HO11421

Decitabine Followed by Bone Marrow Transplant and High-Dose Cyclophosphamide for the Treatment of Relapsed and Refractory Acute Myeloid Neoplasms

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PROTOCOL SYNOPSIS

Decitabine Followed by Bone Marrow Transplant and High-Dose Cyclophosphamide for the Treatment of Relapsed and Refractory Acute Myeloid Neoplasms

Principal Investigator: Mark B. Juckett, MD

Study Design: This study is a Phase II study of decitabine followed by myeloablative conditioning and transplantation of bone marrow from HLA-matched or HLA-mismatched, related donors.

Primary Objective: The primary objective is to determine overall survival at 100 days following decitabine and a bone marrow transplant using a myeloablative preparative regimen and post-transplantation cyclophosphamide.

Secondary Objectives: Patients enrolled in this study will also be followed for the following endpoints: neutrophil and platelet recovery, graft failure, acute graft-versus-host disease (GVHD), chronic GVHD, incidence of infection, treatment-related mortality, time to relapse/progression, overall survival, and progression-free survival.

Study Design: This study is a Phase II study of decitabine followed by myeloablative conditioning, transplantation of bone marrow and post-transplantation cyclophosphamide in patients with acute myelogenous leukemia, and high-risk myelodysplastic syndrome.

Accrual Objective: The target sample size is 20 patients.

Accrual Period: The estimated accrual period is two years.

Eligibility Criteria: Patients 18 - 75 years of age and with at least a partially (≥ 5 of 10) HLA-matched donor.

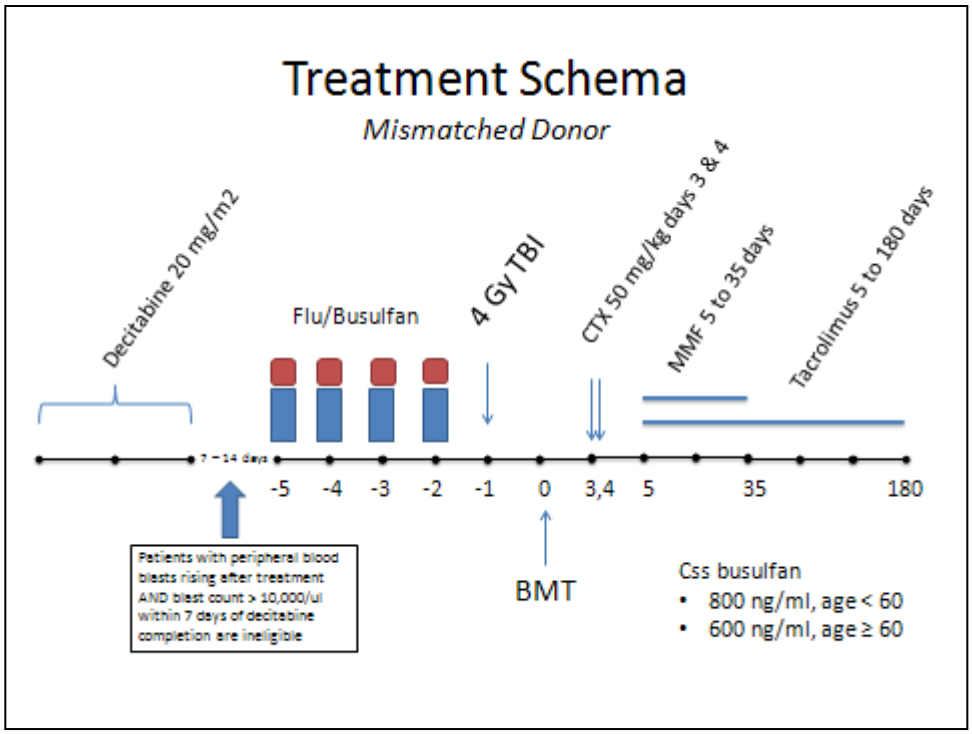
Treatment Description: The preparative regimen will consist of:

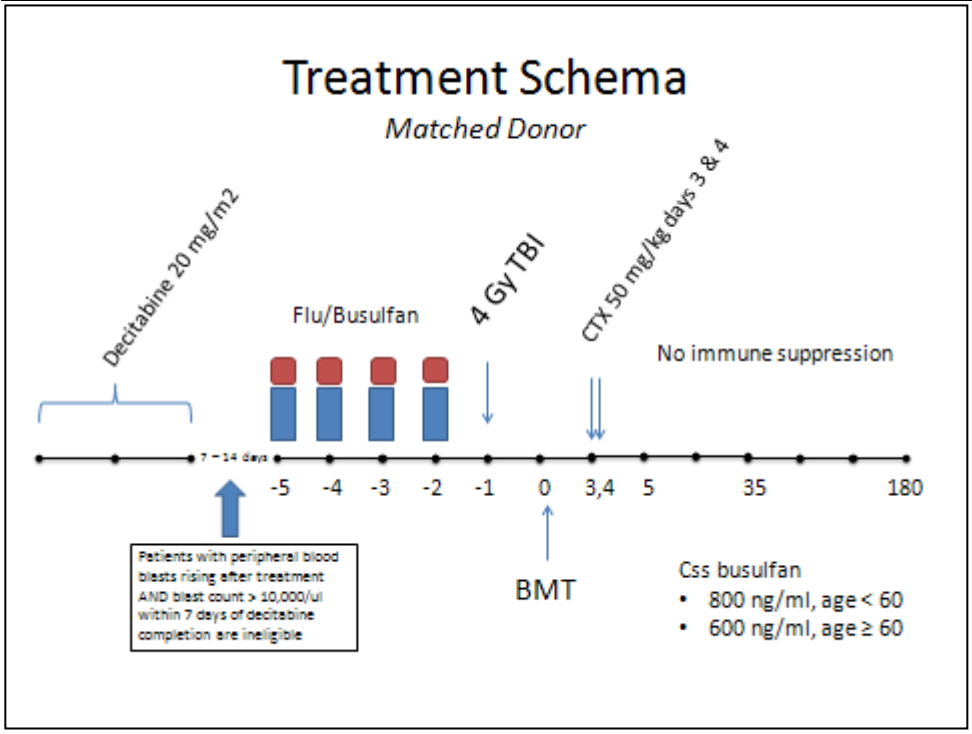
- Decitabine 20 mg/m^2 IV daily for 10 days to begin no sooner than 24 days and no later than 17 days prior to the beginning of the preparative regimen for transplant
- Fludarabine 50 mg/m^2 IV daily over 30 minutes on Days -5 to -2
- Busulfan 3.2 mg/kg IV daily over 3 hours on Days -5 to -2 with dosing modified based on pharmacokinetic modeling to achieve steady state concentration of 800 ng/mL for patients age 18 to 60

or Busulfan 2.4 mg/kg IV daily over 3 hours on Days -5 to -2 with dosing modified based on pharmacokinetic modeling to achieve steady state concentration of 600 ng/mL for patients 61 to 75.
- Total body irradiation (TBI) 4 Gy will be given Day -1 followed by infusion of a bone marrow transplant on Day 0.
- G-CSF 5 mcg/kg/day beginning Day 5 until ANC ≥ 1,000/mm3.

The GVHD prophylaxis regimen will consist of:
- Cyclophosphamide 50 mg/kg IV Days 3, 4 for all patients.
- Patients with an HLA-mismatched donor (at one or more HLA loci) will also receive:
    - Tacrolimus 0.12 mg/kg/day oral in two divided doses (or 0.04 mg/kg/day IV if unable to tolerate oral) with dose adjusted to maintain a level of 5-15 ng/mL beginning Day 5 until Day 180 and Mycophenolate mofetil (MMF) 15 mg/kg oral TID beginning Day 5 until Day 35

Study Duration: Patients will be followed for one year after transplantation.


Treatment Schema
Mismatched Donor
Decitabine 20 mg/m2
Flu/Busulfan
4 Gy TBI
CTX 50 mg/kg days 3 & 4
MMF 5 to 35 days
Tacrolimus 5 to 180 days
7 – 14 days
-5 -4 -3 -2 -1 0 3,4 5 35 180
Patients with peripheral blood blasts rising after treatment AND blast count > 10,000/ul within 7 days of decitabine completion are ineligible
BMT
Css busulfan
• 800 ng/ml, age < 60
• 600 ng/ml, age ≥ 60


Treatment Schema
Matched Donor
Decitabine 20 mg/m2
Flu/Busulfan
4 Gy TBI
CTX 50 mg/kg days 3 & 4
No immune suppression
7 – 14 days
-5 -4 -3 -2 -1 0 3,4 5 35 180
Patients with peripheral blood blasts rising after treatment AND blast count > 10,000/ul within 7 days of decitabine completion are ineligible
BMT
Css busulfan
• 800 ng/ml, age < 60
• 600 ng/ml, age ≥ 60

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1.0 INTRODUCTION

In Wisconsin, there were approximately 194 patients with a new diagnosis of acute myelogenous leukemia (AML), and 244 patients diagnosed with myelodysplastic syndromes (MDS) in 2010 (http://statecancerprofiles.cancer.gov/). The majority of these patients will die from their disease within two years primarily because of ineffective treatment. Patients with high-risk AML or MDS can only be cured with hematopoietic stem cell transplantation (HSCT), but the relapse rate remains high following HSCT, particularly in those patients who are **not in a remission** or who have overtly chemotherapy **refractory** disease prior to transplantation. In fact, most HSCT centers do not offer HSCT to patients with AML that is not in remission because of the high relapse rate and regimen-related mortality among these patients. A recent retrospective registry study examined in detail the outcome of 2,255 patients with relapsed or refractory AML who underwent HSCT (1). With a median 61 month follow-up, 3-year overall survival rates were 19% with a mortality rate at 100 days after HSCT of 39%. A multivariate analysis identified 5 adverse prognostic variables that influenced survival: length of first CR duration, circulating blasts at the time of HSCT, disease-associated cytogenetics, donor source, and performance status. Patients with no risk factors had OS at 3 years of 42% while those with 3 or more risk factors had OS rate of 6%. The primary cause of failure was relapse, which was present in 42% of deaths, often within the first 3 months after HSCT. Infection and organ failure were the second and third most common reason for early demise, which are likely reflections of prolonged neutropenia and toxicity from repeated attempts at AML control prior to transplantation. The relapses occurred early after HSCT despite the use of so called “myeloablative” preparative regimens.

