	

****HEMATOPOIETIC CELL INFUSION: DAY 0 (see Section 4.4)****

Mycophenolate mofetil (MMF)	IV over 2 hours. Convert to oral when tolerated.	15 mg/**kg**/dose, every 8 hours	+1 to +45	If ≤ Grade I aGVHD, discontinue by Day +45.	

Ht_____cm Wt_____kg BSA_____m²

Date Due	Date Given	Day	Anti-seizure prophylaxis ____mg	BUS ____mg	FLUD ____mg	rATG ____mg	TAC ____mg	MMF ____mg	Studies	Comments
			Enter calculated dose above and actual dose administered below							
		-8	__mg __mg __mg __mg	_____mg		_____mg			(a, b, c, d, g) [1]	
		-7	__mg __mg __mg __mg	_____mg		_____mg				
		-6	__mg __mg __mg __mg	_____mg		_____mg				
		-5	__mg __mg __mg __mg	_____mg	____mg	_____mg				
		-4	__mg __mg __mg __mg		____mg					
		-3			____mg					
		-2			____mg					
		-1					____mg			
		0					____mg			
		+1					____mg	____mg ____mg ____mg		
		+2					↓	↓		
		+3								
		...								
		+30							b, c, d, e, f, g	
		...								
		+45								
		...								
		+60							c, e, f, g	
		...								
		+90							b, c, d, e, f, g	
		...								
		+120							c, e, g	
		...								
		+180							c, e, g	
			See Section 7.2 for evaluations after Day +180.							

[1]Prior to HCT conditioning. **SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 4.5 FOR SUPPORTIVE CARE.**

5.0 DOSE MODIFICATIONS FOR TOXICITIES

Notify the Study Chair of any serious adverse events, protocol violations and/or if a patient is removed from protocol therapy for toxicity.

5.1 Dose Adjustment of Chemotherapy for Patients Whose Weight Exceeds > 125% IBW

Chemotherapy given during the preparative regimen (including rATG) will be dosed based on actual weight for patients ≤ 125% IBW. Those > 125% IBW will be dosed based upon adjusted ideal body weight as follows:

Adjusted ideal body weight = IBW + 0.25 (Actual weight – IBW).

The following formulas for pediatric and adult IBW calculations are recommended, but IBW may be calculated according to institutional SOPs.

5.1.1 Recommended Ideal Body Weight Calculation for Children Age 1- 17 years

$$\text{IBW} = \frac{\text{Height (cm)}^2 \times 1.65}{1000}$$

5.1.2 Recommended Ideal Body Weight Calculation for Adults (≥ 18 years)

IBW (females) = (cm ÷ 2.54 – 60) x 2.3 kg + 45.5 kg

IBW (males) = (cm ÷ 2.54 – 60) x 2.3 kg + 50 kg

5.2 Tacrolimus

Tacrolimus commonly causes mild/moderate hypertension and alopecia and less commonly kidney or liver dysfunction, transplant associated microangiopathy (TAM), and neurological changes associated with significant hypertension. When trough levels are kept in the therapeutic range and patients receive adequate hydration and magnesium replacement, most of these side effects can be minimized. Hypertension should be managed with single or combination antihypertensive therapy per institutional standards. Tacrolimus should be held for severe toxicities thought to be related to its administration (significant neurological changes/malignant hypertension, TAM, kidney failure, etc). Other immune suppressive medications may be substituted if tacrolimus is not tolerated (cyclosporine, etc).

For levels < 5 ng/mL (or < 8 ng/mL for mismatched or unrelated donors), it is suggested that the dose of tacrolimus be increased by approximately 25% increments every 1-2 days, rounded to the nearest 0.5 milligram (when dosing is oral) until the target range is achieved (dose adjustments may vary from the suggested 25% as needed to achieve the therapeutic range based upon the measured level and the clinical status of the patient). Conversely, for levels > 12 ng/mL, it is suggested that the dose of tacrolimus be decreased by approximately 25% every 1-2 days until the target level is achieved. For very high levels (> 20 ng/mL), tacrolimus may need to be held entirely for a period of time. This may be done as long as serum levels are monitored daily and the drug is restarted at an appropriate dosage when the level returns to the therapeutic range.

5.3 Mycophenolate

Mycophenolic Acid (MPA) trough levels should be monitored as clinically indicated and dose modified according to the table below (or as per institutional practice).

Table 2: Mycophenolate Dose Adjustment Guidelines

MPA Concentrations (mcg/mL)	MMF dosing adjustment
<1-3.5	No change
3.6-6	Decrease dose by 25%
>6	Hold one dose, decrease dose by 50%

6.0 DRUG INFORMATION

See the consent document for toxicities. All other information is available on the COG website in the commercial agent monographs manual titled "Drug Information for Commercial Agents used by the Children's Oncology Group." This manual is provided under Standard Sections for Protocols at: https://members.childrensoncologygroup.org/prot/reference_materials.asp.

7.0 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

7.1 Evaluations at Study Entry and Requirements Prior to Stem Cell Transplant

In cases that the patient's diagnosis occurred prior to study entry, data obtained at the diagnosis of JMML prior to study entry must be submitted instead of readings taken at enrollment.

STUDIES TO BE OBTAINED	Time of Diagnosis	Study Entry	Prior to HCT*	Study Requirements
Clinical Evaluations:				
History (incl. cardiac)		X		
Physical exam with VS [1]		X		
NF1 status by clinical exam		X		See Appendix II
Pregnancy Test [2]		X	2 weeks	Negative
IgG level	X	X^		
Direct Coombs Antibody Test	X	X^		
Performance Status			2 weeks	Lansky or Karnofsky performance status score ≥ 60.
Pulmonary Function Tests			2 weeks	FEV1, FVC, & DLCO corrected for Hgb ≥ 60% (or if unable to obtain PFTs: no evidence of dyspnea at rest, no exercise intolerance, & not requiring supplemental oxygen therapy).
Echo/EKG			2 weeks	SF ≥ 27% or EF ≥ 50% by radionuclide angiogram.
Liver Function Tests			1 week	Total bilirubin < 2.5 mg/dL (unless due to Gilbert's Syndrome); Transaminases < 5 x ULN
Creatinine Clearance or GFR or Serum Creatinine			2 weeks	≥ 70 mL/minute/1.73m^2 or serum creatinine by age/gender
CNS Function			2 weeks	CNS toxicity ≤ Grade 2
CBC, differential, HgbF	X	X^	2 weeks	
BMA (biopsy optional) [3]	X	X^	2 weeks	
Cytogenetics & FISH for Monosomy 7[4]	X	X^	X[4]	
BCR/ABL by FISH or PCR	X	X^		
GM-CSF Hypersensitivity	X	X^		Only for patients without mutations in *PTPN11, RAS, CBL*, or *NF1*, and who otherwise do not fulfill the diagnostic criteria in Section 4.3.1.
Spleen & liver measurement[5]	X	X^	X	
Concomitant Therapy		X	X	
HCT Donor Information			X	
Infection Status			X	See Section 4.3.5 for requirements.
HSV and CMV titers			1 month	
Hepatitis C by serology and PCR			1 month	
Hepatitis B surface antigen			1 month	
HIV Test		X	X	Negative
Required Sample Submission: [6]				
Buccal swab / Oragene Saliva Kit		X		
Peripheral blood		X	X	

Bone marrow aspirate		X	X	
Central Review Submissions:				
Cytogenetics Report[7]		X		
Pathology Review Materials[8]		X		
Optional Samples: [9]				
Spleen Tissue		X	X	If splenectomy was performed.
Skin sample		X		Requested only for patients with buccal swabs demonstrating suspicious germline mutations in *PTPN11, RAS*, or *CBL*
Skin rash biopsy		X	X	Only if a rash is biopsied.
Parent buccal swabs		X		
Peripheral blood		X		
Bone marrow aspirate		X		

1 Including NF-1 status, Noonan syndrome, and assessment of hepatosplenomegaly.
2 Women of childbearing potential require a negative pregnancy test prior to starting treatment; sexually active patients must use an acceptable method of birth control.
3 Submit pathology materials for central review.
4 Performed at a COG-approved cytogenetics laboratory. Submit results for central review. Repeat cytogenetic analysis pre-HCT if patient received any pre-HCT chemotherapy OR if there was a delay of ≥ 30 days between initial diagnosis and HCT. See Section 13.
5 By ultrasound.
6 See Section 15 for sample collection & shipping instructions. Samples will be assessed for genotyping in the CLIA-certified laboratory at UCSF. Other research studies will be performed on samples in the Loh Laboratory (results will not be returned for these research studies).
7 See Section 13 for central cytogenetics review submission requirements.
8 See Section 14 for central pathology review submission requirements.
9 See Section 15 for sample collection & shipping instructions.

^ If performed at diagnosis of JMML prior to study entry, does not need to be repeated at study entry.
* Please note that pre-HCT observations reflect recommended timing by the study committee. It will not be considered a protocol violation if there is a deviation from this recommended timing.

7.2 Required & Optional Clinical, Laboratory and Disease Evaluations Post HCT

Evaluations post-HCT may occur within +/- 3 days of the collection day specified below.

STUDIES TO BE OBTAINED	+30	+60	+90	+120	+180	+270	+365	+540	Suspected of Relapse
Clinical Evaluations:									
CBC, differential, HgbF	X	X	X	X	X	X	X	X	X
BMA (biopsy optional) [1]	X		X						X
Cytogenetics & FISH [2]	X		X						X
Spleen & liver measurement [3]	X	X	X	X	X	X	X	X	X
Concomitant therapy	X	X	X	X	X	X	X	X	X
GVHD assessment	X	X	X	X	X	X	X	X	X
Donor chimerism	X	X	X	X	X	X	X	X	X
Complications [4]	X	X	X	X					
Required Samples: [5]									
Peripheral blood [6]	X	X	X	X	X	X	X	X	X
Bone marrow [6]	X	X[7]	X	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]
Optional Samples: [5]									
Spleen Tissue1[8]	X								
Skin rash biopsy [9]	X								

1 Submit pathology materials for central review. See Section 14.
2 Submit Day +30 and +90 bone marrow for cytogenetic analysis if an abnormal cytogenetic clone was identified at initial diagnosis (or pre-HCT). Performed at a COG-approved cytogenetics laboratory. Submit results for central review. See Section 13.
3 By ultrasound.
4 Grade 3-4 non-hematologic toxicities
5 See Section 15 for sample collection & shipping instructions.
6 Samples will be assessed at each time point for repeat genotyping/454 sequencing (MRD) in the CLIA-certified laboratory at UCSF. Qualitative sequencing results will be returned to the treating institution. Other research studies will be performed on samples in the Loh Laboratory (results will not be returned for these research studies).
7 Only if clinically indicated.
8 Only if splenectomy was performed.
9 Only if a rash is biopsied.

This table only includes evaluations necessary to answer the primary and secondary aims. Obtain other studies as indicated for good clinical care.

7.2.1 Suspicion of Relapse

Patients may be suspected of relapse (or "non-response," see Section 10.2 for definition details) based on either clinical findings (such as increasing spleen size), or laboratory features (such as increasing monocytosis). At the discretion of the treating physician, patients should have a bone marrow aspirate performed, in addition to the other evaluations listed in the table in Section 7.2. Furthermore, some patients may have a "weak-positive" mutation analysis following HCT (defined as a 1-14.99% mutant allele burden). It is highly recommended that these patients be considered "suspected of relapse," and that that evaluations

listed in the table in Section 7.2 be performed within a month of the test yielding the first "weak-positive" result.

