Title: Epigenetic priming with 5-Azacytidine prior to in vivo T-cell depleted allogeneic stem cell transplantation for patients with high-risk myeloid malignancies in morphologic remission

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Amendment History

- Original Protocol Date: January 6, 2014
- Amendment #1: April 9, 2015
- Amendment #2: November 2, 2015
- Amendment #3: April 16, 2018

Epigenetic priming with 5-Azacytidine prior to in vivo T-cell depleted allogeneic stem cell transplantation for patients with high risk myeloid malignancies in morphologic remission

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SCHEMA

PROTOCOL TITLE: Epigenetic priming with 5-Azacytidine prior to in vivo T-cell depleted allogeneic stem cell transplantation for patients with high risk myeloid malignancies in morphologic remission	
PROTOCOL NUMBER	1306014009
FINAL PROTOCOL DATE	
STUDY DRUG	5-Azacytidine
INDICATION	Poor risk AML in CR1, High Risk MDS in CR, AML in CR>1
STUDY PHASE	Phase II

STUDY OBJECTIVES:

Primary Objective:

To evaluate 6 month, 1-year and 2-year disease-free survival and overall survival after allogeneic stem cell transplantation with 5-Azacytidine priming immediately prior to conditioning and Alemtuzumab in vivo T- cell depletion in patients with poor risk myeloid malignancies in complete morphologic remission.

Secondary Objectives:

To evaluate the incidence and severity of acute and chronic GVHD after 5-azacytidine priming prior to conditioning for allogeneic stem cell transplantation with Alemtuzumab-based in vivo T-cell depletion in patients with poor risk myeloid malignancies in complete morphologic remission.

To evaluate engraftment and chimerism patterns after 5-azacytdine primed conditioning for stem cell transplantation with Alemtuzumab-based in vivo T-cell depletion in patients with poor risk myeloid malignancies in complete morphologic remission.

To evaluate changes in global gene methylation by 5-azacytidine prior to conditioning for stem cell transplantation with Alemtuzumab-based in vivo T-cell depletion in patients with poor risk myeloid malignancies in complete morphologic remission.

STUDY DESIGN:
This open label two-step phase II study is designed to determine the safety and efficacy of epigenetic priming with the hypomethylating agent 5-Azacytidine immediately prior to reduced intensity conditioning for an in vivo T-cell depleted hematopoietic stem cell transplantation for high risk myeloid malignancies in complete morphologic remission (CR).

The inclusion criteria are AML with poor risk cytogenetics or other poor risk characteristics in CR 1 or beyond, high risk MDS in CR, other AML in CR 2 or beyond (excluding promyelocytic leukemia) and secondary AML after MDS/MPN in CR.
These patients will be given a five day course of subcutaneous 5-azacytidine, followed by a reduced intensity conditioning regimen of fludarabine and melphalan with total body irradiation prior to an allogeneic hematopoietic stem cell transplantation from a related or unrelated HLA matched donor.
The effect of 5-azacytidine on global gene methylation will be assessed. Evaluations for safety, in particular for graft failure, transplant related mortality and acute graft versus host disease will be made on a weekly basis. Efficacy, as defined by disease free survival, will be evaluated with a bone marrow biopsy at the standard time points, which are one-, three-, six-, and twelve-months after transplant and upon clinical suspicion within regular follow-up visits - weekly for the first 3 months, then biweekly for 3 months, then monthly until one-year post-SCT. Thereafter, unless otherwise dictated by the clinical scenario, the follow up visits will be every 3 months.
Patients will be followed for the primary endpoints disease relapse or death and engraftment kinetics, incidence of acute and chronic graft versus host disease will be analyzed.

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1. OBJECTIVES

1.1. Primary Objectives

To evaluate disease free survival and overall survival after allogeneic stem cell transplantation with 5-azacytidine priming immediately prior to conditioning and Alemtuzumab-based in vivo T- cell depletion in patients with poor risk myeloid malignancies in complete morphologic remission.

1.2. Secondary Objectives

To evaluate the incidence and severity of acute and chronic GVHD after 5-azacytidine priming prior to conditioning for allogeneic stem cell transplantation with Alemtuzumab-based in vivo T-cell depletion in patients with poor risk myeloid malignancies in complete morphologic remission.

To evaluate engraftment and chimerism patterns after 5-azacytidine primed conditioning for stem cell transplantation with Alemtuzumab-based in vivo T-cell depletion in patients with poor risk myeloid malignancies in complete morphologic remission.

To evaluate changes in global gene methylation induced by 5-azacytidine prior to conditioning for stem cell transplantation with Alemtuzumab-based in vivo T-cell depletion in patients with poor risk myeloid malignancies in complete morphologic remission.

2. BACKGROUND

2.1 Disease

Poor risk AML and MDS and allogeneic stem cell transplantation

The prognosis of AML with poor risk cytogenetics treated with conventional chemotherapy is dismal with 5 year overall survival rates ranging from 4-14%[1]. Similarly, the outlook for patients with intermediate-2 or high grade MDS is grim, with median survivals of 1.2 and 0.4 years, respectively[2].

Allogeneic stem cell transplantation remains the most promising therapeutic modality and a multitude of trials have confirmed its potentially curative role for these diseases. But even after transplant outcomes are worse for patients with adverse risk factors, particularly patients with monosomal karyotypes[3]. A study by van Straaten of allo transplant for patients with AML or MDS with chromosome 5 and 7 abnormalities showed a 25% 3-year overall survival rate. Benefits were confined to

patients younger than 40 years of age with overall survival of 38% versus 8% for those older than 40[4].

More recent studies for both high risk AML and MDS as well as secondary AML still show a long term overall survival rate between 20% and 45% after allogeneic transplants[5,6,7]. Recurrence of leukemia constitutes the most important cause of treatment failure for patients transplanted in remission. The ultimate objective of our proposed study is to develop strategies to further improve the outcomes of allogeneic transplantation in high risk patients.

Evolution of transplant related mortality and relapse rate

In the past decade, the outcome of stem cell transplantation has significantly improved, in part due to the advances of conditioning regimens as well as improved supportive care. While the risk of non-relapse mortality has been reduced by 52% since the years 1993 through 1997, the rates of relapse have undergone a more modest reduction of 21%[8].Reductions in the intensity of the pretransplant conditioning have opened the option of an allogeneic stem cell transplant to patients previously thought not fit for the procedure but the success of non-myeloablative transplants is compromised by relapse rates ranging from 40-50% (9,10).
A recent retrospective analysis of over 5900 patients transplanted for AML in CR over the last 25 years showed that the improvement in overall survival is mainly due to reductions in NRM while relapse rates of the underlying malignancy have remained unchanged (11). The development of conditioning regimens with increased antileukemic efficacy but limited toxicity represents a major challenge.
It is with this purpose that we propose a novel strategy to enhance the susceptibility of leukemic cells to the conditioning regimen.

