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THE UNIVERSITY OF CHICAGO

DIVISION OF MEDICINE

A phase I-II study of busulfan-fludarabine conditioning and T-cell depleted allogeneic stem cell transplantation for patients with advanced hematologic malignancies

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PROTOCOL ABSTRACT

Protocol:

A phase I-II study of busulfan-fludarabine conditioning and T-cell depleted allogeneic stem cell transplantation for patients with advanced hematologic malignancies

Principal Investgator:

Andrew Artz, M.D.

Patient Eligibility:

Diagnosis:
Phase I portion:

- Relapsed or refractory acute myelogenous or lymphoid leukemia.
- Chronic myelogenous leukemia in accelerated phase or blast-crisis.
- Recurrent or refractory malignant lymphoma or Hodgkin's disease
- Recurrent or refractory multiple myeloma.
- Chronic lymphocytic leukemia, relapsed or with poor prognostic features.
- Myeloproliferative disorder (polycythemia vera, myelofibrosis) with transformation
- Myelodysplastic syndromes with more than 5% blasts.

Phase II portion:

- AML with active disease or beyond CR2
- MDS with more than 5% blasts

Zubrod performance status 2 (See Appendix B).
Life expectancy is not severely limited by concomitant illness.
Adequate cardiac and pulmonary function. Patients with decreased LVEF or PFTS will be evaluated by cardiology or pulmonary prior to enrollment on this protocol.
Calculated creatinine clearance >50 ml/min.
Serum bilirubin 2.0 mg/dl, SGPT <3 x upper limit of normal
No evidence of chronic active hepatitis or cirrhosis.HIV-negative Patient is not pregnant Patient or guardian able to sign informed consent.
Matched sibling donor, 1 Antigen mismatched relative or matched unrelated donor.

Treatment Plan:

See appendix A

PATIENT EVALUATION:

See section 6 and section 7

Miscellaneous Information:

Objectives:

1. To establish the maximally tolerated dose (MTD) of intravenous busulfan (Busulfan®) in combination with fludarabine as conditioning regimen for transplantation with in-vivo T-cell depletion
2. To evaluate disease free and overall survival after this conditioning regimen in patients with advanced AML and MDS.
3. To evaluate potential pharmacogenomic determinants of toxicity of this regimen.
4. To evaluate potential pharmacogenomic determinants of efficacy of this regimen.

Statistics:

The phase I portion of the trial will use the modified continual reassessment method (CRM) to determine the maximum tolerated dose (MTD). The primary endpoint for the phase II trial will be the disease-free survival rate (DFS) at one year, and a Simon two-stage design will be used to test the null hypothesis that the one-year DFS rate is 25% against the alternative that it is at least 40%. Adverse events will be monitored, and multiple regression, logistic regression, and Cox regression analyses will be performed to examine the association between GSTA1 enzymatic activity and busulfan metabolic rate, GSTA1 enzymatic activity and the incidence of toxicity, and pharmaco-genomic predictors of DFS, respectively.

1 OBJECTIVES

4.1 To establish the maximally tolerated dose (MTD) of intravenous Busulfan (busulfex®) in combination with fludarabine as conditioning regimen for transplantation with in-vivo T-cell depletion

4.2 To evaluate disease free and overall survival after this conditioning regimen in patients with advanced AML and MDS.

4.3 To evaluate potential pharmacogenomic determinants of toxicity of this regimen.

4.4 To evaluate potential pharmacogenomic determinants of efficacy of this regimen.

5.0 BACKGROUND

5.1 Allogeneic transplantation in advanced hematologic malignancies.

Allogeneic transplantation has been extensively used for the treatment of advanced hematologic malignancies and results in durable remissions in approximately 30% of patients. Besides patient related factors, the outcome of transplantation depends on (1) the conditioning regimen, (2) type of donor (HLA-matched vs -mismatched or unrelated), (3) Type of GVHD prophylaxis.