7.3 Required Busulfan Pharmacokinetic Studies* (see Section 16).

For Single (once daily) IV Dosing

Time Following Start of 1st Dose
3 hours
3 hours 15 minutes
4 hours
5 hours
6 hours
8 hours

* To be done on 1st dose only.

For Q6 hour IV Dosing

Time Following Start of 1st Dose
2 hours
2 hours 15 minutes
2 hours 30 minutes
3 hours
4 hours
5 hours
6 hours

* To be done on 1st dose only.

For Q12 hour IV Dosing

Time Following Start of 1st Dose
1 hour
2 hours
3 hours
5 hours
6 hours
7 hours
8 hours

* To be done on 1st dose only.

7.4 Follow-up

See COG Late Effects Guidelines for recommended post treatment follow-up: http://www.survivorshipguidelines.org/

Although the primary end-points of the trial are the 100-day TRM and 18 month EFS, patients will be followed until off-therapy for other reasons, or to 5 years from study enrollment.

Note: Follow-up data are expected to be submitted per the Case Report Forms (CRFs) schedule.

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Removal from Protocol Therapy

a) Progressive disease (non-response, as described in Section 10.2.4).

b) Refusal of further protocol therapy by patient/parent/guardian.

c) Completion of planned therapy and post-therapy sample collections.

d) Physician determines it is in patient's best interest.

e) Development of a second malignancy.

f) Criteria to proceed to HCT (Section 4.3) are not met within 1 year of enrollment.

Patients who are off protocol therapy are to be followed until they meet the criteria for Off Study (see below). Follow-up data will be required unless consent was withdrawn.

8.2 Off Study Criteria

a) Death.

b) Lost to follow-up.

c) Patient enrollment onto another COG study with tumor therapeutic intent (eg, at recurrence).

d) Withdrawal of consent for any further data submission.

e) The fifth anniversary of the date the patient was enrolled on this study.

9.0 STATISTICAL CONSIDERATIONS

From June 11, 2001 to October 23, 2006 patients with JMML could enroll on COG AAML0122, a single arm trial evaluating a targeted agent following by HCT. This trial enrolled 88 patients (~18 cases/year); however a survey of transplant physicians indicated that many eligible patients were not enrolled due to concerns regarding trial design that mandated the use of total body irradiation in this very young patient population. In addition, diagnostic testing for JMML is now more advanced to what was previously available and will likely diagnose a greater number of children. We anticipate at least equal, if not superior, accrual to this trial due to the removal of TBI from the conditioning regimen and more precise diagnostic procedures.

Enrollment onto COG study AAML0122 averaged 18 patients per year. This study will benefit from the support of the JMML Foundation so it is estimated that at least 24 patients per year can be enrolled. Enrollment occurs when a clinical diagnosis of JMML is made, but eligibility for the HCT randomization is only determined after 1) genetic assessment of the diagnosis is made and the complete WHO diagnostic criteria are fulfilled; 2) an eligible donor is found; and 3) the patient survives and consents to be randomized. It is estimated that approximately 75% of enrolled patients will be eligible (18 eligible patients/year) for the HCT randomization. Thus, we calculate that up to 145 patients will need to be enrolled in order attain the target 108 eligible patients and, with a 1 year ramp up period, the enrollment period is expected to require 7 years. Given the complexities of diagnosing and treating these patients, precise data-driven calculations are impossible and the study committee would welcome more rapid accrual. Conversely, as the trial progresses, if we find that we are not meeting this metric, we will amend the number of children enrolled accordingly, and have secured funding from the BIQSFP mechanism to genotype up to 220 children.

9.1 Study Design

The study design is a modified randomized phase II trial. Patients will be assigned to BU-FLU or BU-CY-MEL myeloablative conditioning regimen using a randomized block strategy within strata defined by donor type and *PTPN11* mutation status. The outcome of the trial will be determined after all eligible patients have 18 months of follow-up. If the difference in the estimated 18 month EFS is more than 5%, then the regimen with the higher EFS will be declared the superior treatment. If the difference in EFS is less than 5%, then the regimen with the lowest 100 day TRM risk will be declared the superior treatment. This design was chosen to exploit the a-priori belief that relapse probabilities will not influenced by the choice of regimen to increase the probability that the trial will pick the better regimen.

9.1.1 Primary Endpoints:

Each patient will be evaluated for Day 100 treatment related mortality (TRM) and 18 month EFS. A patient who dies prior to relapse or non-response will be considered to be a TRM and time to TRM will be defined as time from transplant to TRM. Patients who die between the start of the conditioning regimen and transplant will be considered a TRM with time to TRM of zero. The estimated 100 day TRM is defined the cumulative incidence of TRM at 100 days as estimated using the method of Aalen and Johannsen.[58] An event is either TRM, primary or secondary graft failure, or relapse/non-response (as defined in Section 10). Time to event will be computed from transplant with patients who die between the start of the conditioning regimen and transplant given a time to event of zero. Estimated 18-month EFS is defined as the Kaplan-Meier estimator of EFS at 18 months. A patient who is in complete remission at transplant (Section 10.2.1) is followed for relapse (Section 10.2.3). A patient not in complete remission at transplant is followed for either non-response (Section 10.2.4) or until the patient meets the complete remission criteria (Section 10.2.2). If non-response, the patient has experienced an event so statistical follow-up ends. If complete remission, the patient is still "event-free" and the patient is further followed for relapse (Section 10.2.3).

9.1.2 Secondary Endpoints:

For the purpose of Objective 1.2.1, relapse will include "relapse" or "non-response" as defined by the criteria described in Section 10. Time to "relapse/non-response" will be from transplant to when all criteria of Section 10.2.3 or 10.2.4 are met.

9.1.3 Stratification

The 1:1 randomization will be block stratified within six strata defined by the cross classification of stem cell source defined as: (1) matched related donor; (2) closely matched unrelated donor; or (3) umbilical cord blood, and mutation in the *PTPN11* gene. We expect the distribution of donors to be 30:35:35 and the prevalence of *PTPN11* mutation to be 35% so that the expected number in each stratum is given in Table 3 below.

Table 3: Expected number in each stratum for randomization

	Donor source			
	Matched Related	Matched unrelated	Cord	Total
PTPN11 WT	21	25	25	70
PTPN11 mutation	11	13	13	38
Total	32	38	38	108

9.2 Sample Size and Study Duration

A sample size of 108 (54 in each arm) was chosen so that in the situation in which the regimens have the same relapse rate and the difference in true 100 day TRM risk is about 5% (7%), the modified phase II design will have over 75% (80%) probability of picking the superior regimen.

9.2.1 Justification for this Design

While EFS is the natural measure for comparing treatment regimens, the modified randomized phase II design, which puts more weight on 100 day TRM risk, was chosen because of an *a priori* belief that the relapse rates will be similar in each. With this design, if there is a large enough difference in observed 18 month EFS, higher EFS determines the winner, whether that difference is because of lower TRM or lower relapse risk. If the difference in EFS is small, TRM risk will determine the winner. This design relies primarily on TRM risk for picking the winner but provides protection against a situation in which there was a large differences in relapse risk that was opposite in direction from the difference in TRM risk. As shown in Section 9.4.2, if the true relapse risks are similar, then this design has much higher probability of picking the regimen that is actually superior to a rule that depends only on the observed EFS.

9.2.2 Trial Outcome Probabilities given TRM and Relapse Probabilities

As originally conceived, the proposed study design was a RPII "pick the winner" design comparing treatment related mortality (TRM) between the two treatment arms. In response to concerns by the COG Scientific Council that the winning treatment could have lower event-free survival (EFS), a modification of the RPII design was developed that picks the treatment that is EFS superior and exploits the Study Committee's *a-priori* belief that only TRM (and not relapse) rates will be influenced by the treatment to increase the probability of picking the regimen that is truly better. Specifically, the modified RPII design for this trial chooses the winning regimen as the one that has (1) an event rate at least 5% lower than the other regimen or (2) a lower TRM rate than the other regimen when the difference in event rates is less than 5%. The trial outcome probabilities are a function of p_{kt} and p_{kr}, the true TRM and relapse rates for regimen k=A,B; δ, the tolerated difference in event rates; and n, the number of patients in each arm of the trial. Following the approach of Simon, Wittes, and Ellenberg for the standard RPII design,[59] let $mult(t,r,p_t,p_r,n)$ be the multinomial probability of t-TRM, r-relapse, and n-t-r "no events" patients out of n in the group. The probability that the trial result is T_A TRM and R_A relapse patients from Regimen A and T_B TRM and R_B relapse patients from Regimen B is

$$p(T_A,R_A,T_B,R_B) = mult(T_A,R_A, p_{At}, p_{Ar},n) \times mult(T_B,R_B, p_{Bt}, p_{Br},n).$$

The probability that Regimen A is selected using this design is the sum over all possible outcomes of T_A,R_A,T_B,R_B in which Regimen A meets criteria (1) or (2). Letting $\Delta=n\delta$, I, the indicator function, and noting that the event rate is given by $T+R$, the probability of Regimen A as winner is

$$\sum[I(T_A+R_A+\Delta \le T_B+R_B) + I(|(T_A+R_A)-(T_B+R_B)| \le \Delta \text{ and } T_A < T_B) + \tfrac{1}{2} I(|(T_A+R_A)-(T_B+R_B)| \le \Delta \text{ and } T_A = T_B)] \text{x } p(T_A,R_A,T_B,R_B)$$

where the sum is over all possible T_A,R_A,T_B,R_B. These probabilities, as well as the probabilities of picking Regimen A using standard RPII designs with TRM or EFS as the outcome, were computed using a macro written by the Study Statistician using the SAS statistical package (Cary, NC) posted on http://hydra.usc.edu/timefactors. The program was validated using a computer simulation study that generated 10,000 studies with the parameters given in the Table below and the winner picked according to the modified RPII design. All estimated Regimen A winner probabilities based on the simulation were within 1% of those calculated analytically.

The table below shows the probability of picking Regimen A (the "superior" regimen). For comparison, the probability of picking Regimen A using a standard RPII design with just estimated 100 day TRM risk or just estimated 18 month EFS probability as the comparison measure. Scenarios 1a to 1d are considered "realistic" situations in which Regimen A has lower true TRM probability than B, and the same true relapse probability. In this situation, inclusion of relapse in the outcome only adds variability to the TRM difference so the standard RPII with TRM outcome has the highest probability of picking Regimen A while the standard RPII with EFS has more than 10% lower probability of picking A. The modified RPII that will be used in this study suffers only a slight loss of relative to the TRM standard design. Under Scenario 2, Regimen A has both lower TRM and relapse risk. In this case, the modified design is the most likely to pick Regimen A. In Scenario 3a, with TRM equal and Regimen A associated with lower relapse risk, the TRM standard design is focused on the wrong outcome and picks either regimen with 50% probability. However, the modified design still has high probability of picking the Regimen A. In scenarios 3b and 3c, the TRM risk from Regimen A is higher than in Regimen B, but relapse risk is lower, such that the true EFS is 10% higher in A than in B (i.e., A is the better regimen). In this case, the TRM-standard design will be more likely to pick (the "wrong" regimen) B, while the modified design picks A with probability close to that using a standard design with EFS as the outcome. In Scenario 3d, the true EFS difference is 5%, equal to the "EFS tolerance." In this situation, the probability that the observed EFS difference is less than 5% is 50% and the probability that the observed TRM risk for A is less than B, conditional on observed EFS difference less than 5%, is 56%. Although the probability of picking the preferable regimen is not much higher than 50%, the modified design is much better in these situations than the TRM standard design which would preferentially pick the inferior regimen with high probability. Finally, in Scenario 3e, there is no difference in EFS so that, arguably, the regimens are equivalent in effectiveness. In this case, the modified design favors regimen B.