DNA methylation and sensitivity to chemotherapy

Epigenetic silencing through hypermethylation of genes that govern apoptosis can confer refractoriness to chemotherapy, a process that may be reversed through hypomethylating agents (12, 13). There is now burgeoning preclinical evidence for a chemosensitizing effect of the hypomethylating agents 5-Azacytidine and Decitabine for a range of hematologic malignancies and solid tumors as well as their ability to restore disrupted proapoptotic pathways (13, 14, 15, 16, 17).
Furthermore, a prospective study conducted at our center by Scandura et al demonstrated the feasibility of priming with decitabine prior to induction chemotherapy for AML (18). Although it was a phase 1 study not designed to assess efficacy there was an encouraging CR rate of 83% for intermediate and poor risk AML patients.
In the current study we plan to use azacytidine based chemosensitization prior to transplant with a well-established conditioning regimen,

2.2 Investigational Agent

5-Azacytidine

5-Azacitidine (4-amino-1-β-D-ribofuranosyl-s-triazin-2(1*H*)-one) is an analog of the naturally occurring pyrimidine nucleoside, cytidine. It differs from cytidine in having nitrogen in the 5-position of the heterocyclic ring
Therapeutic classification: At lower doses acts as DNA-hypomethylating agent. At higher doses acts as a cytidine analog.

Pharmacological data: 5-azacytidine is supplied as a lyophilized powder in single-use vials containing 100 mg of each 5-azacytidine and mannitol as a freeze dried cake or powder. It is reconstituted with 4ml of sterile water per 100 mg of drug.
Dosing information: The usual dose is 75 to 100 mg per m2 by subcutaneous injection daily for 7 days, repeated every 4 weeks.
Drug interactions: none known
Side effects: Myelosuppression, with neutropenia, thrombocytopenia, anemia, and febrile neutropenia is the major dose limiting toxicity of 5-azacytidine. Nausea, vomiting, diarrhea, dyspepsia and abdominal pain are common. Signs of CNS depression, including lethargy, confusion and coma have been reported.
Laboratory abnormalities such as hyperglycemia, hypomagnesemia, hypokalemia, and elevations of liver function tests are common.
Mechanism of action: At lower doses, 5-azacytidine causes hypomethylation of DNA. In neoplastic cells, this hypomethylation can either induce apoptosis or restore normal function to genes that are critical for the control of cellular differentiation, proliferation and apoptosis. At higher doses, it acts as a cytidine analog, becoming incorporated into nucleic acids, and causing DNA transcription and replication errors. Nonproliferating cells are relatively insensitive to 5-azacytidine.
Pharmacokinetics/metabolism: The currently available form of the drug is not orally bioavailable. Following administration, the drug is activated inside cells to its triphosphate form. It is metabolized in the liver and excreted primarily in the urine. The elimination half time is about 4 hours.

2.3 Rationale

We are basing the conditioning regimen and graft versus host prophylaxis on a study previously published by van Besien et al. combining a Fludarabine/Melphalan based reduced intensity conditioning regimen with Alemtuzumab as an in vivo T-cell depleting agent with standard risk AML and MDS as indication. While retaining the efficacy of the prior regimen of Fludarabine/Melphalan without T-cell depletion, this regimen has shown remarkably low rates of grade 3-4 acute GVHD (10%) and extensive chronic GVHD (18%). Overall survival at 18 months was 48% (19,20,21).

The disappointing lack of improvement in relapse rates for AML post allogeneic stem cell transplantation in the last 20 years despite multiple variations of conditioning regimens has called our attention to new ways of increasing the therapeutic efficacy of already existing conditioning regimens (8,9).

Given the wealth of preclinical data on the chemotherapy sensitizing effect of hypomethylating agents on a wide range of tumors (12-17) and the proof of principle of clinical feasibility of this concept in AML (18) there is rationale to employ epigenetic priming before conditioning for an allogeneic stem cell transplant. In the study conducted by Scandura et al, increased hematologic toxicity had been a concern initially, but in fact there was a suggestion of more rapid platelet recovery and no delay in neutrophil recovery in the patients undergoing pre induction Decitabine, dispelling this fear.

Furthermore, in the case of allogeneic transplantation, hematologic toxicity is not a major concern as recovery is based on engraftment of allogeneic cells and given the short half life of 5-Azacytidine (4 hours), there should no longer be any clinically relevant serum drug levels at the time of transplantation.

The dosing of 5-azacytidine is derived from both clinical experience and preclinical efficacy studies comparing the hypomethylating activity of 5-azacyidine and decitabine which showed a 2 to 10 fold stronger hypomethylating activity on AML cells for decitabine compared to 5-azacytidine.(22).

The routine dose for 5-azacytidine to treat MDS is 75 mg/m2 for 7 days. (23). Our epigenetic priming study employed decitabine at 20 mg/m2 /d either as 1h infusion or 24h CVI for 3, 5 or 7 days and no significant difference in the global hypomethylating activity was found.

Given the possibility of administering 5-azacytidine subcutaneously without the need for hospital admission we chose this drug over decitabine. A dosing schedule of 50 mg/m2/d for 5 days appears sufficient to induce global hypomethylation in the genome of hematopoietic cells. This hypothesis will be verified by pre- and post-priming measurements of the methylation status of the HIST1H2AA gene in bone marrow samples (see below).

2.4 Correlative Studies Background

We will use the pharmacodynamic measurement of DNA hypomethylation to assess and efficacy of our 5-azacytidine dosing schedule. At the dose level used in this protocol, 5-azacytidine primarily acts to induce DNA hypomethylation and the site and extent of demethylation is thought to underlie its clinical activity. Previous studies have established that 5-azacytidine is a potent hypomethylating agent capable of decreasing both universal cytosine methylation (at repetitive elements, or in total hydrolyzed genomic DNA) and select CpG Island methylation.

As a measure of 5-azacytidine's ability to induce genome wide hypomethylation we will measure the methylation status of the HIST1H2AA gene in in CD 34+ bone marrow cells, which can serve as a surrogate marker for global gene methylation status (18).