5.2 Conditioning regimens for allogeneic transplantation. Historical and institutional results.

Traditionally, total body irradiation or high doses of busulfan in combination with high doses of cyclophosphamide have been the mainstay for conditioning in allogeneic transplantation. But more recently reduced intensity conditioning regimens have been utilized in an attempt to reduce early regimen related toxicity.[1-3] Such regimens assure reliable engraftment in recipients of HLA-identical transplants, and prolonged disease free survival has been demonstrated in a proportion of patients with end-stage malignancies.
We have evaluated a combination of fludarabine and melphalan as originally pioneered by Giralt et al. [1] Using this regimen, we obtained approximately 30% disease-free survival, 30% treatment related mortality and observed a high incidence of extensive chronic GVHD. Disease free survival was obtained in approximately 20-30% of the patients.[4] In a subsequent study, at the University of Chicago (IRB 11300 A), we have continued to use the same conditioning

regimen, but employed Campath 1H (alemtuzumab), a T-cell depleting agent, instead of methotrexate for GVHD prophylaxis. [5] This conditioning results in excellent engraftment, much decreased toxicity, near absence of chronic GVHD and excellent initial survival. Unfortunately, recurrence rates are high in patients with advanced leukemia and lymphoma and long-term disease free survival in this patient group is likely to be in the range of 20% to 35%.[5-7 (and manuscript submitted)]

Our experience indicates that treatment related mortality not only depends on the conditioning regimen, but also depends on the toxicity of the medications used for GVHD prophylaxis and on the efficacy of GVHD prevention. Indeed, the fludarabine melphalan conditioning regimen has considerably more toxicity when tacrolimus and methotrexate are used for GVHD prophylaxis, than with the use of Campath and a short course of tacrolimus. Vice versa, tacrolimus and methotrexate are less efficient in preventing GVHD than alemtuzumab. So more patients develop acute and chronic GVHD with tacrolimus-methotrexate. This also results in an increase in treatment related mortality.

5.3 Busulfan for conditioning in transplant.

To address the problem of delayed disease recurrence after fludarabine-melphalan conditining and GVHD prophylaxis with alemtuzumab, we are interested in studying the efficacy and treatment related mortality associated with a more intensive conditioning regimen, but similar alemtuzumab based GHVD prophylaxis regimen.

Busulfan (1,4-bis-(methanesolfonoxyl) butane) is a bifunctional alkylating agent, which was first described by Haddow and Timmis.[8] Since the demonstration of its potent antitumor effects, it has been used extensively for the treatment of malignant disease, especially hematologic malignancies and myeloproliferative syndromes. Its use was for a long time limited to low dose oral therapy with palliative intent, and frequent monitoring of the blood counts was routinely recommended. The advent of some 2 to 3% of patients developing busulfan-induced pulmonary fibrosis, as well as occasionally severe, sometimes even irreversible myelosuppression after prolonged administration effectively deterred dose escalation beyond 8-10 mg daily.

In 1974, however, Santos and Tutschka investigated the use of busulfan to create a murine model of aplastic anemia.[9] Subsequently, the experience gained in this model system was used to introduce high-dose combination chemotherapy based on oral busulfan for pretransplant conditioning of non-human primates and thereafter patients undergoing both autologous and allogeneic marrow transplantation.[10-17] Since then, high-dose busulfan, most commonly in combination with cyclophosphamide (Bu/CY), has proven to be an effective antileukemic regimen when used in conjunction with autologous or allogeneic hematopoietic stem cell support and is comparable in efficacy to TBI containing regimens.[18]

High-dose busulfan therapy has several advantages for use in marrow ablation/pretransplant treatment. First, when using chemotherapy alone for conditioning of patients undergoing marrow transplantation, one avoids the dependence on a radiation unit with, usually, limited capacity to deliver the necessary treatment on a fixed schedule. Second, a radiation-based regimen can only be delivered to patients who have not been previously irradiated. Many patients with lymphoma, Hodgkin's disease and leukemia have had extensive radiation previously for control of locally aggressive disease in sites such as the mediastinum, neck, and CNS. Additionally radiation as part of the pretransplantation conditioning regimen may cause irreversible and often fatal toxicity in such cases. However, a majority of previously radiated patients can safely receive a busulfan-based regimen, provided that the previous acute radiation toxicity (usually within the first 2-4 months after radiation therapy) has subsided. Third, in selected patients who suffer recurrent leukemia after allogeneic marrow grafting, a second marrow transplant may still offer a chance for long-term disease control or even cure. [19] Due to subclinical (irreversible) toxicity, a TBI-based regimen can only be utilized once in a patient's lifetime, whereas combination chemotherapy can be employed following a previous TBI-based regimen. Busulfan-based chemotherapy may, therefore, serve as a valid alternative.