Thus, the design focuses on TRM differences but provides some protection if, in fact, there is relapse difference in the opposite direction of the TRM difference. At the planned sample size of 108 patients (54 in each arm), if there is no difference in relapse risk and a true difference of 5% in TRM risk (Scenario 1b, 1c), there is at least an 75% chance of picking the better regimen.

Probabilities of picking Regimen A under the proposed modified randomized Phase II, and standard randomized Phase II designs using estimated 100 day TRM and 18 month EFS as the outcomes as a function of true 100 day TRM and 18 month relapse probabilities with a sample size of 108 (54 in each arm).

Table 4: Probability of Picking Regimen A for the Modified Randomized Phase II, Standard Randomized Phase II with TRM as outcome, and Standard Randomized Phase II with EFS as Outcome for a Range of Hypothetical TRM and Relapse Scenarios.

	TRM probability		Relapse probability				Probability of picking Regimen A		
Scenario	Regimen A	Regimen B	Regimen A	Regimen B	A-B EFS difference	Modified RPII	Standard RPII with TRM outcome	Standard RPII with event outcome	Modified RPII: Simulation[a]
1a	0.08	0.15	0.35	0.35	0.07	0.84	0.87	0.77	0.84
1b	0.05	0.12	0.35	0.35	0.07	0.85	0.91	0.77	0.84
1c	0.10	0.15	0.35	0.35	0.05	0.76	0.78	0.70	0.76
1d	0.05	0.10	0.35	0.35	0.05	0.78	0.84	0.70	0.78
2	0.05	0.10	0.30	0.40	0.15	0.95	0.84	0.94	0.95
3a	0.10	0.10	0.30	0.40	0.10	0.79	0.50	0.85	0.79
3b	0.15	0.08	0.33	0.50	0.10	0.73	0.13	0.85	0.73
3c	0.15	0.10	0.30	0.45	0.10	0.74	0.22	0.85	0.74
3d	0.15	0.10	0.30	0.40	0.05	0.56	0.22	0.70	0.56
3e	0.15	0.10	0.30	0.35	0.00	0.39	0.22	0.50	0.38

[a]Based on 10,000 trials.

9.2.3 TRM and Relapse Probabilities for the Outcome Probability Calculations

To calculate the probability of picking a given regimen using the criteria described in Section 9.3.1, the probabilities of 100 day TRM and 18 month relapsed risk need to be specified for each regimen. These were estimated from reports in the literature. The expected Day 100 TRM for patients receiving BU-CY-MEL can be determined by analysis of the published reports:

Table 5: Expected Day 100 TRM for Arm A Based on Published Reports.

	MRD	URD	UCB
BU-CY-MEL[4]	5%^	8%^	14%^ (n = 7)
BU-CY-MEL[6]	NA	NA	~25%* (n = 42)
Summary	5%	8%	~22%
Expected Proportion of Patients on Trial	0.3	0.35	0.35
Expected Rate of Day 100 TRM	1.5%	2.8%	7.7%
Total = 12%			

^Day 100 TRM not reported, but extrapolated from reported 5-year TRM, that occurred at a median of 2.7 months (range, 1 to 16) from transplant, by dividing reported figure in half.

*Day 100 TRM not reported, but extrapolated from 2-year TRM of 33%, with approximately 75% of this expected to occur within 100 days.

Although there is no data on the BU-FLU regimen in patients with JMML, a careful review of the literature can provide an expected baseline Day 100 TRM in children with non-malignant diseases or myeloid malignancies.

Table 6: Expected Day 100 TRM for Arm B Based on Published Reports.

	MRD	URD	UCB
BU-FLU[26]	0/7	2/11	0/1
BU-FLU[27]	1/12	1/23	NA
BU-FLU[60]	NA	NA	0/30
BU-FLU[61]	0/17	0/3	2/20
BU-FLU^[16]	0/60	1/36	NA
Summary	1/96 = 1%	4/73 = 5.5%	2/51 = 3.9%
Expected Proportion of Patients on Trial	0.3	0.35	0.35
Expected Rate of Day 100 TRM	0.3%	1.9%	1.4%
Total = 3.6%			

^Adults with AML/MDS

Thus, given the expected proportion of stem cell sources used for patients enrolling on this trial, we would expect a TRM of approximately 12% in the BU-CY-MEL arm, and approximately 4% in the BU-FLU arm. Since neither of these approaches has been implemented in a truly multicenter approach, we would expect slightly higher rates, such as 15% for BU-CY-MEL and 7% for BU-FLU.

9.3 Methods of Analysis

9.3.1 Primary objectives

The trial is a modified randomized phase II design. The trial will end when all eligible patients have accrued at least 18 months of follow-up time. The analysis to determine the superior regimen is as follows: If there is at least a 5% difference in 18 month EFS (as estimated using the Kaplan-Meier method), then the regimen with the higher 18 month EFS is superior. If the difference in estimated 18 month EFS is less than 5%, then the regimen with the lower estimated 100 day TRM risk (cumulative incidence as estimated using the Aalen-Johansen method[58]) is superior.

9.3.2 Secondary objectives

9.3.2.1 Relapse / non-response and graft failure rates will be estimated using the cumulative incidence via the method of Aalen and Johannsen.[58] For relapse, death and non-response (per Section 10.2.3) will be treated as competing risks. For graft failure, death will be the competing risk.

9.3.2.2 While we have made a reasonable estimate that we will need to screen 145 patients in order to enroll 108 to the randomized intervention, we nonetheless have funding from the BIQSFP agency to perform genotyping on up to 220 patients if necessary.

9.3.3 Exploratory objectives

9.3.3.1 To determine the rates of severe toxicities (Grade 3/4) at Day 100 post-HCT between the two myeloablative conditioning regimens (BU-FLU vs. BU-CY-MEL).

For Grade 3/4 toxicities (by CTCAEv.4.0), as well as severe SOS (as defined below) occurrence will be compared in a number of ways: proportion of patients experiencing each toxicity and number of Grade 3/4 toxicities per patient. High levels to busulfan, as measured by serum levels, have been associated with excessive rates of liver VOD/SOS and TRM.[62] Thus, severe toxicities (Grade 3/4) at Day 100 post-HCT will be assessed as a function of busulfan dose.

Unacceptable (severe) SOS: The CTC does not include SOS as a single toxicity. Therefore, this study will utilize a composite definition of SOS used in previous studies (NANT N01-02; ANBL0532, ANBL09P1, ANBL12P1) to evaluate SOS: Serum total bilirubin > 2.0 mg/dL, with 2 of the following findings within 21 days of transplantation: hepatomegaly with right upper quadrant pain, ascites, or weight gain >5% above baseline. Severe SOS is defined as an episode of SOS in addition to a specific organ failure:

a. Hepatic encephalopathy (CTC Grade 4 hepatic failure), OR
b. Pulmonary dysfunction: Continuous oxygen support (CTC Grade hypoxia) for > 48 hours, ventilatory support not clearly attributable to another cause,
   OR
c. Renal dysfunction: serum creatinine > 3 times the ULN (CTC Grade 3 creatinine), or the need for dialysis (CTC Grade 4 renal), not clearly attributable to another cause.

9.3.3.2 To determine the rates of acute and chronic (at 18 months post-HCT) graft-versus-host disease (GVHD) following HCT using two different conditioning regimens (BU-FLU vs. BU-CY-MEL) in children with JMML.

The 18 month risk of acute and chronic GVHD will be estimated using cumulative incidence curves with TRM and relapse as the competing risks.

9.3.3.3 To create a JMML-specific pre-HCT co-morbidity index to allow better risk-stratification of future patients.

Constellations of potential prognostic factors that can be assessed pre-HCT, that identify relapse and mortality risk diversity will be identified using Cox regression and semi-parametric survival methods. These factors may include patient gender, age at diagnosis, complete blood count values at diagnosis, genetic karyotype, genetic mutation status, hemoglobin F percentage, bone marrow blast percentage, and spleen size. Standard cross- and other validation approaches will be used to estimate the actual relapse risk in the risk-groups.

9.3.3.4 To determine the feasibility of assessing post-transplant disease burden by donor chimerism measurements and allele specific PCR in mononuclear and sorted cell subsets.

We will perform 454Jr next generation sequencing to assess post-transplant disease burden for patients enrolled on the study (as a quantitative value for research only) and we plan to release qualitative results for unsorted mononuclear cells based on predetermined thresholds (defined and validated in the CLIA lab for the BIQSFP application). In other independently funded work, we will compare the sensitivity of 454 sequencing performed on subsets of sorted/selected cells (CD3, CD14/15, and CD34) against allele specific PCR based assays and donor chimerism to identify the optimal methods to diagnose relapse. Any patient who is followed on protocol therapy for at least 30 days post HCT will be considered in the evaluation of feasibility of post-HCT disease burden assessment. The proportion of patients who are successfully assessed will be calculated as the number of patients with at least one re-sequencing evaluation 30 days post-HCT divided by the number of patients who are evaluable for post-HCT disease burden. This proportion will be compared with 75% using a 1-sided exact test of approximate size 0.05. If this test is rejected, the procedures used for obtaining this specimen will be examined. For example, we expect that 81/108 patients will be evaluable for post-HCT disease burden using 454 sequencing. If 72 or less are successfully evaluated, the procedures for sending the specimens will be examined. If the true rate of successful evaluation is only 60%, the post-HCT disease burden evaluability rate will be considered too low with 95% probability.

9.3.3.5 To determine predictors of overall survival using either gene expression or methylation classifiers determined at study enrollment

A gene expression profile was previously described by Bresolin et al[45] and predicted for poorer survival in those patients that displayed an AML-associated signature. Similarly, Olk-Batz has identified that the constellation of hypermethylation of 4-genes was associated with a higher rate of treatment

failure.[50] Similar studies will be performed at study enrollment for those patients who are ultimately transplanted. Our analysis strategy will be to compare the event (relapse / non-response, and TRM rates) across groups defined by the prognostic levels. For the gene expression profile (GEP) studies, prior work has indicated that a cohort of only 44 patients treated on various protocols, revealed statistically different groups with either an "AML-type" or a "non-AML" like GEP. Those with an AML type GEP had 6% EFS versus 63% EFS for those without ($P = .0010$).[45] On that study, 50% of JMML patients were AML-like. Cause-specific relapse and TRM rates in AML gene expression positive and negative patients will be compared using Cox regression, controlling for other prognostic factors and treatment arms to the extent possible. Relapse/non-response and TRM rates will be estimated using the method of Aalen and Johansen. With 108 patients on this study there is over 70% power to detect EFS differences of 25% and over 90% power to detect DFS differences of 30% when the high-risk group has an EFS rate in the range of 10-50%.

Hypermethylation signatures will be analyzed in a similar manner. For instance, methylation signatures will be summarized into a continuous methylation score based on the four genes defined in the Olk-Batz work that predicted outcome. On this prospective trial there will be at least 65% power to detect DSF differences of 20% and over 80% power to detect DFS differences of 30% between the lowest quartile and the upper three quartiles of the score.