We **hypothesize** that we can improve the outcome of patients with relapsed & refractory AML and high-risk MDS by using a novel strategy with the following features:

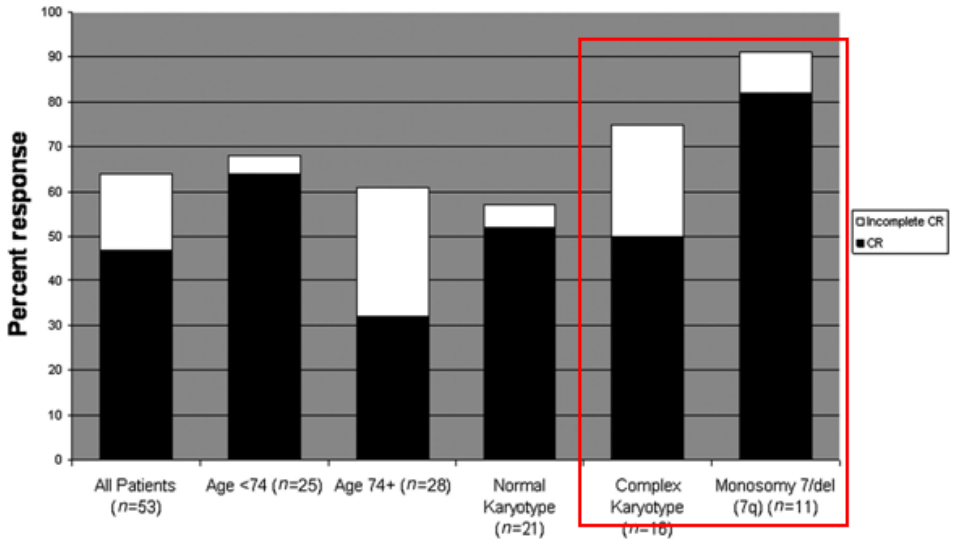
- Administering a 10 day course of decitabine immediately prior to transplantation, which as a single agent, is associated with minimal non-hematologic toxicity, and is an effective leukemia agent via a mechanism that does not depend on direct cytotoxicity
- Selecting a donor based on optimal natural-killer (NK) cell receptor expression to maximize the chance for NK-directed leukemia cell reactivity. This includes selecting partially HLA-matched related donors that may allow for KIR mismatching between donor and patient.
- Using high-dose cyclophosphamide for prophylaxis against GVHD
- Using myeloablative doses of total body irradiation (12 Gy over 8 fractions)

Pretreatment with decitabine

Decitabine is a methyltransferase inhibitor that appears to exert a therapeutic effect by epigenetic modification of suppressed genes associated with oncogenic potential. It is FDA approved to treat MDS using a cumbersome inpatient schedule that was associated with a 17% overall response rate (2). A 5-day outpatient regimen was later developed, which resulted in an overall response rate of 73%, a complete remission rate of 39% (3), and with minimal non-hematologic toxicity. A retrospective comparison suggested that older patients receiving decitabine experience a significant survival advantage compared to intensive chemotherapy (4), primarily because of the reduced toxicity associated with decitabine treatment. The overlap of clinical and pathologic features between high-risk MDS and AML favored studies of decitabine in AML, which to date, suggest that decitabine is an important new agent. In a similar 5-day schedule, Cashen, et al. demonstrated that decitabine is associated with a 24% CR rate with minimal non-hematologic toxicity in patients with AML (5). The improvement in response previously observed after a longer treatment schedule led Blum, et al. to study a 10-day schedule in patients with AML that resulted in a CR rate of 47% (6,7) (figure 1 below). The excellent activity in AML, unique mechanism of action, and minimal non-hematologic toxicity has led to our interest in studying its use prior to HSCT in patients who are not in remission prior to transplantation.

Figure 1.

Response rates for all patients and for selected subsets.

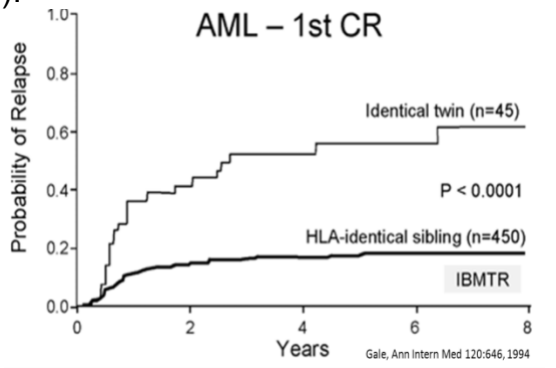


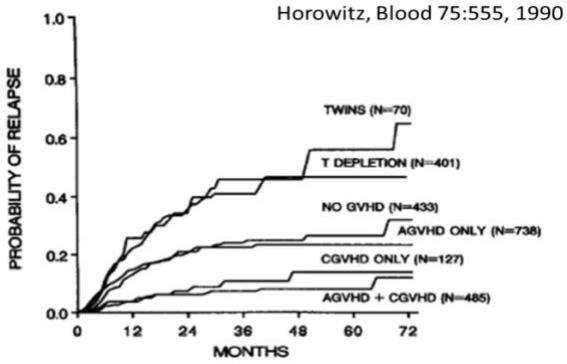
Blum W et al. PNAS 2010;107:7473-7478

PNAS

Selecting donors based on NK receptor expression

The recurrence of AML shortly after transplantation using myeloablative chemotherapy and whole-body irradiation is a common occurrence when patients are not in remission at the start of the process, and this fact is consistent with the notion that treatment of relapsed and refractory AML is unlikely to be improved by dose intensity. In fact, it appears that successful treatment of AML by HSCT is more dependent on factors other than dose-intensity. Several studies have shown that myeloablative regimens perform no better than reduced-intensity regimens in terms of overall survival following HSCT(8,8–10). Furthermore, other studies have supported the concept that successful HSCT is related to immunological factors that help support a graft-vs-leukemia reaction, and it is this process that is most responsible for long-term control of AML. One of the first examples of this phenomenon is a study demonstrating improved outcome among patients receiving allogeneic stem cells from a matched-sibling compared to an identical-twin, and improved outcome among those patients with mild chronic GVHD compared to those who had none after allogeneic HSCT(11) (below).





The immune cell responsible for a so-called “graft-vs-leukemia” (GvL) effect is not well delineated. NK cells are a subset of peripheral blood lymphocytes that are characterized by the expression of CD56 and CD16 and the absence of the T-cell receptor and CD3 (12). NK cells are cytotoxic lymphocytes that are known to lyse various tumor- and virus-infected cells without prior stimulation or immunization (13). There is an array of inhibitory and activating receptors on the surface of the NK cell and it is the complex interplay between activating and inhibitory receptors that ultimately can lead to NK cell activation or

inhibition. Perhaps the best understood NK cell receptors are the killer immunoglobulin receptors (KIRs). KIRs can be activating or inhibiting and the best characterized are those that are inhibitory in nature. KIRs recognize the MHC class I molecule on somatic cells, and this interaction inhibits the activation of the NK cell, but down regulation of MHC I that occurs, for example, after a viral infection, can lead to loss of NK inhibition and NK directed lysis of the infected cell. The absence of the cognate ligand for KIR on a target cell (e.g., tumor cell) is permissive for activation of NK cells by tumor specific antigens, which may lead to subsequent lysis of the target cell.

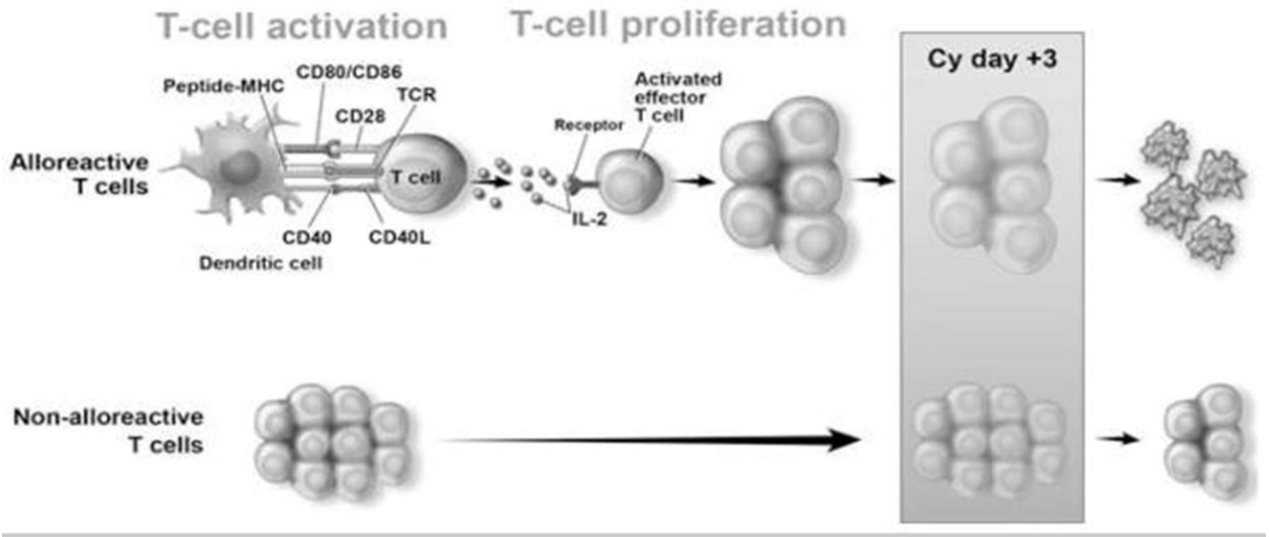
HLA genes are encoded on chromosome 6. The genes for KIR expression are encoded on chromosome 19 and segregate independently from HLA genes. Recently, several investigators have shown that allogeneic NK cells may play an important role in reducing relapse risk among patients with AML and may play an important role in a GvL response(14,15). Ruggeri et al (16). has shown, for example, that patients who received an HSCT from a donor mismatched at certain HLA-C or B loci so that a “KIR-mismatch” occurred had a reduced chance of relapse consistent with the hypothesis that NK KIR ligands may be important mediators in a NK directed anti-leukemia response. In fact, these investigators found that transplantation from NK-alloreactive donors was associated with a significantly lower relapse rate in patients transplanted in CR (3% vs. 47%, p>0.003), better event-free survival in patients transplanted in relapse (34% vs. 6%, p=0.04), and in remission (67% vs. 18%, p=0.02) for 112 patients with high-risk AML with and without NK-alloreactive donors respectively (16). In this study, the NK-alloreactivity results from missing KIR ligands that occur in a HLA-mismatched donor setting. This observation has been replicated in other centers within studies of HLA-mismatched HSCT (17), but more recently, it appears that NK-alloreactivity may occur within the context of an HLA-matched HSCT when a patient receives a transplant from a donor that possesses certain KIR genes that can be broadly defined as a “B”-haplotype (14,15). Taken together, it appears that donors may be selected for optimal KIR expression in a manner that will optimize the occurrence of GvL and a web-site has been created to optimize this strategy(http://www.ebi.ac.uk/ipd/kir/) (17)(18).