5.3.1 Rationale for a Parenteral Formulation of Busulfan in Marrow Transplantation

Oral busulfan has, unfortunately, several serious shortcomings. When used in high-dose combination regimens, serious side effects in the liver and lungs are often encountered [10;20;21] Several investigators have reported veno-occlusive disease (VOD) of the liver leading to fatal liver failure, as the most serious side effect.[11;13;14;17]. Neurologic disturbances such as grand mal seizures, and severe nausea and vomiting are also frequently encountered.[22-25] It is impossible to predict which patients will develop liver failure, and it is further unknown whether the liver failure is due to toxicity from the systemic busulfan or whether it is mainly due to a first-pass phenomenon. Based on the limited information that is available regarding busulfan pharmacokinetics, it appears however that patients who absorb a large fraction of the ingested dose, with a prolonged high busulfan plasma concentration, will be at increased risk for developing serious side effects.[14;17;26;27] Another disadvantage with oral busulfan is that patients who develop severe nausea and vomiting shortly (within one-half to two (1/2-2) hours) after a dose has been delivered will lose part or all of the dose, and it will be virtually impossible to accurately determine how much of the dose has been lost in a vomiting subject. Furthermore, the intestinal resorption of any delivered drug may be influenced by the patient's nutritional status, and by concurrent administration of other drugs affecting the intestinal microenvironment, as well as by whether the patient has eaten in close proximity to ingestion of the administered drug dose and, finally, by the inherent biological variability in intestinal absorption between different patients. Due to these uncertainties, oral administration of high-dose busulfan carries with it an inherent safety problem

both from the potential danger of inadvertent overdosing with a risk for lethal toxicities, as well as from the hazard of suboptimal underdosing the patient with an inadvertently high potential for recurrent or persistent malignancy after the marrow transplant.

Busulfan dissolved in DMA/PEG400/dextrose (Busulfex®) has been recently approved for conditioning in transplantation and has replaced oral busulfan because of its reliable dosing and its more predictable intrapatient pharmacokinetics. A multi-institutional trial of IV Busulfan in combination with cyclophosphamide as pretransplant conditioning therapy in patients with advanced hematologic malignancies indicated that the safety profile is greatly improved when the busulfan is administered IV as compared with the standard oral busulfan formulation. In particular, the incidence of serious, lifethreatening or lethal veno-occlusive disease of the liver was reduced to <20% of what would be expected from using oral Busulfan in high- dose pretransplant conditioning therapy in patients with advanced hematologic malignancies.[28] Additional safety information was obtained a series of CML patients undergoing HSCT after this regimen when the busulfan is given as a standardized dose based on PK parameters. These limited data suggest an "optimal" dose-exposure ("area under the curve" AUC) for Busulfex, below and above which there is increased treatment failure.[29]

5.3.2 Rationale for using fludarabine in combination with IV busulfan

The acute toxicities of high-dose oral busulfan in combination with cyclophosphamide are well known. Chief among the described adverse events is VOD which is dose-related and only rarely seen in patients whose plasma AUC is less than 1,500 µMol-min.[17] Recently, Mcdonald et al demonstrated that the hepatic toxicity of the commonly used cyclophosphamide-TBI regimen is mainly due to cyclophosphamide metabolites and suggested that toxicity may be reduced by replacing cyclophosphamide with less toxic alternatives. [30] It is likely that cyclophosphamide metabolites also contribute to the toxicity of the busulfan-cyclophosphamide regimen, a situation in which cyclophosphamide metabolism and toxicity is influenced by the prior administration of busulfan.[31] Fludarabine is an effective drug against hematologic malignancies and appears less toxic than cyclophosphamide in many studies.[1]
As discussed above, the combination of fludarabine and intravenous melphalan has been actively explored by our section as a less intensive preparative regimen that allows engraftment of allogeneic progenitor cells from both related and unrelated donors and acceptable toxicities in older and medically debilitated patients.[11] We are now interested in establishing the MTD of intravenous busulfan combined with fludarabine in combination with T-cell depleted transplantation. Others have already evaluated this combination in different doses, with different GVHD prophylaxis and with other formulations. Slavin et al, studied the combination of low dose busulfan and fludarabine in patients who were not