9.3.3.6 To comprehensively assess genetic and biochemical alterations amongst patients with JMML who are treated on this transplant protocol.

This exploratory aim will build on previous and simultaneously conducted work to determine the proportion of children with JMML who harbor new mutations that may be discovered in parallel whole exome sequencing approaches, as well as biochemical perturbations using phosphoflow signaling analyses. In addition, biomarkers of autoimmunity (IgG levels and direct Coombs tests) will be measured and assessed descriptively to determine the incidence and effect of these abnormalities in children with JMML enrolled on this trial. Comparison of event rates by genetic and biomarkers will be done using standard Cox regression methods with event risk in patient groups estimated using Kaplan-Meier curves. Changes in the genetic or biochemical measures between diagnosis and transplant will be correlated with treatments received during this period.

9.3.4 Safety Monitoring

Safety monitoring of outcomes will take place two years after the start of the study as part of the Study Progress Report cycles. The rules for each outcome to be evaluated are constructed such that there is no more than a 10% chance of stopping if, in fact, an excess of the event of concern is not elevated above expected.

9.3.4.1 Graft Failure in the BU-FLU Arm

Patients on the BU-FLU arm with follow-up of at least 100 days post-HCT will be assessed for graft failure. A graft failure (primary or secondary) rate in these patients of up to 10% is acceptable. The table below gives the number of graft failures (or greater) that would be observed with probability of 10% given a true graft failure rate of 10%, given the number of BU-FLU patients assessed. If the number of graft failures is greater than or equal to the number shown, the study

will be temporarily suspended for DSMC evaluation. If there are 30 patients and the true graft failure rate is 30%, then the probability of observing six or more graft failures is 92%.

Table 7: Number of BU-FLU Arm Patients with Graft Failure to Trigger DSMC Review by Number of BU-FLU Patients with More than 100 Days Follow-Up.

BU-FLU patients	15	20	25	30
Graft failures for temporary suspension	4	5	5	6

9.3.4.2 Relapse Rate in the BU-FLU Arm

All patients on the BU-FLU arm will be evaluated for relapse and the relapse rate at 18 months, with TRM as a competing risk, will be estimated using the method of Aalen and Johansen. If the estimated relapse/non-response is greater than 40%, the study will be temporarily suspended for DSMC evaluation.

9.3.4.3 TRM Rate in the BU-CY-MEL Arm

Patients on the BU-CY-MEL arm with follow-up of at least 100 days post-HCT will be assessed for TRM. A TRM rate in these patients of up to 20% is acceptable. The table below indicates the number of TRMs (or greater) that would be observed with probability of 10% given a TRM rate of 20%, given the number of BU-CY-MEL patients assessed. If the number of TRMs is greater than or equal to the number shown, the study will be temporarily suspended for DSMC evaluation. If there are 30 patients and the true graft failure rate is 40%, then the probability of observing 10 or TRMs is 82%.

Table 8: Number of BU-CY-MEL Arm Patients with TRM to Trigger DSMC Review by Number of BU-CY-MEL Patients with More than 100 Days Follow-Up.

BU-CY-MEL patients	15	20	25	30
TRMs for temporary suspension	6	7	9	10

9.4 Evaluability for Response

An enrolled patient will be evaluable for response (EFS and TRM) if
1) Diagnosis is confirmed based on criteria in Section 4.3.1,
2) A stem cell donor source, meeting the criteria in Section 4.2 is identified,
3) Other patient HCT requirements are met,
4) The patient provides consent and starts a study preparative regimen.

9.5 Evaluability for Toxicity

An enrolled patient will be evaluable for toxicity if the patient meets the criteria for evaluability for response.

9.6 Gender and Minority Accrual Estimates

The gender and minority distribution of the study population is expected to be:

Accrual Targets			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	10	19	29
Not Hispanic or Latino	63	128	191
Ethnic Category: Total of all subjects	73	147	220
Racial Category			
American Indian or Alaskan Native	2	0	2
Asian	4	11	15
Black or African American	4	12	16
Native Hawaiian or Pacific Islander	2	0	2
White	61	124	185
Racial Category: Total of all subjects	73	147	220

This distribution was derived from the gender/race/ethnicity data from the Statistics & Data Center childhood cancer registry grant, ICD-O morphology codes for JMML (9945/3, 9946/3) between 2005-2012.

10.0 EVALUATION CRITERIA

10.1 Common Terminology Criteria for Adverse Events (CTCAE)

This study will utilize version 4.0 of the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). Additionally, toxicities are to be reported on the appropriate case report forms.

Please note: 'CTCAE v4.0' is understood to represent the most current version of CTCAE v4.0 as referenced on the CTEP website (ie, v4.02 and all subsequent iterations prior to version 5.0).

10.2 JMML Response Criteria

10.2.1 Definition of Complete Remission prior to HCT:

The majority of patients with JMML will enter HCT with some evidence of active disease; however, some patients may enter HCT in complete remission. Complete remission will be determined upon entry to HCT (i.e., start of conditioning regimen) as those patients that achieve ALL of the following criteria:

1. Absence of splenomegaly on exam and imaging, if the spleen is present
2. Absolute monocyte count < 1000/μL
3. Absence of myeloid precursors (including blasts) in peripheral blood

4. Normal HgbF for age
5. Blasts in bone marrow of < 5% with normal cytogenetics
6. For patients with previously identified molecular abnormality, disappearance of molecular abnormality in *NRAS*, *KRAS*, or *PTPN11* (below a level of 1% on 454Jr sequencing).

10.2.2 Definition of Complete Remission after HCT:

The majority of patients with JMML will enter HCT with some evidence of active disease. Complete remission will be defined on Day +90 (for those who enter HCT without remission) as those patients that achieve the following criteria:

Blasts in bone marrow of < 5%; AND EITHER:

1. Achievement of donor chimerism of > 95% in unsorted mononuclear cells from bone marrow;
   OR
2. For patients with previously identified cytogenetic or molecular abnormality, disappearance of clonal cytogenetic abnormality or molecular abnormality in *NRAS*, *KRAS*, *PTPN11* (below a level of 1% on 454Jr sequencing)
   OR
3. For patients without cytogenetic abnormalities or mutations, or with mutations in NF1 or CBL at diagnosis, who do not achieve full donor chimerism (as defined above), ALL of the following features of clinical remission must be achieved:
   a. Absence of splenomegaly on exam and imaging, if the spleen is present
   b. Absolute monocyte count < 1000/µL
   c. Absence of myeloid precursors (including blasts) in peripheral blood
   d. Normal HgbF for age

A small number of patients may not achieve CR at the Day +90 evaluation and may convert to full donor chimerism or meet negative cytogenetic/molecular criteria at a later point. These patients will initially be scored as a "Non-Response" however, if they are alive at Day +540 without the initiation of additional anti-JMML-directed pharmacotherapy following HCT, then their status will be changed to CR.

10.2.3 Definition of Relapse:

Post-HCT relapse can only be determined in patients who meet the criteria for clinical remission prior to or post HCT as defined above, and will require EITHER:

ALL of the following 3 features:

1. Splenomegaly on exam and/or imaging, if the spleen is present, and had previously normalized in size
2. An increase in host chimerism by ≥ 5% (from the highest achieved chimerism) in unsorted mononuclear cells of bone marrow or peripheral blood
3. Re-emergence of an elevated HgbF for age, if initially elevated at diagnosis

OR, for patients with previously identified cytogenetic or molecular abnormality, the following feature:

Re-emergence of a clonal cytogenetic abnormality or molecular abnormality in *NRAS*, *KRAS*, or *PTPN11* as measured by two sequential molecular tests (within one month of each other) if weakly positive at the first time-point followed by another positive (weak or strong) at the second sequential measurement OR a single strong positive at any time ("strong positive" definition corresponds to ≥ 15% mutant allele burden that can be verified by Sanger sequencing, "weakly positive" corresponds to 1-14.99% mutant allele burden)

Time to "relapse" will be from transplant date to the date at which all criteria have been documented, or the initiation of additional anti-JMML-directed pharmacotherapy following HCT, whichever occurs sooner.

10.2.4 Definition of Non-Response:

Post-HCT "Non-Response" is defined as those patients who never achieved CR prior to or after transplant (as measured on Day +90). Time to "Non-Response" will be from transplant date to the date at which the Day +90 bone marrow evaluation was performed, or the initiation of additional anti-JMML-directed pharmacotherapy following HCT, whichever occurs sooner.

10.3 Graft Failure following Allogeneic HCT

10.3.1 Primary Graft Failure

Failure to achieve an ANC ≥ 500/μL after 42 days, determined by three consecutive measurements on different days; or < 5% donor cells in blood or bone marrow by Day +42 (as demonstrated by a chimerism assay), without evidence of JMML.

10.3.2 Secondary Graft Failure:

Initial engraftment followed by severe neutropenia (ANC ≤ 500/μL) that is not caused by recurrent leukemia, or < 5% donor cells in the blood or bone marrow (as demonstrated by a chimerism assay) without subsequent improvement occurring either spontaneously or after growth factor treatment. Improvement is defined as an ANC > 500/μL consistently. Severe neutropenia with bone marrow cellularity ≥ 25% is not secondary graft failure.

Marrow, UCB or peripheral blood stem cell reinfusion carried out any time after Day 0 because of inadequate hematopoietic function will be taken as a definitive indication of graft failure regardless of ANC values and marrow cellularity. Donor lymphocyte infusion is not considered a stem cell reinfusion.

10.4 Definition of Engraftment

Neutrophil engraftment is defined as the first of three consecutive days in which the absolute neutrophil count exceeded 500 per cubic millimeter. Platelet engraftment is defined as the first of seven consecutive days in which the platelet count exceeded 20,000 per cubic millimeter without platelet transfusions. The presence of donor cells is demonstrated by the detection of informative variable-number tandem-repeat

polymorphisms or by fluorescent in situ hybridization with a Y-chromosome–specific probe in cases of sex-mismatched transplants.

10.5 Evaluation and Diagnosis of GVHD

10.5.1 Evaluation and Diagnosis of Acute GVHD

Review of systems and physical examinations should be performed daily, while in hospital, and weekly thereafter to screen for signs and symptoms of acute graft versus host disease (GVHD). Acute GVHD generally develops within the first three months after transplantation and appears as a characteristic dermatitis often accompanied by hepatitis, cholestasis and enteritis. The onset of acute GVHD usually heralds itself as a pruritic macular exanthem of the palms and soles with morbilliform lesions on the extremities, trunk, and face. With progression, the lesions become confluent, leading to generalized erythroderma and bulla formation complicated by bacterial superinfection and exfoliation. The clinical appearance of skin GVHD can be mimicked by toxicity of the hematopoietic cell transplant conditioning regimen and by drug reactions. Therefore, documentation of the diagnosis by skin biopsy is recommended. Severity of liver GVHD is usually described according to the serum bilirubin level. Hepatic GVHD cannot be assessed solely on clinical grounds in patients who have concurrent drug toxicity, viral hepatitis, or toxicity caused by pre-transplant chemotherapy irradiation. Liver biopsy can be helpful but often cannot be done because of clinical contraindications, such as thrombocytopenia or coagulopathy. Gastrointestinal GVHD is indicated by watery diarrhea and anorexia, nausea and vomiting accompanied by abdominal cramps, gastrointestinal hemorrhage, and ileus. Eating often exacerbates symptoms. The volume of diarrhea has been used as an indicator of the severity of gut GVHD, but this can be inaccurate and highly variable from day to day. In many cases, it can be difficult to distinguish GVHD from infectious enteritis, and endoscopic biopsy is often helpful and should be done whenever possible.