It is our **hypothesis** that certain KIR-HLA combinations between a donor and a recipient of a transplant will allow donor derived NK cells to eradicate residual leukemia and improve outcome in patients with AML. We further hypothesize that this form of NK cell based immunotherapy may be accomplished within the setting of HSCT. An additional advantage of NK cell therapy is that while NK cells have similar cytotoxic properties as T cells, they do not seem to cause GVHD.

High-dose cyclophosphamide for GVHD prophylaxis

After HSCT, infused lymphocytes of donor origin recognize tissue antigens of recipient origin and these so-called “alloreactive” lymphocytes have the potential to proliferate and initiate a cytotoxic response against recipient tissues. This process, if left unchecked can lead to a potentially life-threatening condition

called **G**raft-**V**s-**H**ost **D**isease (GVHD). The risk of GVHD can be reduced by eradicating the most alloreactive lymphocytes early after transplantation – a process referred to as "GVHD prophylaxis". GVHD prophylaxis strategies usually include a calcineurin inhibitor (i.e. tacrolimus) to moderate cytokine signaling and a cytotoxic agent intended to cause cell death of stimulated and rapidly proliferating lymphocytes (i.e. methotrexate). Investigators from Johns Hopkins have pursued a more aggressive approach to eliminating alloreactive lymphocytes by using high-dose cyclophosphamide 50 mg/kg given days 3 and 4 after HSCT, and found this to be sufficient for matched-related HSCT alone and for partially-matched HSCT when combined with tacrolimus and mycophenolate(19–21). The rationale for the strategy depends on the sensitivity of alloreactive and therefore, proliferating T-cells to cyclophosphamide, whereas non-reactive lymphocytes will be unaffected (as depicted below).



Luznik, Immunol Res. 2010 July ; 47(1-3): 65–77

A table summarizing three reported studies is listed below. In these studies, performed at Johns Hopkins University and the Fred Hutchison Cancer Research Center, patients did not have HLA-identical donors and had a variety of myeloid malignancies.

	Hopkins A	Hopkins B	Fred Hutch
Patients (N)	20	40	28
Treatment			
Preparative regimen	Cy 29 mg/kg Flu 150 mg/m2 TBI 200 cGy	Same	Same
Cy dosing	Cy 50 mg/kg day +3	**Cy 50 mg/kg day +3 & 4**	Cy 50 mg/kg day +3
GVHD proph	Tac until day 50 MMF BID day 35	**Tac until day 180 MMF TID day 35**	Tac until day 90 MMF TID day 35

Results			
ANC > 500	Day 15	Day 16	Day 12
Plt > 20,000	Day 30	Day 26	Day 18
Graft Failure	6/19	6/39	3/26
Acute GVHD (III – IV)	10%	10%	10%
Chronic GVHD	15%	8%	46%

The consensus drawn from these studies is that partially-HLA-matched HSCT can be performed with relatively little GVHD, with reasonable safety and with excellent overall survival that rivals HLA-matched HSCT. These studies formed the basis for a multi-center clinical trial opened within the NCI-supported Blood and Marrow Transplant Clinical Trial Network (BMTCTN). In the BMTCTN 0603 trial, cyclophosphamide was given 50 mg/kg days 3 & 4, tacrolimus was given for 180 days and MMF was given TID for 35 days (all denoted in bold in the table above). This study was conducted in parallel with a separate BMTCTN 0604 trial using a similar preparative regimen and umbilical cord blood. In a report that included both studies, the outcome was similar between the two graft sources with OS at one year of 54% vs. 46% for cord units vs. partially matched family donors respectively (22). The follow-up randomized study comparing cord units to partially matched family donors is ongoing (BMTCTN 1101). Although there have not been direct comparisons between multiple graft sources, evidence exists supporting the possibility that outcome following the use of a partially-matched donor graft with cyclophosphamide for GVHD prophylaxis will be equivalent or possibly better than using HLA-matched donors. For example, a study examining the relationship between outcome and HLA-disparity showed that patients receiving a graft HLA mismatched at 3 – 4 loci had equivalent survival compared to those receiving grafts with 0 – 2 mismatches (23) if treated with cyclophosphamide. A single institution study of 271 patients receiving transplants from matched-related, matched-unrelated or partially-matched family donors (treated with cyclophosphamide for GVHD prophylaxis) demonstrated no difference in two year survival (76%, 67%, and 64% respectively) (24). A second study presented in abstract form demonstrated among 611 patients with 8-year follow-up that there was no difference in overall survival between patients receiving partially-matched family, matched-related or matched-unrelated donors (although a different method of GVHD prophylaxis was used) (25). In our study, relapse is the expected reason for failure in the majority of patients and given the evidence of similar outcome between partially-matched family donors, umbilical cord units, and matched-unrelated donors combined with the possibility of reducing relapse by using pretransplant decitabine and selecting donors where possible based on optimal NK-KIR interactions, we plan to offer the protocol to all patients eligible regardless of the possibility of finding a matched-unrelated donor through the National Marrow Donor Program.

Purpose of this proposal: Our BMT program at UW does not currently offer HSCT to patients with relapsed or refractory AML or those with high-risk myeloid malignancies who do not have an HLA-matched adult donor. In addition, we do not offer HSCT from mismatched donors other than from umbilical cord blood, which is generally an unsuitable option for patients NOT in remission. We wish to conduct a phase II study that will provide a potentially curative option for patients who do not otherwise have a viable transplant option with high-risk myeloid malignancies, based on the hypotheses mentioned above. If the protocol is successful at meeting the specific objectives mentioned below, we envision using the regimen as a platform to pursue further immunotherapy options such as NK cell infusion after transplantation to improve immune reconstitution and graft-vs-malignancy effects.

2.0 STUDY AIMS/STUDY OBJECTIVES

2.1 Patients with AML that is not in remission at the time of transplantation have a high relapse rate, and high-transplant related mortality following standard transplant protocols. We hope to improve on the outcome of these patients by using decitabine to better control disease prior to transplantation, using a novel donor selection process to optimize a "graft-vs-malignancy" effect, and using a novel GVHD prevention strategy. If this approach improves outcome, we will expect to see improved overall survival after transplantation.

Primary Hypothesis

- The overall survival at day 100 after transplantation is over 70%

Secondary Hypotheses

- The acute GVHD grade III and IV by day 100 is below 20%
- The graft failure rate by day 30 is below 10% (defined by ANC < 500/uL AND platelets < 20,000/uL in the absence of relapse).

To study these hypotheses, we propose to conduct a clinical trial with the following objectives:

Primary Objective: The primary objective is to determine overall survival at 100 days after transplantation following decitabine and a bone marrow transplant using a donor that is at least partially-matched and a myeloablative preparative regimen with post-transplantation cyclophosphamide for GVHD prophylaxis.

Secondary Objectives: Patients enrolled in this study will also be followed for the following endpoints: neutrophil and platelet

recovery, graft failure, acute graft-versus-host disease (GVHD), chronic GVHD, incidence of infection, treatment-related mortality, time to relapse/progression, overall survival, and progression-free survival.

3.0 SELECTION OF STUDY PATIENTS

3.1 Study entry is open to adult patients regardless of gender, race or ethnic background. Every effort will be made to include women and minority patients, but the patient population is expected to be similar to the advanced AML and high-risk MDS population seen at the UWCCC.

3.2 Inclusion Criteria

3.2.1 Age 18 to 75 years

3.2.2 Patients must meet one of two disease criteria listed in 3.2.3 or 3.2.4.

3.2.3 Acute myelogenous leukemia within one of the following categories:

- Primary induction failure (PIF): Patients who have not achieved a complete remission following initial diagnosis and after at least two induction cycles of chemotherapy consisting of cytarabine and an anthracycline or high-dose cytarabine.
- Relapsed AML: Patients are defined as having relapsed disease if they entered a complete remission confirmed with a bone marrow biopsy following initial treatment, and then were found to have morphological or cytogenetic evidence of recurrent disease on a subsequent bone marrow exam.
- Any CR2 or greater: CR must be defined using a bone marrow exam taken at least 21 days since the last chemotherapy (including a methyltransferase inhibitor), and may include CRp (morphologic CR without peripheral platelet recovery).
- CR1 with high-risk features. Includes patients with treatment-related AML, secondary AML (following MDS or MPN), high-risk cytogenetic or molecular phenotype (by NCCN criteria).
- Untreated AML (> 20% blasts on a bone marrow) arising from a previous confirmed diagnosis of MDS or MPN (excluding BCR-ABL positive diseases).

3.2.4 Myelodysplastic syndromes within one of the following categories:

- High-risk MDS at diagnosis as defined by the IPSS or WPSS.
- Transfusion dependent MDS (either RBC or platelet dependent) without a hematologic response to at least 4 months of MTI therapy. Hematologic response is defined as transfusion independence for two or more months.
- Progressive MDS following at least 4 months of MTI therapy. Progression is defined as resumption of transfusion dependence after at least two months of transfusion independence OR increase of marrow blasts by 50% from pretreatment OR overall blasts over 10% of marrow cells at any time after treatment.