We will then compare patient samples obtained before, and immediately following priming treatment to establish the pharmacodynamic activity of 5-azacytidine.

3. PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1 Patients must have histologically or cytologically confirmed AML or MDS as specified below:

Acute myeloid leukemia with poor risk cytogenetics (1) in complete morphologic remission. These include: del (5q)/-5, del (7q)/-7, abn 3q, 9q, 11q, 20q, 21q, 17p, t(6;9), t(9;22) or complex karyotypes (≥ 3 unrelated abnormalities)

Acute myeloid leukemia with either Flt-3-, TET 2-, p53-, DNMT3A- or ASXL 1-mutation, mutations of genes involved in the chromatin/spliceosome category (EZH2, SRSF2, U2AF1, ZRSR2), BCOR and RUNX1, as well as MLL rearrangement EVI1 overexpression in complete morphologic remission

Acute myeloid leukemia with a white blood cell count of ≥50000/mcL at presentation in first complete morphologic remission

Acute myeloid leukemia in first complete morphologic remission, having required more than one course of induction chemotherapy to attain remission status

Acute myeloid leukemia, all types, excluding M3 (Promyelocytic leukemia) in second or higher complete morphologic remission

Myelodysplastic syndromes (MDS) (intermediate-2, high risk and CMML with bone marrow blasts <5%)

Secondary acute myeloid leukemia on the basis of prior MDS or prior myeloproliferative neoplasm (MPN) in complete morphologic remission

3.1.2 Age ≥18 years.

3.1.3 Life expectancy not severely limited by concomitant disease.

3.1.4 KPS at least 70.

3.1.5 Adequate organ function as defined below:
Serum Bilirubin: <2.0 mg/dL
ALT (SGPT): <3X upper limit of normal
Creatinine Clearance: >60 mL/min (eGFR as estimated by the modified MDRD equation)

Patients with decreased LVEF or abnormal PFTs will be evaluated by cardiology or pulmonary prior to enrollment on this protocol and their eligibility will be determined by the compound of their clinical cardiopulmonary status and testing results as patient with abnormal echo and /or PFTs can have adequate cardiopulmonary functioning

3.1.6 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Evidence of chronic active hepatitis or cirrhosis.

3.2.2 HIV infection.

3.2.3 Uncontrolled illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.4 Pregnant and lactating women are excluded from the study because the risks to an unborn fetus or potential risks in nursing infants are unknown.

3.2.5 There are no prior therapies or concomitant medications that would render the patients ineligible.

4. REGISTRATION PROCEDURES

Central Patient Registration

Patients will be centrally registered with the Weill Cornell Medical College (WCMC), Division of Hematology and Medical Oncology Clinical Research Office.

To register a patient, fax the following documents to the Clinical Research Office at (646) 962-1610:

- WCMC Patient registration form
- First and last page of the fully executed informed consent form, plus additional pages if checkboxes for correlative studies are required.
- Fully executed HIPAA research authorization form
- Eligibility checklist signed and dated by investigator and research nurse
- Documentation of any eligibility waivers granted
- For inpatients, signed consent documentation template

Central registration information is reviewed and entered into the HemOnc centralized research database. Documentation of patient registration will be faxed to the Investigational Pharmacy to allow for release of study agent.

5. TREATMENT PLAN, DONOR SELECTION AND EVALUATIONS

5.1 Agent Administration

Treatment will be administered on an *outpatient* basis.

5-Azacytidine priming

5-Azacytidine 50 mg/m2 subcutaneously daily at the same time on days -11, -10, -9, -8 and -7. This will be administered on an outpatient basis.

Hospital admission will usually take place on the first day of the conditioning regimen.

Conditioning with Fludarabine-Melphalan-Alemtuzumab

Fludarabine 40 mg/m^2 intravenously daily at the same time over 30 minutes on days -6,-5,-4,-3.
Melphalan 140 mg/m^2 IV on day -3.
Alemtuzumab, 30 mg subcutaneously on days -4 and -2 for unrelated donors, and on day -2 for related donors.

Total Body Irradiation (TBI)

Given a recent publication indicating better disease control for this condition regimen with the inclusion of low dose TBI (24), patients will receive 2 doses of TBI of 200 cGy each on one day of the conditioning regimen (between days -6 and -3)

Stem cell infusion

On day 0 the stem cell product will be infused according to BMT unit policy.

Allogeneic hematopoietic stem cells (HSC) may be infused fresh or after cryopreservation depending on donor availability.

All HSC procurement through the NMDP will done in strict compliance with the protocols, policies, and procedures established by the NMDP.

Graft versus Host Disease prophylaxis

Tacrolimus 0.03 mg/kg/day IV CI over 24 hr from 4 PM day -2 until engraftment or when patient is able to take PO, then tacrolimus 0.09 mg/kg PO in 2 divided doses. Tacrolimus should be given at full dose to maintain levels of 5-15 ng/mL through day 100. Thereafter, tacrolimus will be tapered by 20% every week.

In recipients of unrelated donor transplants, tacrolimus will be continued until day 180. Thereafter it will be tapered by 20% per week.

Infection, toxicity or other clinical circumstances may prompt earlier discontinuation or adjustment of doses. In the presence of GVHD, a clinical decision by the attending physician will determine if tacrolimus can be tapered or should be continued. PO tacrolimus can be used when IV access for CI tacrolimus is unavailable.

5.2 General Concomitant Medication and Supportive Care Guidelines

Infection prophylaxis, growth factor administration and supportive care will be as per BMT unit policy. CMV prophylaxis with high dose ganciclovir-acyclovir-valacyclovir is strongly recommended.

5.3 Evaluations

Pre-Conditioning Evaluation

Pre-Transplant Evaluation will follow transplant program policies.

Prior to of Azacytidine priming, a bone marrow biopsy and aspiration will be performed to ascertain the patient's status in complete morphologic remission. This is part of the standard of care evaluation for every patient prior to an allogeneic stem cell transplantation.

An additional 3-5 cc of bone marrow aspirate will be set aside for measurement of HIST1H2AA methylation level.

On the first day after completion of the course of 5-Azacytidine, another bone marrow aspiration will be obtained and again there will be sample of 3-5 cc set aside for HIST1H2AA methylation level evaluation. This bone marrow aspiration is solely for research purposes and will serve to evaluate the changes in global gene methylation in response to 5-Azacytidine as well as the impact of 5-Azacytidine on the morphology and cellularity of the bone marrow.