ORGAN STAGING OF GVHD

Stage	Skin	Liver (bilirubin)	Gut (stool output/day)
0	No GVHD rash	< 2 mg/dL (< 34 µmol/L)	Adult: < 500 mL/day Child: < 10 mL/kg/day
1	Maculopapular rash < 25% BSA	2–3 mg/dL (34.1–51 µmol/L)	Adult: 500–999 mL/day Child: 10–19.9 mL/kg/day Or persistent nausea, vomiting, or anorexia, with a positive EGD biopsy.
2	Maculopapular rash 25–50% BSA	3.1–6 mg/dL (51–102 µmol/L)	Adult: 1000–1500 mL/day Child: 20–30 mL/kg/day
3	Maculopapular rash > 50% BSA	6.1–15 mg/dL (102.1–255 µmol/L)	Adult: > 1500 mL/day Child: > 30 mL/kg/day
4	Generalized erythroderma with bullous formation and desquamation > 5% BSA	> 15 mg/dL (> 255 µmol/L)	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 estimated to be 50% of total stool / urine mix.
- 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 colonic 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:

Grade 0	No Stage 1-4 of any organ
Grade I	Stage 1-2 rash and no liver or gut involvement
Grade II	Stage 3 rash, or Stage 1 liver involvement, or Stage 1 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

10.5.2 Evaluation and Diagnosis of Chronic GVHD

Review of systems and physical examinations should be performed weekly to screen for signs and symptoms of chronic graft versus host disease (cGVHD). cGVHD should be described in terms of:

- Extent of skin involvement

- Liver dysfunction: AST, ALT, total bilirubin levels
- Eye involvement: results of Schirmer's test
- Involvement of salivary glands or oral mucosa
- Pulmonary involvement
- Presence or absence of joint contractures or limitation on range of movement
- other organ involvement
- Results of biopsies (skin, liver, intestine), where applicable

cGVHD should be graded according to the criteria set out by Shulman et al.[63] as follows:

Limited cGVHD
Either or both:
1.) Localized skin involvement
2.) Hepatic dysfunction due to chronic GVHD

Extensive cGVHD
Either:
1.) Generalized skin involvement, **or**
2.) Localized skin involvement and/or hepatic dysfunction due to cGVHD

Plus:
3a.) liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis, **or**
3b.) involvement of eye (Schirmer test with < 5 mm wetting), **or**
3c.) involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, **or**
3d.) involvement of any other target organ

11.0 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents.

11.2 Determination of Reporting Requirements

Reporting requirements may include the following considerations: 1) the characteristics of the adverse event including the *grade* (severity); 2) the *relationship to the study therapy* (attribution); and 3) the *prior experience* (expectedness) of the adverse event.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. In some cases an agent obtained commercially may be used for indications not included in the package label. In addition, NCI may on some occasions distribute commercial supplies for a trial. Even in these cases, the agent is still considered to be a commercial agent and the procedures described below should be followed.

Determine the prior experience Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered

unexpected, for reporting purposes only, when either the type of event or the severity of the event is not listed in:

- ***the current known toxicities for each commercial agent as provided in the Drug Information for Commercial Agents Used by the Children's Oncology Group posted on the COG website; or***
- ***the drug package insert.***

Secondary Malignancy

A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (eg, treatment with investigational agent/intervention, radiation or chemotherapy). A metastasis of the initial neoplasm is not considered a secondary malignancy.

All secondary malignancies that occur following treatment need to be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy
- Myelodysplastic syndrome
- Treatment related secondary malignancy

11.3 Reporting of Adverse Events for Commercial Agents – via CTEP-AERS

Expedited AE reporting must use CTEP-AERS (Adverse Event Expedited Reporting System), accessed via htt;s://eapps-ctep.nci.nih.gov/ctepaers

Commercial reporting requirements are provided in Table B. The commercial agent(s) used in this study are listed in the front of this protocol immediately following the Study Committee roster.

- COG requires the CTEP-AERS report to be submitted **within 7 calendar days** of learning of the event.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

CTCAE term (AE description) and grade: The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting and are located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. All appropriate treatment areas should have access to a copy of the CTCAE.

Table B

Reporting requirements for adverse events experienced by patients on study who have NOT received any doses of an investigational agent on this study.

CTEP-AERS Reporting Requirements for Adverse Events That Occur During Therapy with a Commercial Agent or Within 30 Days[1]

Attribution	Grade 4		Grade 5
	Unexpected	Expected	
Unrelated or Unlikely			CTEP-AERS
Possible, Probable, Definite	CTEP-AERS		CTEP-AERS

[1]This includes all deaths within 30 days of the last dose of treatment with a commercial agent, regardless of attribution. Any death that occurs more than 30 days after the last dose of treatment with a commercial agent which can be attributed (possibly, probably, or definitely) to the agent and is not due to cancer recurrence must be reported via CTEP-AERS.

11.4 Routine Adverse Event Reporting

Note: The guidelines below are for routine reporting of study specific adverse events on the COG case report forms and do not affect the requirements for CTEP-AERS reporting.

The NCI defines both routine and expedited AE reporting. Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database. For this study, routine reporting will include all toxicities reported via CTEP-AERS and all Grade 3 and higher non-hematological Adverse Events. In addition, routine reporting will include unacceptable (severe) SOS as defined in Section 9.3.3.

12.0 STUDY REPORTING AND MONITORING

The Case Report Forms and the submission schedule are posted on the COG web site with each protocol under "*Data Collection/Specimens*". A submission schedule is included.

12.1 CDUS

This study will be monitored by the Clinical Data Update System (CDUS). Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31. This is not a responsibility of institutions participating in this trial.

12.2 Data and Safety Monitoring Committee

To protect the interests of patients and the scientific integrity for all clinical trial research by the Children's Oncology Group, the COG Data and Safety Monitoring Committee (DSMC) reviews reports of interim analyses of study toxicity and outcomes prepared by the study statistician, in conjunction with the study chair's report. The DSMC may recommend the study be modified or terminated based on these analyses.

Toxicity monitoring is also the responsibility of the study committee and any unexpected frequency of serious events on the trial are to be brought to the attention of the DSMC. The study statistician is responsible for the monitoring of the interim results and is

expected to request DSMC review of any protocol issues s/he feels require special review. Any COG member may bring specific study concerns to the attention of the DSMC.

The DSMC approves major study modifications proposed by the study committee prior to implementation (eg, termination, dropping an arm based on toxicity results or other trials reported, increasing target sample size, etc.). The DSMC determines whether and to whom outcome results may be released prior to the release of study results at the time specified in the protocol document.

13.0 CYTOGENETICS ANALYSIS GUIDELINES AND REQUIREMENTS

13.1 Cytogenetic Analysis Overview

It is required that specimens be sent for cytogenetic analysis at the time of initial diagnosis and at relapse (if applicable). These require submission of karyotypes, any applicable FISH images and corresponding forms for central review. Additionally specimens should be sent for cytogenetic analysis pre-HCT if there was a delay of 30 days or more between initial diagnosis and HCT, or if the patient received any pre-HCT chemotherapy. Full central review of karyotypes, FISH images and forms from pre-HCT studies is required ONLY for those cases in which there is a change in cytogenetic status from the initial diagnostic study. If pre-HCT cytogenetic studies reveal the same results as those of initial diagnosis, only the forms (G-banding and FISH) need to be submitted. Day +30 and Day +90 (following HCT infusion date) must be submitted for those patients who had an abnormal clone identified by cytogenetics at initial diagnosis. Cytogenetic studies must be performed by a COG-approved laboratory.

The institutional CRA must inform the cytogenetics laboratory that the patient is anticipated to enroll in a COG JMML study and that the cytogenetics/FISH data must be submitted within 2 weeks after enrollment on the ASCT1221 protocol.

13.2 Local Cytogenetic Analysis and Central Review Submission Guidelines

13.2.1 Specimen Collection for Local Cytogenetics Analysis

A minimum of 2 mL (optimal volume, 3 mL) of fresh whole bone marrow aspirated through a needle into a syringe containing sodium heparin (preservative-free is preferable) is recommended in all cases. A first or second draw, or a draw from a repositioned needle, is best to ensure a sufficient number of leukemic cells in the aspirate. The specimens should be kept at **room temperature** and transported to the institutional cytogenetics laboratory as quickly as possible (**always within 24 hours of collection**). It is critical for the laboratory to know that the specimen is for a COG registered patient, the registration number of the patient, and the COG study on which the patient is enrolled.

If bone marrow cannot be aspirated, a core biopsy, or peripheral blood can be submitted. These are much less favorable for diagnosis of a clone than is a bone marrow aspirate, and should only be sent in exceptional situations.

13.2.2 Data Submission for Central Cytogenetics Review at Diagnosis and Relapse

Submit the following for central review following completion of local cytogenetic studies:

- The COG G-banding Reporting Form along with two G-banded karyotypes (and their associated metaphase spreads) for each clone identified.
- The COG FISH Reporting Form along with images of two cells demonstrating any abnormal FISH pattern observed. This protocol requires only a single FISH assay: a dual probe set (for example, CEP7, D7S486) for monosomy 7 that has been validated by the Clinical Laboratory. The probe set must include a probe to the centromere of chromosome 7 (CEP 7).

In addition, BCR/ABL1 testing is required, either by PCR or FISH. If PCR has not been performed, then the cytogenetics laboratory is required to perform FISH testing utilizing a probe set validated in the clinical laboratory.

***NOTE**: Some laboratories will perform additional FISH testing as part of the diagnostic work-up. All FISH testing should be recorded on the COG FISH Reporting Form. Only abnormal FISH results need be documented with images for review.

Use the COG Microarray Reporting Form for results of genomic microarray (SNP or array CGH).

*** NOTE**: This protocol does NOT require microarray testing by the local laboratory. However, if such testing was performed as part of the diagnostic work-up, results should be summarized on the Microarray Reporting Form and submitted for central review.

13.2.3 Data Submission for Central Cytogenetics Review

Pre-HCT cytogenetics is performed locally if: 1) the patient received pre-enrollment chemotherapy, or 2) there was a delay between initial diagnosis and HCT of ≥ 30 days. Full central review of karyotypes, FISH images and forms from pre-HCT studies is required ONLY for those cases in which there is a change in cytogenetic status from the initial diagnostic study. If pre-HCT cytogenetic studies reveal the same results as those of initial diagnosis, only the forms (G-banding and FISH) need to be submitted.

For patients with an abnormal cytogenetics clone at initial diagnosis (or pre-HCT as described above), Day +30 and +90 samples must be submitted for local cytogenetics analysis. Central review materials must be submitted if the abnormal clone is shown to have evolved.

13.2.4 Central Cytogenetics Review Submission Guidelines

Label all documents with the patient's COG ID number and the protocol ID number.

Please send above materials by e-mail (preferably as a PowerPoint file) to one of the following COG Cytogenetics Reviewers:

WEST OF MISSISSIPPI RIVER	EAST OF MISSISSIPPI RIVER
(Include Minnesota and Wisconsin), Australia, New Zealand, Western Canada	(Exclude Minnesota and Wisconsin), Europe, Eastern Canada
SEND TO: **Betsy Hirsch, PhD**	SEND TO: **Susana C. Raimondi, PhD, FACMG**
Telephone: (612) 273-4952/3171	Telephone: (901) 595-3537/3536
E-mail: hirsc003@umn.edu	E-mail: susana.raimondi@stjude.org

14.0 PATHOLOGY GUIDELINES AND SPECIMEN REQUIREMENTS

This study requires a peripheral blood and bone marrow specimen to be submitted to your local institution to make the diagnosis of JMML. See Section 14.1 for central pathology review submission requirements.