3.2.5 Available related donor that is at least an allele level haplotype-match at HLA- A, B, C, DRB1 and DPB1 loci (DPB1 matching according to the "permissive – non-permissive" dichotomy as stated by UW Histocompatibility Laboratory). A minimum match of 5/10 loci is required. An unrelated donor search is not required for a patient to be eligible for this protocol.

3.2.6 Karnofsky score of 60% or better ("Requires occasional assistance, but is able to care for most of his/her needs").

3.2.7 Pulmonary: DLCO (corrected for hemoglobin) > 40%; and FEV1 > 50%

3.2.8 Cardiac: EF ≥ 50% and no uncontrolled angina, symptomatic ventricular arrhythmias, or ECG evidence of active ischemia.

3.2.9 Renal: serum creatinine within normal range for age, or if serum creatinine outside normal range, then renal function (estimated GFR by MDRD formula) > 40 mL/min/1.73m2

3.2.10 Women of child bearing potential must have a negative pregnancy test within 14 days prior to study registration and agree to use adequate birth control during study treatment.

3.2.11 Voluntary written consent

3.2.12 Patients must be 28 days from the end of the last induction course or at least 14 days from completion of previous methyltransferase inhibitor therapy (azacitidine or decitabine) at the time of registration.

3.3 Exclusion Criteria

3.3.1 Active CNS leukemia within two weeks of registration. Patients with a history of CNS leukemia must have adequate treatment as defined by at least two negative spinal fluid assessments separated by at least one week. Patients who

have received cranial XRT must still be eligible to receive total body irradiation to 4 Gy.

3.3.2 New or active infection as determined by fever, unexplained pulmonary infiltrate or sinusitis on radiographic assessment. Infections diagnosed within 4 weeks of registration must be determined to be controlled or resolving prior to treatment.

3.3.3 Active HIV, hepatitis A, B or C infection

3.3.4 Allergy or hypersensitivity to agents used within the treatment protocol.

3.4 Donor selection

3.4.1 Inclusion criteria

- Donors must be at least HLA-haploidentical first degree relatives of the patients. Eligible donors include biological parents, siblings, half-siblings or children.
- Age ≥ 18 and ≤ 60 years.
- Donors must meet the selection criteria prior to the start of the recipient's pre-transplant conditioning regimen as defined by the Foundation for the Accreditation of Cell Therapy (FACT) and will be screened according to the American Association of Blood Banks (AABB) guidelines and UW BMT program SOP.

3.4.2 Exclusion criteria

3.4.2.1 Recipient derived anti-donor high-titer (> 3000 MFI) HLA antibody as determined by Luminex assay.

3.4.2.2 Not suitable for donation according to UW BMT program donor selection SOP.

3.5 Donor prioritization schema

While an objective of this trial is to optimize donor selection with respect to KIR genotype, the single most important determinant in predicting outcome following allogeneic transplantation is the HLA match between the donor and recipient (26). To assure the greatest likelihood of favorable outcome following transplantation, we will prioritize HLA match over KIR selection in choosing donors. All potential donors will undergo HLA typing. Among two or more of the best HLA-matched donors, KIR typing will also be performed. The selection of donors will occur with the following priorities:

1. HLA-genotypic match
2. KIR mismatch based on the "receptor-ligand" model (see appendix A)
3. KIR genotype according to "B" content (see appendix A)

When the donor search is initiated, all available potential donors will undergo HLA typing according to the current UW SOP. The best available donors based on HLA match with the recipient will then be chosen for further genotypic KIR typing. If there is more than one possible donor of a given HLA match, narrowing the selection will be performed according to the priorities listed above. If there is more than one donor available after working through the selection priorities above, then the donor will be selected based on CMV serology, ABO matching and age (in that order) according to our standard UW guidelines.

4.0 RESEARCH DESIGN AND METHODS:

The study is a phase II study intended to study outcome and toxicity of a novel transplant approach for patients with high-risk myeloid malignancies. Research subjects will be identified among patients referred to the UWCCC for treatment of advanced myeloid malignancies. The patients will be treated and followed within the UW BMT program according to our approved standard-operating-procedures for clinical management of patients undergoing HSCT. All clinical care will be provided by the UW BMT program and follow-up thereafter according to the clinical needs of the patients and what is considered standard of care. There will be no scheduled visits or evaluation on dates that do not correspond to those indicated by best practice. Scheduled evaluations may be performed within 3 days from the targeted date prior to day 28 and within 7 days thereafter.

Study Data Collection and Monitoring
The study will report clinical data using the OnCore database using electronic case report forms. Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions and all measures to maintain confidentiality. In addition, specific transplant related information will be collected and recorded in the UW BMT program database according to the program standard operating procedures, which will assure confidentiality according to HIPAA regulations.

5.0 REGISTRATION PROCEDURES

Patients cannot begin protocol treatment prior to registration.
Registration will occur after the patient has signed the subject consent and eligibility is confirmed, but before any treatment has been administered. Any patient signing consent, but not enrolled in the study, will be recorded as a screen failure. To be eligible for registration to this study, the patient must meet each criteria listed on the eligibility checklist based on the eligibility assessment documented in the patient's medical record.

6.0 TREATMENT / INTERVENTION PLAN

Day -29 to -22	Start decitabine 20 mg/m2 IV daily for 10 days
Day -5 to -2	Fludarabine 50 mg/m2 IV daily over 30 minutes
Day -5 to -2	Busulfan 3.2 mg/kg (or 2.4 mg/kg) IV daily over three hours following fludarabine
Day -1 (or Day 0)	Total Body Irradiation (TBI) 4 Gy in 2 fractions
Day 0	Bone marrow transplant
Day 3, 4	Cyclophosphamide 50 mg/kg IV, Mesna 40 mg/kg IV
Day 5	Begin tacrolimus, MMF (for partially matched donors only)
Day 5	Begin G-CSF

The protocol is intended for patients with active AML or high-risk MDS at the time of registration. We expect that decitabine will succeed in controlling AML prior to transplantation in most but not all patients, and a subset will experience progressive disease during and after the 10 day period of decitabine treatment. Patients who have evidence of rapidly progressing AML while on treatment are felt to be at particularly high-risk for early death during the transplant process and are unlikely to derive any long-term benefit from transplantation. **Accordingly, patients with a peripheral blast count that is rising during decitabine treatment and the week following to over 150% of pre-treatment count AND have a absolute blast count that exceeds 10,000 blasts per microliter of blood within 7 days of the completion of decitabine will be ineligible to proceed.** While there are no restrictions on the use of growth-factors, these are discouraged as they may influence the blast count and render a patient ineligible for transplantation.

6.1 Weight Calculations

For all medications, actual body weight will be used for patients when actual body weight is less than ideal body weight. Unless otherwise stated, if the actual body weight is above ideal body weight, then adjusted ideal body weight will be used.

Ideal Body Weight (IBW) Formulas:

Males IBW = 50 kg + 2.3 kg/inch over 5 feet
Females IBW = 45.5 kg + 2.3 kg/inch over 5 feet

Adjusted Ideal Body Weight Formula:

AIBW = IBW + [(0.25) x (ABW - IBW)]

6.2 Decitabine

Actual body weight is used to calculate decitabine dose. Patients will receive decitabine 20 mg/m2 IV over one hour daily for 10 consecutive days according to current UW chemotherapy council approved treatment plan that is available in Beacon. The decitabine must start by 29 days but no later than 22 days prior to transplant (be completed within 14 days but not sooner than 7 days from the start of the preparative regimen).

6.3 Preparative Regimen

Fludarabine 50 mg/m2/day will be administered over 30 minutes intravenous infusion on Days -5 through -2 for a total dose of 200 mg/m2. For patients who have an estimated or measured GFR < 70 mL/min/1.73 m2, the fludarabine dose should be reduced by 20%. Fludarabine dosing is based on the last calculated GFR prior to the start of conditioning. The fludarabine dose should be the same on Days -5 to -2, even if the patient's creatinine changes. Fludarabine must be administered before busulfan on each day.

Busulfan 3.2 mg/kg/day given intravenously over 3 hours on Days -5 to -2 following fludarabine for patients up to the age of 60. For patients 61 to 75, the busulfan dose is 2.4 mg/kg/day. Following the first busulfan infusion, the IV administration line will be flushed with normal saline and blood samples (3 mL/sample) will be collected in sodium heparin tubes. The samples will be immediately placed on ice, centrifuged at 4°C and the plasma collected and frozen in a labeled plastic tube. Samples will be collected at the end of the infusion (180 min), and 195 min, 4, 5, 6 and 8 hours after the infusion began. The frozen plasma samples will be sent to the Seattle Cancer Care Alliance Pharmacokinetics Laboratory, Seattle Washington, for the analysis of busulfan pharmacokinetics.

After quantitation of busulfan samples, the individual patient's concentration-time data are fit using WinNonlin (version 5.2). The AUC is estimated and the steady state concentration calculated. After calculation of the patient's clearance, the target dose for subsequent doses is calculated linearly to achieve the target Css (27).

Target Concentration of busulfan at steady state (Css)
Age 18 to 60: 800 ng/mL
Age 61 to 75: 600 ng/mL

Drug Interactions: Busulfan is metabolized in the liver primarily through a reaction with glutathione-S-transferase, but clearance also depends on the CYP3A4 enzyme system, although the mechanism is not well described. It is important to avoid administering drugs that compete with busulfan for clearance via the glutathione pathway or interact with the CYP3A4 enzyme system. The most important interactions include those with phenytoin, acetaminophen, metronidazole and extended-spectrum antifungal azoles. It is recommended that the patient not take these medications within one weeks of the first dose of busulfan and until 3 days following the completion of busulfan.

Total-body irradiation will be administered at a dose of 4 Gy in 2 fractions on Day -1 according to the current UW standard operating procedure. If needed because of scheduling requirements, TBI may be given on Day 0 but Day -1 is the preferred day of treatment.

6.4 Bone marrow transplant

On Day 0, patients will receive unprocessed marrow unless there is a major ABO incompatibility, in which case red blood cells will be depleted from the donor marrow using the current UW SOP. Donor bone marrow will be harvested with a target yield of 4×10^8 nucleated cells/kg recipient ideal body weight (IBW). A sample of the product to be infused will be sent for flow cytometry to determine the content of CD34+, CD3+, CD4+ and CD8+ cells.