Post-transplant Evaluation

Post-transplant Evaluation will follow the routine for the allogeneic transplant patient. The tests outlined in Table 1 are strongly recommended, but can be adjusted as dictated by clinical circumstances.

Table 1: The follow-up schedule for scheduled study visits is outlined below. These tests may be adjusted as warranted by clinical circumstances and evolving transplant policy. Please also refer to institutional transplant work up guidelines.

	Prior to aza (between day-53 and -11)	After aza Day -6	14	21	28	60	70	100	180	365	q365
Physical exam, height, weight, and KPS performance status	X				X			X	X	X	
GVHD and other morbidity assessments[5]					X			X	X	X	
Toxicity assessments	X				X			X	X	X	
Electrocardiogram	X										
Infectious disease titers[3]	X										
Chest CT or x-ray[4]	X										
LVEF, or shortening fraction[4]	X										
DLCO, FEV I and FVC and 02-saturation[4]	X										
HLA typing[6]	X										
B-HCG serum pregnancy test (pre-menopausal females only) within 4 weeks of conditioning	X										

	Prior to aza (between day-53 and -11)	After aza Day -6	14	21	28	60	70	100	180	365	q365
CBC[1] , differential, platelet count, and blood chemistries[2]	X	X	X	X	X			X	X	X	
Bone marrow biopsy and aspirate for pathology	X	X			X			X	X	X	X
Chimerism	X		X		X	X	X	X	X	X	
Lymphocyte Subsets and Ig Levels	X				X			X	X	X	
Pneumococcal antibody levels[5]	X								X	X	
HLA Antibodies[7]	X										
Research Blood and Marrow Samples	X (this will occur within 10 days prior to Aza priming)	X			X	X		X	X	X	X

Note:
**The exact day of the tests is approximate. Tests can be scheduled several days before or after. The window is up to five days before and after in the first three weeks. Up to seven days before and after in the first 100 days and up to one month before and after at subsequent time points.*
***Baseline tests: see also section 7.0*

1. CBC performed at least three times a week from Day 0 until ANC >500 mcL for three days after nadir. CBC performed twice weekly until Day 28. CBC performed approximately weekly after Day 28 until 12 weeks post-transplant.
2. Blood chemistries to include a standard complete metabolic panel, LDH, and magnesium.
3. Infectious disease titers include: CMV, Hepatitis panel (HepBSAb, HepBSAg, HepB Core Ab, HepCAb), herpes simplex virus, syphilis, HIV and HTLV I/II antibody.
4. CT scan and PFTs can be obtained up to 2 months prior to conditioning, unless there have been intercurrent infections affecting the respiratory tract.
5. Echocardiogram and ECG can be obtained up to 2 months prior to conditioning, unless the patient received cardiotoxic chemotherapy or had any cardiac event (ie MI) in the interim.
6. HLA Typing can occur at any time prior to the administration of 5-azacytidine as HLA Typing does not change.

7. Testing for HLA antibodies can be performed at any time prior to the administration of 5-azacytidine as the frequency of testing will vary depending on the donor being used for transplant.

Blood and bone marrow samples

Samples of bone marrow will be obtained at the following time points:

Prior to priming with 5-Azacytidine (bone marrow) within 10 days before therapy – this is research-specific
After priming with 5-Azacytidine on day -6 (bone marrow) – this is research-specific
Day +28 +/- 4 days **(BM and blood)**
Day +60 +/- 7 days **(Blood)**
Day +100 +/- 7 days **(BM and blood)**
Day +180 +/- 7 days **(BM and blood)**
At relapse (BM and blood)
One year and yearly thereafter (BM and blood)

The 2 bone marrow samples will be examined for global gene methylation status by measuring the methylation status of HIST1H2AA by Dr. Joseph Scandura (see section 9).

5.4 Duration of Therapy and Criteria for Removal from Study

As the stem cell transplantation is a non-repetitive intervention there will be no cycles of treatment. Patients will be followed for disease free and overall survival as well as for secondary endpoints. They will remain on study until one of the following occurs

- Disease recurrence
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.5 Duration of Follow Up

Patients will be followed for 5 years after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6. DOSING DELAYS/DOSE MODIFICATIONS

Given the higher than expected rate of grade 3 or 4 renal toxicity after the first 12 patients were treated with azacytidine 75m/m2 daily x 5 days (2 with grade 3, 2 with grade 4) we will reduce the dose of Azacytidine to 50 mg/m2 daily x 5 days. Should renal toxicity grade 3 or 4 occur by day 15 in 2 or more of the first six patients, then the dose of azacytidine will be further reduced to 32 mg/m2 / day x 5 days. If 2 or more grade 3 or 4 toxicities occur among the first six patients at the 32 mg/m2/d level, the trial will be considered too toxic and closed for accrual. If one or fewer grade 3 or 4 renal toxicities occur, accrual will continue at this dose level with close continuous observation for renal toxicity.

7. ADVERSE EVENT REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The investigator will be required to provide appropriate information concerning any findings that suggest significant hazards, contraindications, side effects, or precautions pertinent to the safe use of the drug under investigation. Safety will be monitored by evaluation of adverse events reported by patients or observed by investigators or research staff, as well as by other investigations such as clinical laboratory tests, x-rays, electrocardiographs, etc.
For the purposes of this study, the pre-conditioning 5-azacytidine administration is the only investigational component of the treatment. All other components (conditioning therapy, gvhd prophylaxis, transplant etc.) are considered standard interventions.

7.1 Investigational Agent Risks:

5-Azacytidine:

Myelosuppression, with neutropenia, thrombocytopenia, anemia, and febrile neutropenia is the major dose limiting toxicity of 5-azacytidine.
Nausea, vomiting, diarrhea, dyspepsia and abdominal pain are common.
Signs of CNS depression, including lethargy, confusion and coma have been reported.
Laboratory abnormalities such as hyperglycemia, hypomagnesemia, hypokalemia, and elevations of liver function tests are common.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events

(CTCAE) version 4.0 will be utilized for AE reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Recording and Reporting of Adverse Events and SAE

- All infections, acute GVHD and any other grade 3 or higher adverse events will be recorded on a patient specific adverse event log. The AE log will be maintained by the research staff and kept in the patient's research chart.

- Stem Cell transplant is a complex procedure with prolonged initial admission and numerous immediate and delayed complications as well as frequent readmissions.

- Expected adverse events are those listed in the consent form and include regimen-related toxicities, myelosuppression, opportunistic infections and GVHD. Expected adverse events of grade III and higher CTC severity will be captured in the transplant database and reported to the IRB upon request.