14.1 Central Pathology Review Submission Guidelines

Central review specimens must be submitted at study entry, Day +30, Day +90 and at relapse (if applicable). Diagnostic specimens should be submitted within 2 weeks of enrollment. Other timepoints should be submitted within 2 weeks of the procedure.

Minimum specimen requirements for central review are as follows:

1. Stained Slides (cover-slipped):
   - 2 well-stained representative bone marrow aspirate smears
   - 1 well-stained peripheral blood smear
   - 1 H&E stained clot section and/or core biopsy (if performed)
2. Unstained Slides:
   - 2 BMA smears
   - 4 recuts from clot section and/or core biopsy (if performed)
3. Other H&E stained and unstained surgical specimen slides (e.g., spleen, others) as applicable.
4. Copies of the following documents:
   A. local institution's pathology reports
   B. peripheral blood counts (preferably the most recent)
   C. hemoglobin F level (from a pre-transfusion sample, if the patient was blood transfused)
   D. cytogenetics/molecular genetics studies
   E. immunophenotyping by flow cytometry report
   F. Copy of GM-CSF hypersensitivity report (if performed)
   G. Completed COG Specimen Transmittal Form

Please label all materials (slides and reports) for central review with the patient's surgical pathology identification number (SPID) and block number found on the corresponding report and the COG patient identification number.

The above material along with contact information of contributing institutions should be forwarded to the Biopathology Center for central review by mail or using your institutional courier account.

Biopathology Center – ASCT1221
Nationwide Children's Hospital
700 Children's Drive, WA1340
Columbus, OH 43205
Phone: (614) 722-2865
Email:BPCParaffinTeam@nationwidechildrens.org

The BPC will send central pathology review materials to the study pathologist (Dr Samir Kahwash).

15.0 SPECIAL STUDIES SPECIMEN REQUIREMENTS

Specimens for mutation analysis (genotyping) should be obtained at study enrollment. The result of the mutation analysis will be used to support the diagnosis of JMML and in the case of a positive *PTPN11* mutation, will serve as a variable for blocked randomization, in conjunction with donor type.

15.1 NIH-Funded Laboratory for Mutation Analysis at Diagnosis

In order to provide uniform mutation analysis that is used for risk-based therapy, all *NRAS, KRAS, CBL,* and *PTPN11* mutation analysis will be performed as part of the protocol therapy at a single site through NIH funding by the Center of Diagnostic Excellence for Pediatric Myeloproliferative Neoplasms at the UCSF Benioff Children's Hospital in San Francisco, CA **free of charge to patients enrolled onto ASCT1221.** If samples for mutational analysis were processed at UCSF prior to patient enrollment (as fee for service), it is acceptable to use these results to fulfill the requirements in Section 4.3 (confirmation of JMML diagnosis according to Chan et al[33]).

15.2 *NRAS*, *KRAS*, *CBL*, and *PTPN11* Mutation Analysis

As previously described, diagnostic DNA samples from each patient will be used to amplify appropriate regions of the genes of interest using pyrosequencing on the Roche 454Jr platform. The 454Jr utilizes bead-based emulsion PCR target amplification coupled with pyrosequencing on a PicoTiterPlate to produce up to 35 million bases of sequencing data per run. The JMML test panel is a target-based approach, for which the 454JrJr is capable of sequencing up to 70,000 amplicons of approximately 300-400bp in length.

15.3 Minimal Residual Disease (MRD) Analysis

Specimen submission for MRD analysis is required and must be collected at the defined timepoints in Section 7.2. All patients that do not have adequate specimens submitted will be followed based on their other clinical and laboratory criteria. Qualitative results prior to and post HCT will be returned to the institution.

15.3.1 CLIA/CAP-Approved Laboratory for Mutant Allele Testing after Diagnosis

In order to provide uniform MRD analysis, qualitative MRD testing in mononuclear cells will be performed as part of the protocol therapy the Center of Diagnostic Excellence for Pediatric Myeloproliferative Neoplasms at the UCSF Benioff Children's Hospital in San Francisco, CA. MRD analysis will be performed at the timepoints listed in Section 7.2 **free of charge to the patient.** Investigators are encouraged to call the laboratory (415 514-8488).

15.3.2 Description of MRD Analysis

All post remission specimens will undergo the identical genotype screening as performed at initial diagnosis based on the genotype identified. Reporting out of weakly positive and strongly positive qualitative results will only be disclosed. Weakly positive are those results on the 454Jr sequencing platform that correspond to 1-14.99% mutant allele while strongly positive correspond to > 15% mutant allele. Note, due to the germline nature of CBL mutations, the interpretation of such results is complex enough that these are not currently factored into the response and relapse criteria; however studying these patients will be critical for future trials.

15.4 Specimen Collection Overview

This section describes the sample submission requirements for the genetic characterization of JMML cells in the UCSF CLIA/CAP-approved laboratory as well as other correlative research studies.

Table 9: Study Specimens at Enrollment

The specimens described below are to be submitted at the time of enrollment.

Specimen	***Required*: UCSF Sample Submissions**		***Optional*: Banking[2]**
	CLIA-Certified Laboratory (genotyping)[1]	**Loh Laboratory[2]**	**COG Leukemia Reference Lab[3]**
Peripheral blood	3 mL purple top	3-5 mL green top[5]	3-5 mL purple top[5]
Bone marrow aspirate	3 mL green top	3-5 mL green top[5]	5 mL purple top[5]
Buccal swab or Oragene saliva kit	X[6]		
Skin biopsy	X[4]		

[1] Label with patient name, COG ID, Date of Birth, type of specimen (BMA or PB), draw date and time. Samples without a name will be discarded.

[2] Label with BPC number, COG ID, collection date and time, and type of specimen (BMA or PB).

[3] Optional banking specimens will be submitted at diagnosis with appropriate consent. Label with the COG ID, BPC number, collection date and time, and type of specimen (BMA or PB). When submitting bone marrow also state collection site (L or R).

[4] A skin biopsy will be requested only if the buccal swab or saliva DNA is either inadequate or demonstrates an unexpected suspected germline lesion.

[5] Volume of blood or bone marrow may need to be adjusted based on the appropriate weight-based volumes described in Section Section 15.5.

[6] Optional parental buccal swabs to be submitted at study entry with appropriate consent. Label with Mother/Father (as applicable) of [*patient name*], COG ID, Date of Birth and type of specimen.

Table 10: Study Specimens pre- and post-HCT

Specimen	***Required*: UCSF Sample Submissions**	
	CLIA-Certified Laboratory (genotyping) [1, 3]	**Loh Laboratory[2, 3]**
Peripheral blood	3 mL purple top	5 mL green top[4]
Bone marrow aspirate	3 mL green top	5 mL green top[4]

[1] Label with patient name, COG ID, Date of Birth, type of specimen (BMA or PB), draw date and time. Samples without a name will be discarded.

[2] Label with BPC number, COG ID, collection date and time, and type of specimen (BMA or PB).

[3] Required within 2 weeks prior to HCT and at defined timepoints in Section 7.2.

[4] Volume of blood or bone marrow may need to be adjusted based on the appropriate weight-based volumes described in Section 15.5.

15.5 Specimen Details

The total weight-based volume of blood and bone marrow to be collected at each time point is provided below.

Peripheral blood: 10-15 mL for patients weighing > 10 kg
5-10 mL for patients weighing ≤ 10 kg

Collected in tubes indicated in Tables 9 and 10 above. Mix well.

Bone marrow: 10 mL for patients weighing > 10 kg
5 mL for patients weighing ≤ 10 kg

Collected in tubes indicated in Tables 9 and 10 above. Mix well.

Buccal swab: Use swab from local institutional genetics service or obtain from the CLIA-approved Diagnostic Laboratory at UCSF (call 415-514-8488 to request a kit). Gently but firmly swab the inner buccal mucosa. Submit 2 swabs (at the time of enrollment only). The buccal swab will allow somatic versus germline mutations to be distinguished in some cases of JMML or NS/MPN.

Oragene saliva kit: Use saliva kit if child is deemed mature enough to collect enough saliva (obtained from the CLIA-approved Diagnostic Laboratory at UCSF; call 415-514-8488 to request a kit).

Skin biopsy A skin biopsy will be requested only if the buccal swab or saliva DNA is either inadequate or demonstrates an unexpected germline lesion. At the time of bone marrow aspirate/biopsy, a small skin sample from the exact spot that the bone marrow aspirate or biopsy needle is inserted is encouraged to be submitted. Place sample in a tube containing RPMI with fetal calf serum. If unavailable, the Loh laboratory will provide the media upon request.

The following samples are optional and should be submitted at the time of enrollment, if patient has consented to these studies:

Additional Specimens for Patients with Extramedullary Involvement (optional):
These samples are optional and should be submitted at any point during study participation, if the patient has consented to submission of these materials:

Skin rash biopsy If a skin rash is biopsied, a sample is encouraged to be submitted.

Spleen specimen If a splenectomy is performed, investigators are strongly encouraged to submit the fresh specimen to the Loh laboratory. Please contact the Loh laboratory up to one week prior to surgery to allow adequate time to prepare to receive the specimen.

15.6 Specimen Shipping Details

15.6.1 Shipments to the University of California, San Francisco
Specimens for genotyping at the CLIA-certified laboratory and specimens for correlative biology studies at the Loh laboratory should be batched for shipment. Specimens must be shipped on the day of collection. Do not ship fresh specimens on Friday or during weekends or holidays. Shipments should arrive at the laboratory between Tuesday and Friday.

Place samples in a sterile inner container and sealed in a light-tight outer container. Shipment in small Styrofoam containers is preferred, to insulate samples from extreme temperature changes. However, shipment in standard biological specimen envelopes (with inner padding) is a suitable alternative.

All specimens should be shipped at room temperature or cooled with a cold pack (not frozen) via Federal Express PRIORITY OVERNIGHT using the COG Federal Express Account number (https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf). Include a COG specimen transmittal form.

Loh laboratory
HSE 302
513 Parnassus Ave
San Francisco, CA 94143

Notify the Loh Lab: (415) 514-9389 of the time of specimen shipment to prepare to receive research samples.

Leftover specimens will be sent to the Biopathology Center (BPC) if the patient has consented to specimen banking.

15.6.2 Shipments to the COG Leukemia Reference Laboratory

An optional bone marrow and peripheral blood specimen will be collected at the time of enrollment for specimen banking, if patient consent if obtained. Specimens should be shipped at room temperature or cooled with a cold pack (not frozen) priority overnight using the COG courier account number (https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf). Include a COG specimen transmittal form with each shipment.