6.5 Graft-vs-Host Disease Prophylaxis

6.5.1 Post-transplantation cyclophosphamide with mesna – hydration with normal saline at 2 mL/kg/hr IV will be started 2 hours prior to cyclophosphamide and continued at 2 mL/kg/hr for 8 hours post-cyclophosphamide.

Mesna will be given in divided doses IV over 15 - 30 min at the following time points: Prior to the start of cyclophosphamide and at

3, 6, and 8 hours post-cyclophosphamide. Mesna dose will be 80% of the total daily dose of cyclophosphamide.

Cyclophosphamide (50 mg/kg if the actual body weight is above ideal body weight, then ideal body weight will be used)
will be given on Day +3 (between 60 and 72 hours after marrow infusion) and on Day +4 (approximately 24 hours after Day +3 dose) post-transplant. Cyclophosphamide will be given as an IV infusion over 2 hours. **No corticosteroids may be given as anti-emetics with the cyclophosphamide.**

6.5.2 Tacrolimus and Mycophenolate For those patients receiving a transplant from a partially matched donor (including one or more mismatches at HLA A, B, C, DR or non-permissive DP) **tacrolimus and mycophenolate** will be started on Day +5.

Tacrolimus – will be started no earlier than 24 hours after the completion of cyclophosphamide. The management of tacrolimus will reflect standard operating procedures for the UW BMT Program. Tacrolimus 0.12 mg/kg/day oral in two divided doses with each dose rounded to the nearest 0.5 mg (or 0.04 mg/kg/day IV if unable to tolerate oral) beginning Day 5 with dose adjusted to maintain a level of 5-15 ng/mL beginning Day 5 until Day 180 and Mycophenolate mofetil (MMF) 15 mg/kg oral PO TID beginning Day 5 until Day 35. Tacrolimus dosing is calculated using actual body weight unless the patient is >25% above ideal body weight in which case adjusted actual body weight should be used to calculate the dose. Serum levels will be measured no later than Day +7 and should be checked biweekly to maintain a level between 5-15 ng/mL. Patients will remain on tacrolimus until day 180, when, in the absence of GVHD, tacrolimus will be discontinued.

Mycophenolate Mofetil (MMF) – will be given at a dose of 15 mg/kg PO TID (based upon actual body weight and rounded up to the nearest pill size) with the maximum total daily dose not to exceed 3 grams (1g PO TID). MMF prophylaxis will be discontinued after the last dose on day 35, or may be continued if there is active GVHD present.

6.6 Indwelling Central Venous Catheter – patients must have a peripherally inserted central catheter (PICC) for the administration of IV medications and transfusion of blood products.

6.7 Growth Factor Support –

will be given according to UW standard for engraftment following BMT. Specifically, G-CSF
will be given subcutaneously beginning Day +5 and daily until the ANC is > 1,000 for one day. The dose will be based on actual body weight as follows: for weight ≤ 80 kg give 300 mcg and for weight > 80 kg, give 480 mcg.

6.8 Supportive Care

Patients will receive the standard supportive care provided to HSCT patients according to the UW BMT SOPs.

7.0 ADVERSE EVENT REPORTING REQUIREMENTS

Purpose

An adverse event (AE) is any untoward medical occurrence in a clinical investigation subject that is enrolled in a clinical trial. An AE may be any unfavorable or unintended sign, symptom, or disease temporally associated with participation in a clinical trial. While some events may not initially appear to be associated with the use of the study treatment, a relationship may not emerge until sufficient numbers of reports accumulate over the course of the study. 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. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner for timelier monitoring of patient safety and care. The following sections provide information about expedited reporting.

General AE reporting requirements

The study period during which all AEs and SAEs must be reported begins upon initiation of study treatment and ends at the 12 month post-transplant follow-up visit, or at the time of initiation of non-protocol treatment for the underlying primary malignancy or death, whichever comes first. After this period, investigators should only report SAEs that are attributed to prior study treatment. Both AEs and SAEs will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE Version 4.0). In general, HSCT is performed by first administrating myelotoxic and immunosuppressive medications followed by infusion of allogeneic stem cells. There is a predictable and expected period of severe hematologic toxicity followed by recovery as the allogeneic stem cells engraft (grow) and produce blood cells. This early post-transplant toxicity is predictable, expected and typically resolved by one month after transplantation. Consequently, reporting requirements for expected AEs and SAEs until

day 30 will follow the guidelines outlined in Table 1. Beyond day 30, patients are followed as outpatients in the clinic and the AE and SAE reporting will be determined according to Table 2below.

Serious Adverse Events (SAE)

Defining an SAE and Reporting Responsibilities:

Adverse events that qualify as serious are defined in the list below (see **Serious Adverse Event Definition)**. Using the list, first determine whether the adverse event is considered an SAE. Depending on the nature and severity of the serious adverse event, an SAE report will be submitted according to the reporting timeline specified in Table I and Table 2 (below). All serious adverse events must be reported to the UWCCC Data and Safety Monitoring Committee Chair. SAEs will be submitted to the UW-IRB per institutional guidance. SAEs will be reported to the FDA if deemed appropriate by the UWCCC Sponsor-Investigator Determination of FDA Reporting for Institutional Trials.

The UWCCC Principal Investigator assumes responsibilities of the study sponsor in accordance with FDA 21 CFR 312.32. In this capacity, the UWCCC PI reviews all reports of SAE occurring on this study at the UWCCC and makes a determination of 1) suspectedness (i.e., whether there is a reasonable possibility that the AE is caused by transplantation process); and 2) whether the event is unexpected (the event is not listed in the list of expected transplant associated AEs) in the context of this study. SAEs will be submitted to the DSMC, IRB, and FDA as appropriate.

Serious Adverse Event Definition

Following FDA guidance, Serious Adverse Events are events that have any of the following outcomes:

1. Death
2. A life-threatening adverse event.
3. An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. A congenital anomaly/birth defect.
6. Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based on medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

TABLE 1 – Serious Adverse Event Reporting Requirements for Events That Occur Within 30 days after HSCT

	Grade 1 – 3	Grade 4	Grade 4	Grade 5	Grade 5
		Unexpected	**Expected**	**Unexpected**	**Expected**
Unrelated Unlikely	Not required	Not required	Not required	10 Calendar days	10 Calendar days
Possible Probable Definite	Not required	10 Calendar days	Not required	24-Hrs; 5 Calendar Days	10 Calendar days

TABLE 2 – Serious Adverse Event Reporting Requirements for Events That Occur 31 to 365 days after HSCT

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in hospitalization ≥ 24 hrs	10 Calendar Days		24-Hour; 5 Calendar Days
Not resulting in hospitalization ≥ 24 hrs	Not Required	10 Calendar Days	

NOTE: Expedited AE reporting timelines are defined as:

- 24-Hour; 5 Calendar Days – The AE must initially be reported via MedWatch within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report. Note: MedWatch form only required to be completed and submitted as indicated by the UWCCC Sponsor-Investigator Determination of FDA Reporting for Institutional Trials form.
- 10 Calendar Days – A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE. Note: MedWatch form only required to be completed and submitted as indicated by the UWCCC Sponsor-Investigator Determination of FDA Reporting for Institutional Trials form.

Exceptions to SAE Reporting
Specific protocol exceptions to expedited reporting (SPEER) are listed in the CAEPR Table below.

Risks and Toxicities

Total Body Irradiation – nausea and vomiting, diarrhea, parotitis, generalized mild erythema, hyperpigmentation, fever, mucositis, alopecia, infertility, amenorrhea, late secondary malignancies. Late effects may include cataract formation, growth retardation, pulmonary damage, carcinogenesis, and sterilization.

Busulfan – Severe myelosuppression with marrow ablation, alopecia, and mild nausea/vomiting are expected. Alopecia may not be completely reversible. Liver toxicity including severe or fatal veno-occlusive disease (<5%) may occur.

Pulmonary toxicity is rare in this schedule. It is expected that patients will require mouth care including narcotic analgesia, and may require parenteral nutrition. Busulfan may cause skin toxicity including painful desquamation, and this may require local care and narcotic analgesia. Darkening of the skin may occur and may last several months. Seizures may occur (<5%). Busulfan causes immunosuppression and risk of opportunistic infection even after resolution of neutropenia. Busulfan is expected to cause nearly universal infertility in the doses used, although men may occasionally father children.

Fludarabine – Myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, autoimmune hemolytic anemia, autoimmune thrombocytopenia, paresthesias, peripheral neuropathy, renal and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine monophosphate. Such toxicity has only been rarely demonstrated at the 25-30 mg dosage of fludarabine monophosphate used in this regimen. Tumor lysis syndrome, complicating fludarabine monophosphate therapy has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed in both pre-treated patients receiving fludarabine and in individuals receiving fludarabine combined with other agents.

Tacrolimus – In patients receiving tacrolimus, 5% to 47% experienced anemia, 8% to 32% experienced leukocytosis, and 14% to 24% experienced thrombocytopenia. Rare cases of microangiopathic hemolytic anemia have been reported. Mild to moderate hypertension was reported in 38% to 50% of patients receiving tacrolimus. Mild to moderate hypertension is a common adverse effect associated with tacrolimus therapy. Chest pain was reported in 19%. Antihypertensive therapy may be required. The most common adverse effects of tacrolimus have involved the central nervous system, and include headache (37% to 64%), tremors (48% to 56%), insomnia (32% to 64%), paresthesia (17% to 40%); and dizziness (19%). Tremor and headache may respond to a dosage reduction. Agitation, anxiety, confusion, seizures, depression, hallucinations, myoclonus, neuropathy, psychosis, incoordination, and abnormal dreams have been reported in 3% to 15% of tacrolimus-treated patients. Hyperkalemia (13% to 45%), hypokalemia (13% to 29%), hypophosphatemia (49%), and hypomagnesemia (16% to 48%) have been associated with tacrolimus therapy. In addition, hirsutism occurs only rarely with tacrolimus. Hyperuricemia has been reported in greater than 3% of tacrolimus-treated patients. Gastrointestinal adverse effects of tacrolimus have included nausea (32% to 46%), vomiting (14% to 29%), anorexia (7% to 34%), constipation (23% to 35%) and diarrhea (37% to 72%). Nephrotoxicity was reported in 36% to 40% and 52% of liver and kidney transplant patients receiving tacrolimus. Overt nephrotoxicity is usually seen early after transplantation and is characterized by an increased serum creatinine

and a decrease in urine output. Hematuria has been reported in greater than 3% of tacrolimus-treated patients (Prod Info Prograf®, 1997). Abnormal liver function tests have been reported in 6% to 36% of patients receiving tacrolimus; ascites was reported in 7% to 27% of these patients. Other miscellaneous effects that have occurred in clinical trials include pain (24% to 63%), fever (19% to 48%), asthenia (11% to 52%), back pain (17% to 30%), and peripheral edema (12% to 36%). The incidence of hyperglycemia is 17% and may require therapy with insulin. Other less frequently occurring effects (greater than 3%) include abscess, chills, peritonitis, and photosensitivity reactions. Anaphylaxis has been reported in a few patients receiving intravenous tacrolimus. Tacrolimus contains castor oil which has been associated with anaphylaxis in other drugs containing castor oil derivatives. The incidence of bloodstream infection is 22%. Most infections are due to bacteria (81%), followed by candidemia (14%), and cryptococcemia (5%). The source of bloodstream infection was primarily intravascular catheter, accounting for 39% of cases.