- Grade three or higher adverse events that are judged to be unexpected and possibly related to the investigational procedure, will be reported to the study chairman within 48 hours of investigator notification. Such events will be reported to the local IRB within the institution's prescribed time period.

- All fatal Adverse Events will also be reported to the study chairman within 48 hours and reported to the local IRB within the institution's prescribed time period.

	Immediate Reporting	**Report quarterly**
Hematopoietic Toxicity	**Graft failure and death**	**Time to Hematopoietic recovery**
Extramedullary Toxicity	**Fatal toxicity. Grade III-IV Toxicity deemed unexpected and possibly related to the investigational procedure**	**Grade III-IV toxicities not deemed expected/unrelated**
Infections	**Fatal**	**Grade II-IV**

Acute GVHD	**Fatal or Grade IV**	**Grade II-III**
Chronic GVHD	**Fatal**	**Limited and extensive**

7.4 Reporting and Exclusions

7. 4.1 Evaluation of toxicity. All patients will be evaluable for toxicity from the time of their first treatment with *5-Azacytidine.*

7. 4.2 *Evaluation of efficacy.* All patients included in the study will be assessed for the specified endpoints if they have proceeded through stem cell transplantation

7.5 Reporting of SAE to FDA

If a reportable AE occurs on this study, the event will be filed on a MedWatch form with the FDA.

CDER INDs:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with *Investigational Agent* can be found in Section 7.1.

8.1 Investigational Agent

5-azacytidine is supplied as a lyophilized powder in single-use vials containing 100 mg of each azacytidine and mannitol as a freeze dried cake or powder. It is reconstituted with 4ml of sterile water per 100 mg of drug.

8.2 Availability

5-azacytidine is an approved drug and will be commercially available.

8.3 Agent Ordering

See above

8.4 Agent Accountability

The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and disposition of all agents on a Drug Accountability Record Form (DARF).

8.5 Other agents and procedures:

Melphalan:

Therapeutic Classification: Alkylating agent.

Pharmaceutical data: Supplied as 50 mg vials reconstitute in 10 ml of diluent.

Stability and storage: Reconstituted solution retains 90% potency for about 3 hours at 30° C. Storage at 5° C results in precipitation. Intact packages can be stored at room temperature.

Route of Administration: intravenous, oral, intraperitoneal.

Side effects: Dose limiting toxicity is hematological. Possible delayed toxicity in patients with irradiated bone marrow. Gastrointestinal toxicity is infrequent and usually mild; occasional azotemia has been reported in certain cases. Tissue necrosis may result if infiltration occurs.

Mechanism of action: Interferes with DNA replication and transcription of RNA and ultimately results in the disruption of nucleic acid function.

Human Pharmacology: Melphalan is incompletely and erratically absorbed, has a prolonged plasma half-life, and is excreted largely unchanged in the urine. Intravenous melphalan result in very high peak concentration, plasma levels followed by an initial rapid decay curve (half-life 30-60 minutes). Most of the administered dose is recovered in the urine, almost none in the feces, and no significant quantity of any metabolite has been identified.

Human Toxicology: Myelosuppresion is the major toxicity and is dose related. Nausea and vomiting is almost uniformly observed, mucositis and diarrhea have also been observed. Transient rises in BUN and creatinine have occurred with high dose melphalan as also acute renal failure. Hypersensitivity reactions to high dose melphalan have been reported.

Fludarabine:

Fludarabine is the 2-fluoro, 5-phosphate derivative of vidarabine.

DOSING INFORMATION: Doses of 25 mg/mL(2)/day (30-minute infusion) for 5 days every 4 weeks has been effective previously treated patients with chronic lymphocytic leukemia; in non-Hodgkin's lymphoma, a loading dose of 20 mg/m(2) intravenously followed by a continuous intravenous infusion of 30 mg/m(2)/24 hours for 48 hours, has been effective; dose reductions are suggested in renal insufficiency.

PHARMACOKINETICS: Following intravenous administration, fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-vidarabine, which subsequently enters tumor cells and is phosphorylated to the active triphosphate derivative; peak plasma levels of 2-fluoro-vidarabine have ranged from 0.3 to 0.9 mcg/mL following a short infusion of 25 mg/m(2) fludarabine; 24% of a dose of fludarabine is recovered in the urine as 2-fluoro-vidarabine; the elimination half-life of 2-fluoro-vidarabine is 9 hours.

CAUTIONS: Myelosuppression, particularly neutropenia, is the predominant adverse effect; a severe neurotoxicity has been observed, mainly with higher doses; other adverse effects include nausea, vomiting, diarrhea, stomatitis, skin rash, and somnolence; pneumonitis has been reported in 1 patient.

CLINICAL APPLICATIONS: Intravenous fludarabine has been highly effective in heavily pretreated patients with chronic lymphocytic leukemia; the drug has also produced responses in patients with non-Hodgkin's lymphoma and acute leukemia; however, neurotoxicity has been a major concern, even with low doses, and more studies are needed to clarify its ultimate place in therapy.

Alemtuzumab:

Therapeutic Classification: Humanized monoclonal antibody

Side effects: The infusion of Alemtuzumab has been associated with fever, nausea, headache, vomiting, rash, chills and rigor. Occasionally hypotension and bronchospasm have been reported. This can be managed by adequate premedication.

Pharmaceutical Data: Alemtuzumab is supplied as a purified preparation diluted in PBS with 0.05 mm EDTA. Each Ampoule contains 30 mg of Alemtuzumab.

Administration: 20 mg of Alemtuzumab is given intravenously or subcutaneously for 3 to 5 days.

Mechanism of Action: Alemtuzumab is a monoclonal antibody directed against CD52, an epitope that is abundantly expressed on T- and B- lymphocytes, but not on NK cells. It has

been extensively used for the prevention of GVHD both in vitro and in vivo.

Tacrolimus:

TACROLIMUS is a macrolide compound with potent immunosuppressant properties. DOSING INFORMATION: TACROLIMUS is usually given intravenously initially in doses of 0.03 mg/kg/day for 3 days, followed by conversion to oral therapy (0.09 mg/kg twice daily); dose adjustments are required in patients with hepatic dysfunction.

PHARMACOKINETICS: The oral absorption of TACROLIMUS is erratic and incomplete; absolute bioavailability is approximately 25%; peak serum levels are seen 1 to 4 h after an oral dose, and therapeutic serum concentrations have ranged from 0.2 to 6 ng/mL; TACROLIMUS is extensively metabolized in the liver, with only small amounts of unchanged drug (2% or less) being recovered in the urine; the elimination half-life of TACROLIMUS is approximately 10 h.