Specimens should be sent to the following address:

COG Leukemia Reference Laboratory
Nationwide Children's Hospital
Protocol ASCT1221
700 Children's Drive, C1961
Columbus, OH 43205

Phone: (614) 722-2866
Fax: (614) 722-2887
Email: MGLab@nationwidechildrens.org

15.7 Description of Correlative Biology Studies

While correlative biology studies will develop over the course of the clinical trial, a brief discussion of some of the intended studies are presented but will be contingent upon successful acquisition of independent funding. Importantly, the UCSF CLIA laboratory will remove any name, DOB, or COG number prior to distributing the research samples to the appropriate laboratories. A unique specimen identifier (USI) generated by COG will be assigned to the sample prior to delivery to the appropriate reference lab. In the Loh laboratory, aliquots of cells will have DNA and RNA selected from whole mononuclear and sorted cell subsets. In addition, one aliquot of cells will have

phosphoflow cytometry performed in increasing concentrations of GM-CSF[53] and potentially exposed to selected drugs for assessment of novel therapies that may be used in the future.

RNA from selected cell subsets will be archived for future gene expression studies for those patients participating in the HCT randomization. DNA from selected and unselected cell subsets will be used for the assessment of mutant allele burden using either pyrosequencing methodologies or allele specific PCR.[51] At the current time CD3, CD14/15, and CD34 selection is planned for post HCT research studies. In addition, DNA from diagnosis will also be archived and assessed for genome wide methylation studies in those patients participating on the randomized arm. Together, the gene expression and methylation assays will be used to define classifiers of relapse, which will be a critical step forward in advancing care for children with JMML. DNA from selected cell subsets may also be subjected to additional genotyping for novel genes discovered to be mutated in JMML. Finally, such strategies including genome wide association studies may be performed on specific cell subsets or buccal or fibroblast DNA. In addition to the aforementioned studies, other strategies to maximize the use of this precious human tissue may be employed in order to gain additional reagents that will ultimately be used to improve outcome. Such strategies include, but are not limited to, creation of cell lines or induced pluripotent cells that will be used for additional genetic and biochemical studies.

16.0 PHARMACOKINETICS FOR BUSULFAN DOSE ADJUSTMENT

16.1 1st Dose Pharmacokinetics

Please note: Pharmacokinetics studies must be performed in a CLIA or NATA certified Laboratory.

Target first dose pharmacokinetics should be performed in all patients regardless of busulfan dosing schedule.

Once daily dosing should target an area under the curve (AUC) of 3,600 to 6,000 (micromole/liter)*minute per dose.

Every 6 hour dosing should target an AUC of 900 to 1500 (micromole/liter)*minute per dose.

Every 12 hour dosing should target an AUC of 1800 to 3000 (micromole/liter)*minute per dose.

Instructions for Q24 hr dosing: Busulfan concentrations will be determined in plasma by collecting blood into a green top sodium heparin tube. Samples should be collected at the end of the first 3 hour infusion, 195 minutes after start of infusion (or end of infusion plus 15 minutes), and 4, 5, 6, and 8 hours after the start of the first infusion. Samples should not be drawn from the lumen used to infuse busulfan. See table in Section 7.3.

Institutional pharmacokinetic studies are acceptable and sample submission requirements per the local laboratory should be followed. If pharmacokinetics cannot be performed at a local laboratory, sites may opt to use the Seattle Cancer Care Alliance laboratory (as fee for service) or other regional laboratories experienced in this assay. Results are usually available in time for adjusting doses 3 and 4.

Instructions for Q6hr dosing: Busulfan concentrations will be determined in plasma by collecting blood into a green top sodium heparin tube. Samples should be collected at the end of the first 2 hour infusion, 135 minutes after start of infusion, 150 minutes after start of infusion, and 3, 4, 5, and 6 hours after the start of the first infusion. Samples should not be drawn from the lumen used to infuse busulfan. See table in Section 7.3.

In case infusion runs more or less than 2 hours, draw 1 sample immediately when infusion ends. Then, draw the next 2 samples 15 minutes apart and continue to draw samples at 3, 4, 5, and 6 hours samples by counting from beginning of the infusion.

Instructions for Q12 hr dosing: Busulfan concentrations will be determined in plasma by collecting blood into a green top sodium heparin tube. Samples should be collected at 1 hour following start of infusion, and then at 2 hours (at the end of the busulfan infusion), 3, 5, 6, 7, and 8 hours after the START of the infusion. Samples should not be drawn from the lumen used to infuse busulfan. See table in Section 7.3.

In case infusion runs more or less than 2 hours, draw 1 sample immediately when infusion ends and another 15 minutes later. Then, draw the next samples as scheduled at 3, 5, 6, 7, and 8 hours after the START of the infusion.

Sample collection instructions for institutions that opt to use the SCCA Pharmacokinetics Laboratory are as follows:

Collect 1-3 mL of blood into sodium heparin tubes (green top) according to the schedule above. Place labeled samples immediately on wet ice and refrigerate. Centrifuge samples as soon as possible at 4°C. Separate plasma from RBCs. Store plasma at –20°C. Plasma tubes must be labeled with the patient's name, medical records number, and the date and actual clock time that the sample was drawn. If using the SCCA Pharmacokinetics Laboratory, sample requisition form, collection schedule, and instructions can also be found at: http://www.seattlecca.org/busulfan-lab-samples.cfm. Sites will be required to set up an institutional account with the SCCA PK laboratory for billing.

16.2 Shipping

For institutions that opt to use the SCCA Pharmacokinetics Laboratory, samples must be shipped on a minimum of 3 kg of dry ice the day they were drawn using an urgent overnight carrier. Please include IV Busulfex PK requisition form and ship using your institutional courier account.

Send samples to:
Pharmacokinetics Laboratory
Seattle Cancer Care Alliance
Room G7-405
825 Eastlake Ave. East
Seattle, WA 98109
Tel: (206) 288-7389
Fax: (206) 288-7397
Email: pklab@seattlecca.org

Note: Notify the lab staff of sample shipment at least 48 hours prior to arrival. Samples can be received Tuesday through Friday, or on Saturdays with at least 3 days advance notice. Coordinate the taking of samples so that delivery will occur Tuesday through Saturday and there will be time for a possible dose adjustment. Thus, the dose must be administered to the patient Monday through Friday. Please provide the lab with a tracking number for the package at the email (preferred) or phone listed above.

Provide fax and phone numbers for the attending physician or pharmacist so that results can be verbally reported and faxed in time for targeted dose adjustment.

16.3 Guidelines for Adjusting Busulfan Dosing Based on Results of First Dose Pharmacokinetic Results

Once the results of the area under the curve (AUC) analysis of the first busulfan dose are available, subsequent doses will be adjusted to achieve an overall exposure target AUC of 3600-6000 (micromole/liter)*minute (or as listed in Section 16.1 if using q6 hour or q12 hour dosing). The amount by which the dose is increased or decreased should be decided by the patient's attending physician in conjunction with the institutional toxicology laboratory director, or the Seattle Cancer Care Alliance Pharmacokinetics Laboratory (see above).

In cases where the pharmacokinetics are performed locally, a second AUC analysis should be performed after administration of the first modified dose, and further dose adjustments made accordingly, whenever feasible.

APPENDIX I: JMML GENE MUTATIONS

List of reported mutations in *CBL/KRAS/NRAS/PTPN11* for JMML patients

gene	chr	exon	cDNA coordinates	nt change	AA change	notes/het or hom
CBL	11	7	c. 1096-1G>C	1096-1G>C	splice site variant	Homo
CBL	11	8	c. 1096-1G>C	1096-1G>C	splice site variant	Homo
CBL	11	8	c. 1106_1171del	1106 del	splice site variant	Homo
CBL	11	8	c. 1111T>C	1111T>C	Y371H	Homo
CBL	11	8	c. 1111T>C	1111T>C	Y371N	Homo
CBL	11	8	c. 1111T>G	1111T>G	Y371D	Homo
CBL	11	8	c. 1112A>G	1112A>G	Y371C	Homo
CBL	11	8	c. 1112A>C	1112A>C	Y371S	Homo
CBL	11	8	c. 1139T>C	1139T>C	L380P	Homo
CBL	11	8	c. 1141T>C	1141T>C	C381R	Homo
CBL	11	8	c. 1150T>C	1150T>C	C384R	Homo
CBL	11	8	c. 1150T>G	1150T>G	C384G	Homo
CBL	11	8	c. 1186T>C	1186T>C	C396R	Homo
CBL	11	8	c. 1190_1199del	1190 del 99	deletion	Homo
CBL	11	8	c. 1202G>C	1202G>C	C401S	homo
CBL	11	8	c. 1210T>C	1210T>C	C404R	Homo
CBL	11	8	c. 1222T>C	1222T>C	W408R	Homo
CBL	11	8	c. 1227+4C>T	1227+4C>T	splite site variant	Homo
CBL	11	8	c. 1228-2A>G	1228-2A>G	splice site variant	Homo
CBL	11	9	c. 1244G>T	1244G>T	G415V	Homo
CBL	11	9	c. 1254C>G	1254C>G	F418L	Homo
PTPN11	12	3	c. 155C>G	155C>G	T52S	hetero
PTPN11	12	3	c. 178G>C	178G>C	G60R	hetero
PTPN11	12	3	c. 179G>T	179G>T	G60V	hetero
PTPN11	12	3	c. 179_181delGTG	179delGTG	del60G	hetero
PTPN11	12	3	c. 181G>T	181G>T	D61Y	hetero
PTPN11	12	3	c. 181G>A	181G>A	D61N	hetero
PTPN11	12	3	c. 182A>T	182A>T	D61V	hetero
PTPN11	12	3	c. 182A>G	182A>G	D61G	hetero
PTPN11	12	3	c. 205G>A	205G>A	E69K	hetero
PTPN11	12	3	c. 214G>A	214G>A	A72T	hetero
PTPN11	12	3	c. 215C>T	215C>T	A72V	hetero
PTPN11	12	3	c. 215C>G	215C>G	A72G	hetero
PTPN11	12	3	c. 218C>T	218C>T	T73I	hetero
PTPN11	12	3	c. 226G>A	226G>A	E76K	hetero
PTPN11	12	3	c. 226G>C	226G>C	E76Q	hetero
PTPN11	12	3	c. 227A>T	227A>T	E76V	hetero
PTPN11	12	3	c. 227A>G	227A>G	E76G	hetero
PTPN11	12	3	c. 227A>C	227A>C	E76A	hetero
PTPN11	12	3	c. 226_227delGAinsAT	226-227GA>AT	E76M	hetero
PTPN11	12	4	c. 417G>C	417G>C	E139D	hetero
PTPN11	12	13	c. 1505C>T	1505C>T	S502L	hetero
PTPN11	12	13	c. 1508G>C	1508G>C	G503A	hetero

PTPN11	12	13	c. 1508G>T	1508G>T	G503V	hetero
PTPN11	12	13	c. 1508G>A	1508G>A	G503E	hetero
PTPN11	12	13	c. 1517A>C	1517A>C	Q506P	hetero
KRAS	12	1	c. 34G>A	34G>A	G12S	hetero
KRAS	12	1	c. 34G>T	34G>T	G12C	hetero
KRAS	12	1	c. 34G>C	34G>C	G12R	
KRAS	12	1	c. 35G>A	35G>A	G12D	hetero
KRAS	12	1	c. 35G>T	35G>T	G12V	hetero
KRAS	12	1	c. 37G>T	37G>T	G13C	hetero
KRAS	12	1	c. 38G>A	38G>A	G13D	hetero
KRAS	12	2	c. 90C>T	90C>T	V34L	synonmous SNP
KRAS	12	2	c. 112-12C>T	112-12C>T (intronic)		
KRAS	12	2	c. 182A>T	182A>T	Q61L	hetero
KRAS	12	2	c. 182A>C	182A>C	Q61P	hetero
NRAS	1	1	c. 34G>T	34G>T	G12C	hetero
NRAS	1	1	c. 34G>A	34G>A	G12S	hetero
NRAS	1	1	c. 35G>A	35G>A	G12D	hetero
NRAS	1	1	c. 35G>T	35G>T	G12V	hetero
NRAS	1	1	c. 35G>C	35G>C	G12A	hetero
NRAS	1	1	c. 36G>C	36G>C	G13R	hetero
NRAS	1	1	c. 37G>T	37G>T	G13C	hetero
NRAS	1	1	c. 38G>A	38G>A	G13D	hetero
NRAS	1	2	c. 181C>A	181C>A	Q61K	hetero
NRAS	1	2	c. 181A>T	181A>T	Q61L	hetero
NRAS	1	2	c. 182A>G	182A>G	Q61R	hetero
NRAS	1	2	c. 183A>C	183A>C	Q61H	hetero
NRAS	1	2	c. 183A>T	183A>T	Q61H	hetero
NRAS	1	2	c. 183A>G	183A>G	Q61P	hetero