Cyclophosphamide – cardiomyopathy, skin rash, mucositis, sterility, fluid weight gain/edema, alopecia and hemolytic/anemia. Hematologic including leukopenia, thrombocytopenia, anemia, pancytopenia; gingivitis, glossitis, pharyngitis, stomatitis, enteritis; nausea/vomiting, anorexia, diarrhea; hematemesis, melena; photosensitivity; nephropathy: hemorrhagic cystitis, dysuria, azotemia, hematuria, renal failure.

Mycophenolate Mofetil – pancytopenia, nausea, vomiting, diarrhea, hypertension, headache, dizziness, insomnia, hyperglycemia, electrolyte imbalances, rash, and leg cramps/bone pain, reversible renal insufficiency, hypertension, hyperglycemia, hypomagnesemia, hypokalemia, and neurologic toxicity.

Comprehensive Adverse Events and Potential Risks (CAEPR) List for BMT.

Adverse Events with at least possible relationship to allogeneic stem cell transplantation			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (> 20%)	**Less Likely (<20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Pancytopenia			Anemia, thrombocytopenia, neutropenia (gr.3)
Febrile neutropenia			Febrile neutropenia (gr. 3,4 before day 30)
	Immune		

	Hemolytic anemia		
		Microangiopathic hemolytic anemia	
CARDIAC DISORDERS			
	Atrial fibrillation		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		Diarrhea (gr. 3)
	Constipation		Nausea/vomiting (gr.3)
Diarrhea			
Oral mucositis			
Nausea and vomiting			
Anorexia			Anorexia (gr. 4 before day 30)
Colitis			
GENERAL DISORDERS			
Edema			
Fever			
		Multi-organ failure	
Pain			
	Non-cardiac chest pain		
HEPATOBILIARY DISORDERS			
		Hepatic failure	
		Veno-occlusive disease	
IMMUNE SYSTEM DISORDERS			
Graft-vs-host disease			
		Cytokine release syndrome	
INFECTIONS AND INFESTTIONS			
	Bladder infection		
	Catheter related infection		
	Bacteremia		
		Sepsis	
	Lung infection		
	Pustular rash		
	Sinusitis		
INVESTIGATIONS			
	AST, ALT, bili, Alkaline		

	phosphatase increased		
	Creatinine increased		
	Lymphocytes decreased		Lymphocytosis (gr. 3)
	Weight gain/loss		
	WBC, HGB, PLTs decreased		Anemia, thrombocytopenia, neutropenia (gr.3)
METABOLISM AND NUTRITIONAL DISORDERS			
	Multiple electrolyte abnormalities		Dehydration (gr.3), Hyponatremia (gr.3), hypokalemia (gr.3)
	Iron overload		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
		Avascular necrosis	
		Fibrosis deep connective tissue	
	Muscle weakness		
	Osteoporosis		
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
	Proteinuria		
REPRODUCTIVE SYSTEM			
Azoospermia			
Dysmenorrhea			
Infertility			
RESPIRATORY DISORDERS			
		ARDS	
		Bronchiolitis obliterans (bronchial obstruction)	
		Pneumonitis	
		Pulmonary edema	
		Pulmonary fibrosis	
		Respiratory failure	

SKIN AND SUBCUTANEOUS DISORDERS			
	Erythroderma		
	Skin induration		
	Rash maculo-papular		
VASCULAR DISORDERS			
	Capillary leak syndrome		
	Hypertension		
	Thromboembolic event		

Investigator Reporting to the FDA
The FDA does not need to be automatically notified of every SAE that occurs during the clinical trial. For every SAE, the PI (or Sub-I in absence of the PI) must complete the UWCCC Sponsor-Investigator Determination of FDA Reporting for Institutional Trials. The PI or Sub-I will evaluate the SAE against certain criteria and determine if reporting to the FDA is necessary.

Adverse drug reactions that are **Serious, Unlisted/Unexpected, and at least possibly associated to the drug** and that have not previously been reported in the Investigators brochure, or reference safety information document should be reported promptly to the Food and Drug Administration (FDA) in writing by each investigator/physician engaged in clinical research. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

The investigator/physician shall notify the FDA by telephone or by fax of any unexpected fatal or life threatening experience associated with the use of the drug as soon as possible, but no later than 7 calendar days after the sponsor's initial receipt of the information. All other events can be reported within 15 days.

Reporting a 24 SAE : A Summary

Serious Adverse Events requiring expedited reporting within 24 hours will be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu within one business day.

A 24 hr initial "SAE Details" Report, generated in the UWCCC database, OnCore, must be attached to the email along with the UWCCC Sponsor-Investigator Determination of FDA Reporting for Institutional Trials and any additional pertinent information available at the time of initial reporting. The DSMC Chair will review the information and determine if immediate action is required.

Within 10 working days, all subsequent SAE documentation must be submitted electronically along with a 24 hour follow-up or final "SAE Details" Report to saenotify@uwcarbone.wisc.edu. Final reports will also be submitted to appropriate individuals listed on the UWCCC SAE Routing Form, however, the Routing Form itself should not be sent with the final SAE. All information is entered and tracked in the UWCCC database.

Procedures for Reporting a 24 Hour SAE that Occurs at the UWCCC

a. **To the FDA:**

   Consult the UWCCC Sponsor-Investigator Determination of FDA Reporting for Institutional Trials to determine if the event requires reporting to the FDA. If so, report the SAE using the FDA MedWatch form available at http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

b. **To the UW HS-IRB:**

   Consult the UW-IRB website for reporting guidelines.

c. **To the UWCCC:**
   Reference the **SAE SOP** (Standard Operating Procedure) and the **SAE Reporting Workflow for DOWGs** on the UWCCC website (http://www.uwccc.wisc.edu) for specific instructions on how and what to report to the UWCCC for 24 hour initial and follow-up reports. Include the UWCCC Sponsor-Investigator Determination of FDA Reporting for Institutional Trials and the FDA MedWatch form with this submission if applicable. **A follow-up report is required to be submitted to the UWCCC within 10 business days of the initial 24 hour report.** The "SAE Details" Report and related supporting documents should be e-mailed to the DSMC at saenotify@uwcarbone.wisc.edu. Final reports will also be submitted to appropriate individuals listed on the UWCCC SAE Routing Form, however, the Routing Form itself should not be sent with the final SAE.

Reporting a 10 Day SAE : A Summary

Serious Adverse Events requiring reporting within 10 working days (as described in the protocol) will also be sent to the UWCCC DSMC Chair via email to saenotify@uwcarbone.wisc.edu. A 10 day "SAE Details" report, generated in the UWCCC database, OnCore, must be attached to the email along with the UWCCC Sponsor-Investigator Determination of FDA Reporting for Institutional Trials and any additional pertinent information regarding the SAE. Final reports will also be submitted to appropriate individuals listed on the UWCCC SAE

Routing Form, however, the Routing Form itself should not be sent with the final SAE. The DSMC Chair will review the information and determine if further action is required. This information is entered and tracked in the UWCCC database.

Procedures for Reporting a 10 Day SAE that Occurs at the UWCCC

a. To the FDA:

Consult the UWCCC Sponsor-Investigator Determination of FDA Reporting for Institutional Trials to determine if the event requires reporting to the FDA. If so, report the SAE using the FDA MedWatch form available at http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

b. To the UW HS-IRB:

Consult the UW-IRB website for reporting guidelines.

c. To the UWCCC:

Reference the **SAE SOP** (Standard Operating Procedure) and the **SAE Reporting Workflow for DOWGs** on the UWCCC website (http://www.uwccc.wisc.edu) for specific instructions on how and what to report to the UWCCC for 10 day initial and follow-up reports. Include the UWCCC Sponsor-Investigator Determination of FDA Reporting for Institutional Trials and the FDA MedWatch form with this submission if applicable. The "SAE Details" Report and related supporting documents should be e-mailed to the DSMC at saenotify@uwcarbone.wisc.edu. Final reports will also be submitted to appropriate individuals listed on the UWCCC SAE Routing Form, however, the Routing Form itself should not be sent with the final SAE.

Study Progress Review

Cumulative reports of serious adverse events requiring expedited reporting and any new serious adverse event requiring expedited reporting are also reviewed at the DSMC bimonthly meetings.

An overall assessment of accrual, toxicities as described in the protocol, and responses will enable the committee members to assess whether significant benefits or risks are occurring that would warrant study closure. This information is provided by Disease Group meeting minutes, internal audit and/or response review reports. The committee may request external DSMB reports or further information from the Disease Groups, or Study Chair.

The Data and Safety Monitoring Committee recommendations for modifications to the trial are forwarded to the Clinical Research Committee (CRC), composed of Cancer Center senior leaders that oversee all aspects of clinical research conducted at the UWCCC and makes final decisions on all issues related to clinical trials. The Study Chair is notified of this recommendation in order that he/she may alert all investigators, at the UWCCC and at external sites involved in the trial, about the potential action. At this time the Study Chair may submit to the CRC additional information that could affect the Committee's decision. The CRC will notify the Study Chair if they concur with the Data and Safety Monitoring Committee recommendations, including suspension or closure. The Study Chair will notify all investigators involved with the study at UWCCC and external sites, the IRB, the sponsor and the funding source.