CAUTIONS: Common adverse effects of TACROLIMUS are headache, hyperesthesia, tremors, circumoral numbness, insomnia, nausea, abdominal discomfort, and appetite insomnia, nausea, abdominal discomfort, and appetite changes; all of these effects occur primarily with IV TACROLIMUS and are more frequent during combined use of TACROLIMUS and CYCLOSPORINE; other adverse effects include nephrotoxicity, hyperkalemia, hyperuricemia, hyperglycemia, dysphasia, photophobia, flushing, and lymphoproliferative disorder; unlike CYCLOSPORINE, hirsutism, gingival hyperplasia, and hypertension are generally not seen with TACROLIMUS; combined therapy with CYCLOSPORINE has resulted in increases in cyclosporine serum levels and more severe nephrotoxicity.

CLINICAL APPLICATIONS: TACROLIMUS is primarily indicated for prophylaxis of organ rejection in patients receiving an allogeneic liver transplant.

Bone marrow biopsy and aspiration:

-Bleeding

-Pain

-Infection

All these risks are minimized by application of sterile technique, local anesthesia and pressure dressing.

Stem Cell infusion:

Chest pain
Chills

Drop in blood pressure
Fever
Flushing
Headache
Hives
Nausea
Pain
Shortness of breath

The risks are minimized by premedication with Acetaminophen and Diphenhydramine and close monitoring during the stem cell infusion enabling the team to intervene immediately should an adverse event occur.

Stem Cell Transplantation- generic risks and complications:

Graft failure
Acute and chronic graft versus host disease
Hemorrhage to vital organs
Venoocclusive disease of the liver (Sinusoidal obstruction syndrome – SOS)
Anemia, leukopenia, thrombocytopenia
Infections (bacterial, viral, fungal, protozoal)
Mucositis of oral cavity, esophagus, stomach and bowels
Damage to kidneys, liver, lungs and heart
Nausea, vomiting and resulting malnutrition
Pain
Cataracts
Premature menopause and infertility
Osteoporosis
Avascular necrosis of bones due to steroid therapy
Thyroid dysfunction
Death

The patients are monitored on a continuous basis for any of these events and standard prophylaxis for graft versus host disease, infections, organ damage is provided through our standard operating procedures.
Furthermore, the risk of organ damage is minimized by pre transplant organ function testing, such as echo, pulmonary function tests and laboratory testing for renal and hepatic function.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Laboratory Correlative Studies

Bone marrow samples (3 to 5 mL) will be obtained by the Investigator, or co-investigator, within 10 days of the first 5-azacytidine dose and on the day after the last

dose of 5-azacytidine (day -6). Samples will be processed in the laboratory of Dr. Joseph Scandura at Weill Medical College of Cornell University.
These samples will serve to measure global gene methylation status through HIST1H2AA methylation evaluation.

10. MEASUREMENT OF EFFECT

Disease-free survival is the primary endpoint. Bone marrow biopsies at days +30, +100, +180, +365 and then annually will serve as the means to detect recurrent disease. As the patients will be monitored after stem cell transplantation on a very regular basis (at least weekly for the first 3 months), it will be at the investigators discretion to perform a bone marrow biopsy outside of these time points if a change in blood counts raises the suspicion of recurrence or graft failure. Relapse will be recorded by the day of initial detection of malignant cells, if these cells were on subsequent testing confirmed to be increasing in number. The diagnosis of disease recurrence will be based on clinical and pathological criteria.

Toxicity will be scored according to the Bearman criteria,(25, 26) (Appendix B) and acute GVHD will be scored according to the criteria proposed by Przepiorka et al.(24) Chronic GVHD will be scored according to appendix D. Limited Chronic GVHD is defined as GVHD with limited skin involvement only or presenting with liver function abnormalities only. All other presentations of chronic GVHD are defined as extensive and will require treatment.

High risk extensive chronic GVHD is characterized by the presence of thrombocytopenia (<100,000/mm3). (12)

Myeloid engraftment will be defined as the first day in which the ANC is > 500/mm^3 for three consecutive days. Cytogenetic and chimerism studies will be performed to confirm donor origin.

Platelet engraftment will be defined as the first day the platelet count is > 20,000/mm^3 without transfusion support for seven consecutive days.

Failure to engraft will be defined as lack of evidence of hematopoietic recovery (ANC < 500/mm^3 and platelet count < 20,000/mm^3) by day +35, confirmed by a biopsy revealing a marrow cellularity < 5%. Graft failure will be defined as initial myeloid engraftment by day +35, documented to be of donor origin, followed by a drop in the ANC to < 500/mm^3 for more than three days, independent of any myelosuppressive drugs, severe GVHD, CMV, or other infection.

Graft rejection will be defined as graft failure with documentation of return of recipient hematopoiesis as determined by cytogenetic and/or chimerism studies.

11. DATA REPORTING / REGULATORY CONSIDERATIONS

11.1 Data Collection

The data collection plan for this study is to utilize REDCap to capture all treatment, toxicity, and efficacy data for all enrolled patients.

11.1.1 REDCap

REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill-Cornell Medical Center CTSC. It is a tool for the creation of customized, secure data management systems that include Web-based data-entry forms, reporting tools, and a full array of security features including user and group based privileges, authentication using institution LDAP system, with a full audit trail of data manipulation and export procedures. REDCap is maintained on CTSC-owned servers that are backed up nightly and support encrypted (SSL-based) connections. Nationally, the software is developed, enhanced and supported through a multi-institutional consortium led by the Vanderbilt University CTSA.

11.1.2 Regulatory Considerations

All protocol amendments and consent form modifications will be made by the Principal Investigator.

12. STATISTICAL CONSIDERATIONS

12.1 Statistical Design

The primary endpoint will be disease-free survival (DFS), as measured from the start of the treatment to the date of either documentation of disease recurrence or death. The diagnosis of disease recurrence will be based on clinical and pathological criteria. All patients will be observed for a minimum of one year. The 1-year DFS for patients in the study cohort has been estimated to be 35%; therefore, the target 1-year DFS for the regimen under evaluation will be 50%. We will define "evaluable" patients as patients who met eligibility requirements, have initiated therapy, and were not removed from the study for non-compliance or patient withdrawal within the first 12 months. Sample size recommendations for the phase II design are determined according to A'Hern's exact single-stage phase II design (28). We project a DFS at 1 year of 35%, below which the regimen will be unacceptable, and a DFS at 1 year of 50%, above which the regimen will be considered worthy of further exploration. The null hypothesis that the 1-year DFS proportion is less than or equal to 35% will be tested against the alternative hypothesis that the 1-year DFS proportion is greater than or equal to 50%.