APPENDIX II: WHO CRITERIA FOR NEUROFIBROMITOSIS-1

The World Health Organization (WHO) defines neruofibromitosis-1 (NF-1) as two or more of the following:[64]

1. Six or more café-au-lait spots 1.5 cm or larger in post-pubertal individuals, 0.5 cm or larger in pre-pubertal individuals
2. Two or more neurofibromas of any type or one or more plexiform neurofibroma
3. Freckling in the axilla or groin
4. Optic glioma (tumor of the optic pathway)
5. Two or more Lisch nodules (benign iris hamartomas)
6. A distinctive bony lesion: dysplasia of the sphenoid bone or dysplasia or thinning of long bone cortex
7. A first-degree relative with NF1

APPENDIX IIIA: DRUGS KNOWN TO MODULATE THE ACTIVITY OF CYP450 ISOENZYMES 3A4, CYP2C19 AND CYP1A2

Note: The use of the agents listed below should be limited due to concerns of drug interactions.
Avoid using these medications if possible. Every attempt should be made to switch from the medications marked with an asterisk (*) to an acceptable alternative. Anticonvulsants that induce CYP3A4/5 are not allowed (**). This may not be a complete list; refer to http://medicine.iupui.edu/clinpharm/ddis/ClinicalTable.aspx for additional information.

	CYP 3A4 Inhibitors	CYP3A4 Inducers	CYP 2C19 Inhibitors	CYP 2C19 Inducers	CYP 1A2 Inhibitors	CYP 1A2 Inducers
amiodarone*	X				X	
aprepitant	X					
barbiturates		X				
carbamazepine**		X				
chloramphenicol	X					
cimetidine*	X		X		X	
ciprofloxacin					X	
clarithromycin*	X					
diethylthiocarbamate	X					
diltiazem	X					
erythromycin*	X					
felbamate**		X				
fluconazole	X					
fluoroquinolones					X	
fluoxetine*			X			
fluvoxamine*	X		X		X	
gestodene	X					
grapefruit juice*	X					
Imatinib	X					
itraconazole*	X					
ketoconazole*	X		X			
lansoprazole			X			
mibefradil*	X					
mifepristone	X					
modafinil		X				
nefazodone*	X					
norfloxacin	X					
norfluoxetine	X					
omeprazole			X			
oxcarbazepine**		X	X			
pantoprazole			X			
pioglitazone		X				
phenobarbital**		X				
phenytoin**		X				
posaconazole*	X					
primidone**		X				
rifampin*		X				
star fruit	X					
St. John's wort*		X				
telithromycin*		X				
ticlopidine			X		X	
tobacco						X
troglitazone*		X				
verapamil*	X					
voriconazole	X					

APPENDIX IIIB: LIST OF ANTICONVULSANTS BASED ON CYP3A4/5 ENZYME INDUCTION

Anticonvulsant drugs with little or no enzyme induction: ELIGIBLE	
Generic Name	*Trade Name*
Gabapentin	Neurontin
Lamotrigine	Lamictal
Levetiracetam	Keppra
Tigabine	Gabitril
Topiramate	Topamax
Valproic Acid	Depakote, Depakene
Zonisamide	Zonegran
Enzyme inducing anticonvulsant drugs (EIACD): <u>NOT ELIGIBLE</u>	
Generic Name	*Trade Name*
Carbamazepine	Tegretol
Felbamate	Felbatol
Phenobarbital	Phenobarbital
Phenytoin	Dilantin
Primidone	Mysoline
Oxcarbazepine	Trileptal

APPENDIX IV: PRE-TRANSPLANT RECOMMENDATIONS

Pre-transplant chemotherapy for JMML has not demonstrated any benefit upon EFS or OS. AAML0122 evaluated the efficacy of a pre-transplant window therapy with a farnesyl transferase inhibitor followed by two cycles of intensive AML-type chemotherapy. The unpublished 2-year EFS from COG AAML0122 was 40% and 2-year OS was 55%. The EWOG-MDS/EBMT JMML trial also demonstrated that no matter what type of therapy patients had prior to HCT (no therapy and low-dose chemotherapy vs. high-dose chemotherapy) the 5-year EFS (52% vs. 50%), RI (35% vs. 38%), and TRM (13% vs. 13%) were nearly identical.[4] However, it is difficult to interpret this data, given that patients with higher disease burdens were likely more intensively pre-treated. Thus, given the heterogeneity of disease burden at diagnosis in children with JMML, it is not feasible to be prescriptive about specific pre-HCT therapies.

Pre-transplant splenectomy is also controversial. The previous COG trial for JMML mandated splenectomy for all children prior to HCT. However, the EWOG-MDS/EBMT JMML trial demonstrated that a comparison of children with: 1) a spleen size < 5 cm at HCT; or 2) spleen > 5 cm at HCT; or 3) post-splenectomy at HCT, showed no statistical benefit in event-free survival (5-year EFS; 61% vs. 44% vs. 48%, respectively), relapse incidence (5-year RI, 24% vs. 45% vs. 39%, respectively), or treatment-related mortality (5-year TRM; 15% vs. 11% vs. 13%, respectively).[4] Therefore, splenectomy is not mandated prior to HCT unless clinically indicated for symptomatic relief.

There is no data to suggest that a lifetime or yearly maximum amount of fludarabine is required to safely administer without the risk of neurotoxicity, and so pre-HCT fludarabine is allowed regardless of eventual treatment assignment. Finally, there may be targeted therapies against JMML available on a clinical trial, and their use would be allowed prior to but not following HCT, unless a post-HCT relapse or progression was to occur.

The following pre-HCT guidelines are provided for physician consideration.

1. Splenectomy is not mandated but may be performed at the discretion of the clinician for control of severe hypersplenism and organomegaly.

2. For asymptomatic patients, no pre-transplant therapy is recommended.

3. For patients with very high WBC counts, pulmonary problems, and/or prominent organomegaly, oral 6-mercaptopurine (50 mg/m^2/day) +/- cis-retinoic acid (100 mg/m^2/day) is recommended.

4. For severely ill children, low-dose intravenous cytarabine (40 mg/m^2/day x 5 days) administration is recommended. If this fails to improve the patient's status, the combination of high-dose cytarabine (2 g/m^2/day x 5 days) plus fludarabine (30 mg/m^2/day x 5 days) may also be utilized.

5. Chloromas may be treated with radiotherapy per physician discretion.

Patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to start of protocol therapy (HCT conditioning). Specific prior therapy wash-out periods are detailed in Section 4.3.2.

APPENDIX V: YOUTH INFORMATION SHEETS

INFORMATION SHEET REGARDING RESEARCH STUDY
(for children from 7 through 12 years of age)

ASCT1221

1. We have been talking with you about a condition you may have called Juvenile Myelomonocytic Leukemia (JMML). JMML is a type of cancer that grows in the center of your bones. The center of your bones is called bone marrow. After doing tests, we think you may have this type of cancer.

2. We are asking you to take part in a research study because you may have JMML. A research study is when doctors work together to try out new ways to help people who are sick. In this study, we are trying to learn more about how to treat JMML.

3. Children who are part of this study will first have some tests to check that JMML is the correct condition. If the condition of JMML is confirmed, children will be treated with a stem cell transplant. The first part of a stem cell transplant involves treatment with cancer-fighting drugs. Then, the patient is given new stem cells from a healthy donor. A donor is a healthy person who gives their stem cells to a person who needs a stem cell transplant. Children on this study will be treated with one of two types of stem cell transplant. Study doctors want to see which stem cell transplant works better in children with JMML. We do not know which one will be better. That is why we are doing this study.

4. Sometimes good things can happen to people when they are in a research study. These good things are called “benefits.” A benefit to you of being part of this study may be having a transplant that works better than another kind of transplant. However, we don’t know for sure if there is any benefit of being part of this study.

5. Sometimes bad things can happen to people when they are in a research study. These bad things are called “risks.” The risks to you from this study are receiving a stem cell transplant that does not work as well or causes more side effects than another type of stem cell transplant. Other things may happen to you that we don’t yet know about.

6. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. There may be other treatments for your illness that your doctor can tell you about. Make sure to ask your doctors any questions that you have.

7. Children who are part of the study will have extra bone marrow, blood, saliva and possibly other samples collected for testing. We want to learn more about JMML and see if there are ways to tell how the cancer will respond to treatment. These samples would be taken when other standard blood and bone marrow tests are being performed, so there would be no extra procedures.

INFORMATION SHEET REGARDING RESEARCH STUDY
(for teens from 13 through 17 years of age)

ASCT1221

1. We have been talking with you about a condition you may have called Juvenile Myelomonocytic Leukemia (JMML). JMML is a type of cancer that grows in the center of your bones. The center of your bones is called bone marrow. After doing tests, we think you may have this type of cancer.

2. We are asking you to take part in a research study because you may have JMML. A research study is when doctors work together to try out new ways to help people who are sick. In this study, we are trying to learn more about how to treat JMML.

3. Children and teenagers that are part of this study will first have some tests performed to check the JMML diagnosis. If the diagnosis is confirmed, children and teenagers will be treated with a stem cell transplant. The first part of a stem cell transplant involves treatment with cancer-fighting drugs. Then, the patient is given new stem cells from a healthy donor. A donor is a healthy person who gives their stem cells to a person who needs a stem cell transplant. Children and teenagers on this study will be treated with one of two types of stem cell transplant. The stem cell transplant you get will be decided by chance, like flipping a coin for “heads” or “tails”. Study doctors want to see which stem cell transplant works better for children and teenagers with JMML. We do not know which one will be better. That is why we are doing this study.

4. Sometimes good things can happen to people when they are in a research study. These good things are called “benefits.” A benefit to you of being part of this study may be having a transplant that works better than another kind of transplant. However, we don’t know for sure if there is any benefit of being part of this study.

5. Sometimes bad things can happen to people when they are in a research study. These bad things are called “risks.” The risks to you from this study are receiving a stem cell transplant that does not work as well or causes more side effects than another type of stem cell transplant. Other things may happen to you that we don’t yet know about.

6. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. There may be other treatments for your illness that your doctor can tell you about. Make sure to ask your doctors any questions that you have.

7. Children and teenagers that are part of the study will have extra bone marrow, blood, saliva and possibly other samples collected for testing. We want to learn more about JMML and see if there are ways to tell how the cancer will respond to treatment. These samples would be taken when other standard blood and bone marrow tests are being performed, so there would be no extra procedures.

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