8.0 STUDY ENDPOINTS

8.1 Primary Endpoint

The primary endpoint is overall survival at 100 days from the time of transplantation.

8.2 Secondary Endpoints

8.2.1 Neutrophil recovery – is defined as achieving an ANC ≥ 500/uL for three consecutive measurements on different days. The first of the three days will be designated the day of recovery. The only competing event for neutrophil recovery is death without neutrophil recovery.

8.2.2 Platelet recovery – is defined as the first day of a platelet count > 20,000/mm3 with no platelet transfusions in the preceding 7 days. The first day of the sustained platelet count will be the designated day of platelet engraftment.

8.2.3 Primary graft failure – is defined as < 5% donor chimerism in the CD3 and CD33 selected cell populations at any time after transplantation.

8.2.4 Acute GVHD – The cumulative incidence of grade II – IV GVHD will be determined. The GVHD grade will be determined by the standard BMT Clinical Trials Network (BMTCTN) criteria (Appendix B). The time to onset of grade II-IV and III-IV aGVHD will be recorded and maximum grade achieved.

8.2.5 Chronic GVHD – The cumulative incidence of chronic GVHD will be recorded and defined according to the BMTCTN criteria (Appendix C).

8.2.6 Complete remission after transplantation – is determined when there is neutrophil and platelet engraftment, a bone marrow with < 5% blasts (and no morphologically abnormal

blasts seen on aspirate), and resolution of any disease related cytogenetic marker of the original disease.

8.2.7 Relapse will be diagnosed when there is any of the following:

- Reappearance of leukemia blasts in the peripheral blood
- 5% blasts in the marrow, not attributable to another cause (e.g. bone marrow regeneration)
- The reappearance of dysplastic changes in the bone marrow aspirate accompanied by cytogenetic findings or chimerism testing to confirm disease.
- The finding of extramedullary leukemia including in the cerebral spinal fluid.

9.0 SCHEDULE OF EVALUATIONS

All evaluations are considered part of the standard of care evaluation of a patient prior to allogeneic stem cell transplantation. The pretransplant evaluations must be completed within 28 days of registration unless otherwise stated below.

	Pre-study Evaluation[7]	Pre-BMT Evaluation	Day 1 to neutrophil engraftment	Day 28	Weekly day 28 to 100	6, 9, 12 months
Informed Consent	X					
Medical History	X	X		X	X	X
Toxicity Assessment	X	X	weekly		X	X
Physical Exam	X	X		X	X	X
Performance Status	X	X		X	Every 4 weeks	X
GVHD evaluation[1]			Twice weekly	X	X	X
CMV PCR[2]			X	X	X	X
EBV PCR					Every two weeks	X
CBC	X[5]	X[5]	Twice weekly		X	X
LFT's Total bilirubin, Alkaline Phosphatase, AST, ALT	X	X	weekly		X	X
Electrolytes, Mag, Creatinine, BUN,	X	X	Twice weekly		X	X
Uric acid, INR, Vitamin D, ferritin, calcium, phosphate	X					
Total cholesterol, triglycerides	X					
Tacrolimus			Twice weekly		X	
Pre-transplant viral panel[3]	X					
Urinalysis with microscopy and culture if indicated	X	X				
Pregnancy test[4]	X	X				
Luminex screen[6]	X	X				
BM aspirate and biopsy	X[5]	X[5]		X	X (day 100 only)	X
CD3, CD33 STR (PB)	X			X	Every 4 weeks	X
ECG	X					
Cardiac ECHO	X					
CT scans – sinus plus C/A/P, panorex	X					
CXR[8]		X				
PFT's	X					
CD4/8				X	X (day 100 only)	X
Quant immunoglobulins	X				X (day 100 only)	X

1. Refer to appendix B for acute GVHD grading scales
2. Weekly CMV PCR starting day 14 for those at risk for CMV infection (CMV positive donor or recipient) until day 100 and then 6, 9 and 12 months
3. Viral panel according to the UW standard operating procedure for pre-HSCT evaluation
4. Urine or serum within 14 days of registration
5. A bone marrow and CBC with differential must be performed within 14 days of beginning decitabine and again within 14 days before beginning the preparative regimen.

6. Recipients will be screened for anti-donor HLA antibodies prior to final selection of donors. Testing will be repeated if more than 30 days have elapsed between the results and the start of decitabine.
7. Unless otherwise specified, pre-study evaluation assessments to be done within 28days of registration.
8. CXR will be performed one the day of or within one week of admission to begin the preparative regimen.

10.0 STATISTICAL CONSIDERATIONS

10.1 Objectives:

As a phase II trial, the objective is to generate preliminary data on clinical outcome such as overall survival, acute GvHD (aGvHD), and graft failure (GF) after transplant, non-hematologic toxicity, and blast content of the bone marrow before and after decitabine from the use of decitabine prior to HSCT, the use of a partially-matched but KIR-HLA selected donor, and the incorporation of high-dose cyclophosphamide for GVHD prophylaxis.

10.2 Endpoints:

The primary endpoint of the study is overall survival at day 100 after transplant. Any patient who is enrolled will be accounted for in the final assessment. Secondary endpoints include aGvHD grade III-IV by day 100, graft failure by day 30 after transplant, and grade 3 or worse non-hematologic toxicity. These analyses will include only those patients who survive to Day 0 (the day of transplantation). Lastly, the blast content of the bone marrow before and after decitabine will be analyzed among those patients who have completed the decitabine treatment and the bone marrow analyses before and after decitabine treatment.

10.3 Analysis plan:

Overall survival and other times to event data such as aGvHD and GF will be analyzed using Kaplan-Meier (KM) method, and day 100 OS and aGVHD grade III-IV and 30 day GF rates will be obtained from the KM estimates along with 95% confidence intervals. Binary outcome variables such as grade 3 or worse non-hematologic toxicity, will be summarized with a proportion and a 95% confidence interval. The blast content of the bone marrow before and after decitabine and its change will be summarized with mean and standard deviation or median and interquartile range, and the change will be tested using a one-sample paired t-test at a two-tailed significance level of 0.05.

10.4 Sample size justification:

The sample size is limited by the feasibility over 2 years. Twenty subjects will be enrolled into the trial. With 20 subjects, the trial will have 0.71 (0.93) power to detect the 100 day OS ≥ 0.7 according to a one-tailed test of the null hypothesis that it is ≤ 0.5 (0.4) at a significance level of 0.1. The trial will have 0.76 (0.94) power to detect the 100 day aGvHD grade III-IV ≤ 0.2 according to a one-tailed test of the null hypothesis that is is ≥ 0.4 (0.5) at a significance level of 0.1. Also the trial will have 0.85 (0.98) power to detect the 30 day GF ≤ 0.1 according to a one-tailed test of the null hypothesis that is is ≥ 0.3 (0.4) at a significance level of 0.1.

10.5 Sequential safety monitoring:

Because of safety concern, OS, aGvHD grade III-IV, and GF will be monitored continuously using a sequential probability ratio test (SPRT). For example, each time death is observed before day 100 or patient survives beyond day 100, a sequential probability ratio test will be performed to test the null hypothesis that the 100 day OS is less than or equal to 0.5 against the alternative hypothesis that it is at least 0.7. Likewise each time grade III or IV aGvHD is observed before day 100 or patient does not experience grade III or IV aGvHD by day 100, a sequential probability ratio test will be performed to test the null hypothesis that the 100 day aGvHD grade III-IV is greater than or equal to 0.4 against the alternative hypothesis that it is no more than 0.2. Also each time GF is observed before day 30 or patient is free of GF by day 30, a sequential probability ratio test will be performed to test the null hypothesis that the 30 day GF is greater than or equal to 0.3 against the alternative hypothesis that it is no more than 0.1. The trial will be suspended if the SPRT boundary for the null hypothesis is crossed for any of the three outcome measures and the UWCCC DSMC will be notified. After investigation of the clinical outcomes as of the SPRT boundary crossing, the PI in consultation with other investigators and study statistician and the UWCCC DSMC will make the decision whether to continue the trial or to terminate for patient safety concern.

11.0 RECORDS TO BE KEPT

The UW BMT Program maintains a HIPAA compliant clinical database that is used to comply with FACT and Center for International Blood and Marrow Transplant Research (CIBMTR) reporting requirements. Patient, disease, treatment and outcome data are collected and reported as required by regulatory and quality reporting requirements. Patients will also be registered in the OnCore database within the UWCCC.

12.0 PATIENT CONSENT AND PEER JUDGMENT

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

13.0 DATA AND SAFETY MONITORING

The UWCCC Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and subject safety for all UWCCC clinical studies. A summary of DSMC activities follows:

- Review of all clinical trials conducted at the UWCCC for data integrity and safety
- Review of all serious adverse events requiring expedited reporting as defined in the protocol (see section 7)
- Review of reports generated by the UWCCC data quality control review process
- Submit recommendations for corrective action to the CRC
- Notify the Study Chair of the DSMC's recommendation to the CRC
- Work in conjunction with the Health Sciences IRB in the review of protocol deviations, violations and unanticipated problems reported by the UWCCC DOWGs.
- The committee ensures that notification is provided to all external sites participating in multiple-institutional clinical trials coordinated by the UWCCC of serious adverse events requiring expedited reporting.

Monitoring And Reporting Guidelines

Data related to these trials are discussed at regularly scheduled Disease Oriented Working Group meetings where the result of each subject's treatment is discussed. The discussion will include for each treatment arm/dose level, the number of subjects, significant toxicities as described in the protocol, dose adjustments, and responses observed. Twice yearly, Protocol Summary Reports are required for submission to the Data and Safety Monitoring Committee for review. Any unanticipated problems, complications, and adverse events will be reported to the investigator upon occurrence, and reported to the IRB in accordance with posted guidance.