The sample size computations were performed assuming a 10% level of significance and 80% power. Patients will be continuously accrued throughout the study up to a maximum of 49 patients. The new regimen will be declared effective and worthy of

further testing if 22 or more patients are disease-free after 12 months of follow-up among the 49 patients entered into the study. This exact single-stage design yields a ≥ 0.80 probability of a positive result if the true DFS proportion at 12 months is ≥ 50%. It yields a ≥ 0.90 probability of a negative result if the true DFS proportion at 12 months is ≤ 35%. A 95% confidence interval constructed around the expected 12-month DFS proportion of 50% can be estimated to be within ± 14.0% of the observed DFS proportion. Assuming 10% are unevaluable/ineligible, we anticipate that a total of 55 patients will be enrolled in the study.

12.2 Stopping Rules for Graft Failure, Transplant-related Mortality and Acute Graft Versus Host Disease (Acute GVHD)

Our hypothesis is that the graft failure rate is ≤5%. The stopping rule to be employed is if 2 of the first 7, 3 of the first 10, or 4 of the first 20 patients have graft failure, the trial will be terminated.

The expected transplant related mortality should not exceed 25%. The trial will be considered for termination for safety concerns if by day +180, 4 of the first 10, 6 of the first 15 or 7 of the first 20 patients die of any cause other than disease relapse. With regard to grade 3 and 4 acute graft versus host disease, we will be operating under the hypothesis that the rate is ≤30%. The trial will be considered for termination if by day +100 4 of the first 10, 6 of the first 15, or 8 of the first 20 patients develop grade 3-4 acute GVHD.

12.3 Sample Size/Accrual Rate

The planned sample size will be 55 patients and we expect an accrual rate of 2-3 patients per month

12.4 Analysis of Endpoints

Analysis Plan for Endpoints:
Primary Endpoint:
The primary endpoint is the DFS proportion at 1 year; A 95% confidence interval will be estimated for the 1-year DFS proportion via binomial proportions.

Secondary endpoints:
Secondary endpoints include graft failure, acute graft versus host disease, high risk extensive chronic graft versus host disease, and overall survival (OS). Median DFS and OS, including survival curves, will be estimated using Kaplan-Meier methodology. Greenwood's formula will be used to calculate 95% confidence intervals for the Kaplan-Meier estimates.

All analyses will be performed in SAS Version 9.3 (SAS Institute, Inc., Cary, North

Carolina) and STATA Version 13.0 (Stata Corporation, College Station, Texas).

REFERENCES

1. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties.
Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, Rees J, Hann I, Stevens R, Burnett A, Goldstone A.
Blood. 1998 Oct 1;92(7):2322-33.

2. International scoring system for evaluating prognosis in myelodysplastic syndromes.
Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, Sanz M, Vallespi T, Hamblin T, Oscier D, Ohyashiki K, Toyama K, Aul C, Mufti G, Bennett J.
Blood. 1997 Mar 15;89(6):2079-88. Erratum in: Blood 1998 Feb 1;91(3):1100.

3. Monosomal karyotype provides better prognostic prediction after allogeneic stem cell transplantation in patients with acute myelogenous leukemia.
Oran B, Dolan M, Cao Q, Brunstein C, Warlick E, Weisdorf D. Biol Blood Marrow Transplant. 2011 Mar;17(3):356-64. Epub 2010 May 27.

4. Allogeneic stem cell transplantation for patients with acute myeloid leukemia or myelodysplastic syndrome who have chromosome 5 and/or 7 abnormalities.
van der Straaten HM, van Biezen A, Brand R, Schattenberg AV, Egeler RM, Barge RM, Cornelissen JJ, Schouten HC, Ossenkoppele GJ, Verdonck LF; Netherlands Stem Cell Transplant Registry "TYPHON". Haematologica. 2005 Oct;90(10):1339-45.

5. Reduced-intensity conditioning followed by allogeneic hematopoietic cell transplantation for adult patients with myelodysplastic syndrome and myeloproliferative disorders.
Laport GG, Sandmaier BM, Storer BE, Scott BL, Stuart MJ, Lange T, Maris MB, Agura ED, Chauncey TR, Wong RM, Forman SJ, Petersen FB, Wade JC, Epner E, Bruno B, Bethge WA,

Curtin PT, Maloney DG, Blume KG, Storb RF.
Biol Blood Marrow Transplant. 2008 Feb;14(2):246-55.

6. Allogeneic transplantation for therapy-related myelodysplastic syndrome and acute myeloid leukemia.
Litzow MR, Tarima S, Pérez WS, Bolwell BJ, Cairo MS, Camitta BM, Cutler CS, de Lima M, Dipersio JF, Gale RP, Keating A, Lazarus HM, Luger S, Marks DI, Maziarz RT, McCarthy PL, Pasquini MC, Phillips GL, Rizzo JD, Sierra J, Tallman MS, Weisdorf DJ.
Blood. 2010 Mar 4;115(9):1850-7. Epub 2009 Dec 23.

7. Sustained remissions of high-risk acute myeloid leukemia and myelodysplastic syndrome after reduced-intensity conditioning allogeneic hematopoietic transplantation: chronic graft-versus-host disease is the strongest factor improving survival.
Valcárcel D, Martino R, Caballero D, Martin J, Ferra C, Nieto JB, Sampol A, Bernal MT, Piñana JL, Vazquez L, Ribera JM, Besalduch J, Moraleda JM, Carrera D, Brunet MS, Perez-Simón JA, Sierra J.
J Clin Oncol. 2008 Feb 1;26(4):577-84. Epub 2007 Dec 17.

8. Reduced mortality after allogeneic hematopoietic-cell transplantation.
Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M, Martin PJ, Sandmaier BM, Marr KA, Appelbaum FR, Storb R, McDonald GB. N Engl J Med. 2010 Nov 25;363(22):2091-101.

9. Long-term outcomes among older patients following nonmyeloablative conditioning and allogeneic hematopoietic cell transplantation for advanced hematologic malignancies.
Sorror ML, Sandmaier BM, Storer BE, Franke GN, Laport GG, Chauncey TR, Agura E, Maziarz RT, Langston A, Hari P, Pulsipher MA, Bethge W, Sahebi F, Bruno B, Maris MB, Yeager A, Petersen FB, Vindeløv L, McSweeney PA, Hübel K, Mielcarek M, Georges GE, Niederwieser D, Blume KG, Maloney DG, Storb R.
JAMA. 2011 Nov 2;306(17):1874-83.