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Appendix A

I. Donor Selection

Related donors that are at least haploidentical with respect to HLA class I and II antigens defined genotypically will be used as donors. The first priority in choosing donors will be based on HLA match. If there are multiple donors of the same degree of HLA-match, then further selection will be based on KIR-HLA ligand mismatching as described below. Using the table below, for each donor, the KIR expressed by the donor will be indicated across the top of the table. The HLA type of the patient will be indicated along the first column and a point will be given for each "match" that occurs within a shaded area on the table.

HLA type of **Patient**	KIR genotype of **Donor**			
	KIR 2DL1 ("A")	KIR 2DL2 ("B")	KIR 2DL3 ("A")	KIR 3DL1 ("A")
HLA-C2				
HLA-C1				
HLA-Bw4				

Donor with lowest score (number of shaded boxes checked) is preferred. If there are more than one donor with the same KIR-HLA ligand score, then a further determination will be made based on the "B" haplotype content as determine by the calculator on this site: http://www.ebi.ac.uk/ipd/kir/donor_b_content.html. The donor with the most "B" haplotype content with be chosen. If there remain options between donors, then usual selection criteria will apply that is based on age, CMV status and ABO blood type.

Donor selection priority:

1. HLA-match
2. KIR-ligand mismatch
3. KIR B haplotype content

TABLE of KIR Genotypes

Group C1 Ser77 Asn80	Cw*01 Cw*03 Cw*07 Cw*08 Cw*12 Cw*13 Cw*14 Cw*16	2DL2 2DL3
Group C2 Asn77 Lys80	Cw*02 Cw*04 Cw*05 Cw*06 Cw*15 Cw*17 Cw*18	2DL1
Bw4	B5 B13 B17 B27 B37 B38 B44 B47 B49 B51 B52 B53 B57 B58 B59 B63 B77 B*1513 B*1516 B*1517 B*1523 B*1524	3DL1

APPENDIX B

ACUTE GRAFT-VS-HOST DISEASE (GVHD)

Introduction

Investigators should document on a weekly basis (beginning with the day of transplant) the raw data for the GVHD target organs either in the medical record directly or on a trial-specific worksheet. This should include the extent of skin rash, if any; the bilirubin; the daily stool output; or number of stools per day for an outpatient. The weekly record should reflect the worst representative days of the preceding week for each target organ involvement. Biopsy confirmation of target organs is recommended in most circumstances to confirm the diagnosis acute GVHD.

In addition to the raw data record to verify acute GVHD organs staging, the relevant differential diagnoses should be recorded (e.g., drug rash, GI infection such as C. difficile, veno-occlusive disease (VOD), total parenteral nutrition (TPN), etc.) each week for target organ involvement. The record should indicate whether a biopsy was diagnostic, not diagnostic, or not done of each organ involved and should indicate when systemic GVHD therapy was initiated.

Grading

Acute GVHD grading should be performed by the consensus conference criteria (Przepiorka, et. al., 1994). See Acute GVHD Staging and Grading Tables**Figure 1.3.1 – Sample Data Sheet**

Clinical Acute GVHD Assessment

Date _____ Patient ID _____ Karnofsky/Lansky _____

	Code							Differential Diagnosis						
	0	1	2	3	4	5		GVHD	Drug Rxn	Cond Reg	TPN	Infect	VOD	Other
Skin	☐	☐	☐	☐	☐		% body rash:____	☐	☐	☐		☐		_____
Lower GI *	☐	☐	☐	☐	☐	☐	Vol: _____	☐	☐	☐	☐	☐		_____
Upper GI	☐	☐						☐	☐	☐	☐	☐		_____
Liver	☐	☐	☐	☐	☐		Max bili: _____	☐	☐	☐	☐	☐	☐	_____

Treatment: ☐ CSA ☐ Tacrolimus ☐ Pred ☐ Methylpred ☐ Ontak
☐ Pentostatin ☐ MMF ☐ Etanercept ☐ Other _____

Code Definitions:

Skin:
0 No rash
1 Maculopapular rash, <25% of body surface
2 Maculopapular rash, 25-50% of body surface
3 Generalized erythroderma
4 Generalized erythroderma with bullous formation and desquamation

Lower GI * (Diarrhea): (* GUT)
0 None
1 ≤500 mL/day or <280 mL/m^2
2 501-1000 mL/day or 280- 555 mL/m^2
3 1001-1500 mL/day or 556- 833 mL/m^2
4 >1500 mL/day or >833 mL/m^2
5 Severe abdominal pain with or without ileus, or stool with frank blood or melena

Upper GI:
0 No protracted nausea and vomiting
1 Persistent nausea, vomiting or anorexia

Liver (Bilirubin):
0 <2.0 mg/dl
1 2.1-3.0 mg/dl
2 3.1-6.0 mg/dl
3 6.1-15.0 mg/dl
4 >15.1 mg/dl

Acute GVHD STAGING and GRADING Tables

Staging*

	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4
Skin	No rash	Rash < 25% BSA	25-50%	> 50% Generalized erythroderma	Plus bullae and desquamation
Gut	< 500 mL diarrhea/day	501-1000 mL/day	1001–1500 mL/day	> 1500 mL/day	Severe abdominal pain & ileus
UGI		Severe nausea/vomiting			
Liver	Bilirubin ≤ 2 mg/dl	2.1-3 mg/dl	3.1-6mg/dl	6.1-15mg/dl	> 15 mg/dl

Grading Index of Acute GVHD*

	Grade A	Grade B	Grade C	Grade D
Skin	1	2	3	4
Gut	0	1-2	3	4
Upper GI	0	1		
Liver	0	1-2	3	4

Data Collection

Weekly GVHD raw data, staging, and grading should be collected until 60-70 days post-transplant for all patients and, if feasible, to Day +100. Subsequently, monthly data involving acute GVHD symptomatology should be collected for all patients remaining on immunosuppressive therapy until two months after discontinuation of immunosuppressive treatment for acute GVHD. If acute GVHD develops later or flares, the data collection should be continued frequently (every 1, 2, or 4 weeks as feasible) in sufficient detail to monitor the progress of the disease.

APPENDIX C

Definitions of Chronic GVHD

The diagnosis of chronic GVHD is based on both clinical and histopathologic findings for each organ system. Pathognomonic and possible manifestations of chronic GVHD are outlined below; possible manifestations should be further evaluated to rule out other potential non-chronic GVHD etiologies. For example, pancreatic enzyme insufficiency may cause malabsorption and weight loss independent of chronic GVHD.

Time since transplant will not be used to distinguish acute from chronic GVHD (e.g., chronic GVHD may occur before Day 100, and acute GVHD may occur after Day 100). If acute GVHD is suspected after Day 60 or chronic GVHD is suspected before Day 100, biopsies are strongly encouraged to confirm the diagnosis. Additionally, biopsies are encouraged to confirm the diagnosis of chronic GVHD in patients with "possible" manifestations (see Table 2.3.1).

Table 2.3.1 – Definite and Possible Manifestations of Chronic GVHD

Organ System	Definite manifestations of chronic GVHD	Possible manifestations of chronic GVHD
Skin	Scleroderma (superficial or fasciitis), lichen planus, vitiligo, scarring alopecia, hyperkeratosis pilaris, contractures from skin immobility, nail bed dysplasia	Eczematoid rash, dry skin, maculopapular rash, hyperpigmentation, hair loss
Mucous membranes	Lichen planus, non-infectious ulcers, corneal erosions/non-infectious conjunctivitis	Xerostomia, keratoconjunctivitis sicca
GI tract	Esophageal strictures, steatorrhea	Anorexia, malabsorption, weight loss, diarrhea, abdominal pain
Liver	None	Elevation of alkaline phosphatase, transaminitis, cholangitis, hyperbilirubinemia
GU	Vaginal stricture, lichen planus	Non-infectious vaginitis, vaginal atrophy
Musculoskeletal/ Serosa	Non-septic arthritis, myositis, myasthenia, polyserositis, contractures from joint immobilization	Arthralgia
Hematologic	None	Thrombocytopenia, eosinophilia, autoimmune cytopenias
Lung	Bronchiolitis obliterans	Bronchiolitis obliterans with organizing pneumonia, interstitial pneumonitis

Standards of Care

The severity of chronic GVHD reporting and the treatment algorithm will be according to the UW BMT program SOP regarding chronic GVHD.

ADDENDUM

Study Summary 02/17/2017

Study design, primary and secondary objectives remain the same throughout the study. Patient enrollment was completed on December 31, 2016, at which time the study was closed to enrollment.

Twenty patients enrolled on study. Eighteen patients are off study and two remain on study, on treatment. Ongoing treatment is proceeding according to the schedule of evaluations (Section 9.0) for both patients. Pre-study and pre-transplant evaluations are completed for both patients. The remaining schedule of evaluations include post-transplant weekly follow-up to Day 100 and again at 6, 9 and 12 months. Follow up procedures include physical exam, bone marrow aspirate and biopsy, medical history, blood sample collection for routine panel and toxicity assessment.

There is no new information at this time that would impact study rationale and all subject data collected under protocol 2012-0217, including any on-going follow-up or previously enrolled patients will be maintained and analyzed under the auspices of protocol application 2017-0116.

AML remains a rare disease with limited treatment options. Treatment options include:

- Induction therapy with chemotherapy drug with the goal of achieving remission

- Stem cell transplantation (hematopoietic cell transplant (HCT) or bone marrow transplant (BMT))

- Participation in clinical trials. Clinicaltrials.gov lists 144 clinical trials for patients with AML, including those listed below. Trials are listed as completed, active, recruiting, results pending, etc. Four trial titles are listed below.

- 10-day Decitabine, Fludarabine and 2 Gray TBI as Conditioning Strategy for Poor and Very Poor Risk AML in CR1(NCT02252107)

- Decitabine, Donor Natural Killer Cells, and Aldesleukin in Treating Patients With Relapsed or Refractory Acute Myeloid Leukemia (NCT02316964)

- Leukemia SPORE Phase II Randomized Study of Decitabine Versus Decitabine and Carboplatin Versus Decitabine and Arsenic in Relapsed, Refractory, and Elderly AML and MDS(NCT02190695)

- Decitabine, Filgrastim, Cladribine, Cytarabine, and Mitoxantrone Hydrochloride in Treating Patients With Newly Diagnosed, Relapsed, or Refractory Acute Myeloid Leukemia or High-Risk Myelodysplastic Syndrome(NCT02921061)