10. Nonmyeloablative allogeneic hematopoietic cell transplantation in patients with acute myeloid leukemia.
Gyurkocza B, Storb R, Storer BE, Chauncey TR, Lange T, Shizuru JA, Langston AA, Pulsipher MA, Bredeson CN, Maziarz RT, Bruno B, Petersen FB, Maris MB, Agura E, Yeager A, Bethge

W, Sahebi F, Appelbaum FR, Maloney DG, Sandmaier BM.
J Clin Oncol. 2010 Jun 10;28(17):2859-67. Epub 2010 May 3.

11. Reducing the risk for transplantation-related mortality after allogeneic hematopoietic cell transplantation: how much progress has been made?
Horan JT, Logan BR, Agovi-Johnson MA, Lazarus HM, Bacigalupo AA, Ballen KK, Bredeson CN, Carabasi MH, Gupta V, Hale GA, Khoury HJ, Juckett MB, Litzow MR, Martino R, McCarthy PL, Smith FO, Rizzo JD, Pasquini MC.
J Clin Oncol. 2011 Mar 1;29(7):805-13. Epub 2011 Jan 10.

12. 5-Azacytidine and decitabine induce demethylation and re-expression of Fas (CD95) and promote apoptosis in neoplastic cells in acute myeloid leukemia. H. Karlic, R. reitermeier, V. Ghanim, H. Herrmann, R. Thaler, S. Spitzer, F. Varga, I. Sadovnik, K. Blatt, F. Kreil, P. Valent. ASH 2011

13. Chemosensitization of diffuse large B-cell lymphoma by demethylating Nucleoside analogues. T. Clozel, S. Yang, M. Kormaksson, R. Elstrom, A. CVasanthakumar, S. Brennan, B. Culjkovic, M. Leser, P. Martin, K. Borden, L. Wang, S. Cheng, W. Tam, R. Shaknovitch, A. Chadburn, L. Godley, J. Leonard, A. Melnick, L. Cerchietti. ASH 2011

14. 5-azacytidine reverses drug resistance in bladder cancer cells.
Ramachandran K, Gordian E, Singal R.
Anticancer Res. 2011 Nov;31(11):3757-66.

15. Down-regulation of mitochondrial ATPase by hypermethylation mechanism in chronic myeloid leukemia is associated with multidrug resistance.
Li RJ, Zhang GS, Chen YH, Zhu JF, Lu QJ, Gong FJ, Kuang WY.
Ann Oncol. 2010 Jul;21(7):1506-14. Epub 2009 Dec 27.

16. Epigenetic silencing of death receptor 4 mediates tumor necrosis factor-related apoptosis-inducing ligand resistance in gliomas.
Elias A, Siegelin MD, Steinmüller A, von Deimling A, Lass U, Korn B, Mueller W.
Clin Cancer Res. 2009 Sep 1;15(17):5457-65. Epub 2009 Aug 25.

17. Methylation mediated silencing of TMS1 in breast cancer and its potential contribution to docetaxel cytotoxicity.
Gordian E, Ramachandran K, Singal R.

Anticancer Res. 2009 Aug;29(8):3207-10.

18. Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML.
Scandura JM, Roboz GJ, Moh M, Morawa E, Brenet F, Bose JR, Villegas L, Gergis US, Mayer SA, Ippoliti CM, Curcio TJ, Ritchie EK, Feldman EJ.
Blood. 2011 Aug 11;118(6):1472-80. Epub 2011 May 25.

19. Fludarabine, melphalan, and alemtuzumab conditioning in adults with standard-risk advanced acute myeloid leukemia and myelodysplastic syndrome.
van Besien K, Artz A, Smith S, Cao D, Rich S, Godley L, Jones D, Del Cerro P, Bennett D, Casey B, Odenike O, Thirman M, Daugherty C, Wickrema A, Zimmerman T, Larson RA, Stock W.
J Clin Oncol. 2005 Aug 20;23(24):5728-38. Epub 2005 Jul 11.

20. Fludarabine-melphalan conditioning for AML and MDS: alemtuzumab reduces acute and chronic GVHD without affecting long-term outcomes.
van Besien K, Kunavakkam R, Rondon G, De Lima M, Artz A, Oran B, Giralt S.
Biol Blood Marrow Transplant. 2009 May;15(5):610-7.

21. Melphalan and purine analog-containing preparative regimens: reduced- intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Giralt S, Thall PF, Khouri I, I, Wang X, Braunschweig I, I, Ippolitti C et al
Blood. 2001;97:631-37.

22. A comparison of azacitidine and decitabine activities in AML cell lines. Hollenbach, P. W. et al. PLoS ONE 5, e9001 (2010)

23. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B.
Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R, Stone RM, Nelson D, Powell BL, DeCastro CM, Ellerton J, Larson RA, Schiffer CA, Holland JF.
J Clin Oncol. 2002 May 15;20(10):2429-40.

24. The Addition of Low-Dose Total Body Irradiation to Fludarabine and Melphalan Conditioning in Haplocord Transplantation for High-Risk Hematological Malignancies.
Choe HK, Gergis U, Mayer SA, Nagar H, Phillips AA, Shore TB, Smith MJ, van Besien K.

Transplantation. 2017 Jan;101(1):e34-e38

25. Regimen-related toxicity in patients undergoing bone marrow transplantation.

Bearman SI, Appelbaum FR, Buckner CD, Petersen FB, Fisher LD, Clift RA, Thomas ED.
J Clin Oncol. 1988 Oct;6(10):1562-8.

26. 1994 Consensus Conference on Acute GVHD Grading.

Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED.
Bone Marrow Transplant. 1995 Jun;15(6):825-8. Review.

27. Chronic graft-versus-host disease after allogeneic blood stem cell transplantation.

Przepiorka D, Anderlini P, Saliba R, Cleary K, Mehra R, Khouri I, Huh YO, Giralt S,

Braunschweig I, van Besien K, Champlin R.
Blood. 2001 Sep 15;98(6):1695-700.

28. Sample size tables for exact single-stage phase II designs.
A'Hern AP. Statistics in Medicine 2001;20:859-866.

APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B

WCMC IRB SAE Reporting Forms

http://researchintegrity.weill.cornell.edu/institutional_review_board/forms.html