otherwise eligible for myeloablative conditioning therapy and reported encouraging results.[32] The Seattle group combined fludarabine with oral busulfan in myeloablative doses and demonstrated an acceptable toxicity profile.[33] Finally, Russell et al. have used IV Busulfan with fludarabine as conditioning therapy for patients with hematologic disorders undergoing allogeneic HSCT. They used once daily administration of both Fludarabine (50 mg/m2) and IV Busulfan (3.2 mg/kg BW) for four days in combination with cyclosporine-methotrexate based GVHD prophylaxis. The obtained safety information indicated, that this regimen is well tolerated without unexpected side effects, and allowing for consistent engraftment and good antitumor effect.[34] Further, they obtained limited pharmacokinetic (PK) information, which support the previous notion that busulfan in this dosing interval displays linear pharmacokinetics. A similar study was reported by the MD Anderson group.[35]

5.3.3 Rationale for further dose escalation studies of busulfan.

Current conditioning regimens have limited cure rates in patients with advance hematologic malignancies. There are considerable data suggesting a dose-response curve for cure rates with busulfan conditioning in hematologic malignancies.[29;36] Further dose-escalation might therefore be associated with higher cure rates.

In the past dose escalation to an AUC above 1500 mcgr.min/L.dose (when four divided doses were given) was associated with an unacceptable increase in VOD.[14;17;36-39] But there are at several observations that suggest that further dose escalation of the Busulfan component might be possible with the fludarabine-busulfex-alemtuzumab regimen.

1. Intravenous busulfan is less hepatotoxic than oral busulfan. 2. Fludarabine-busulfan combination is likely to be considerably less hepatotoxic than the commonly used cyclophosphamide-busulfan combination, where cyclophosphamide metabolites contribute to regimen related toxicity.
3. Alemtuzumab based GVHD prophylaxis is likely to be less hepatotoxic than the commonly used cyclosporine methotrexate based GVHD prophylaxis.

5.3.4 Rationale for test dose and AUC based dosing.

The use of a test-dose followed by frequent pharmacokinetic sampling will allow the assessment of individual patient pharmacokinetics and calculation of the dose necessary to achieve an intended AUC with subsequent doses.[27;40] IV Busulfex has linear pharmacokinetics[27;29;34;41] and does not accumulate even after repeated daily dosing. On the other hand, there continues to be considerable interpatient variability in pharmacokinetics and the AUC cannot reliably be predicted from the administered doseOn the other hand, there is considerable evidence to indicate that busulfan AUC is closely associated with toxicity and, AUC should guide dose escalation studies rather than administered dose.

In this study, all patients will receive an initial test dose of 0.5 mg/kg IV over 3 hours. Pharmacokinetic sampling will be performed at four time intervals after

completion of the infusion and used to calculates treatment doses necessary to achieve an intended AUC. The treatment doses will be administered as four daily doses. Pharmacokinetic sampling will be performed with the first and fourth dose to confirm the relationship between predicted and obtained AUC. The target starting daily AUC (4800 umol-min) is slightly lower than the median AUC (4897 umol-min) observed in the MD Anderson study with a fixed dose of 130 mg/kg.[35] One should however also take into account the additional one-time busulfan exposure (approximately 600 umol-min) from the test dose in our patients. Therefore, even at the starting target AUC, cumulative busulfan exposure for our patients at 19800 umol-min, should be slightly higher than the median exposure in the MD Anderson study of approximately 19600 umol-min. Blood samples will be stored on each of the patients for subsequent pharmacogenomic studies. In patients with active AML and MDS tumor samples will be stored as well for subsequent pharmacogenomic studies.

5.4 Donor type

The outcome of allogeneic transplantation is determined to a large degree by the choice of stem cell donor. The best outcomes are obtained with the use of syngeneic donors (i.e. identical twins). Grafts from such donors are accepted without risk for graft failure or graft versus host disease.
Among siblings, the probability of being identical for all major histocompatibility (HLA) antigens is approximately 25%. Allogeneic matched (HLA-identical) siblings can be identified for approximately 20-30% of the population. Seventy percent of patients who might otherwise benefit from allogeneic hematopoietic stem cell transplantation lack a suitably matched related donor. Alternative donor sources include partially matched related donors, unrelated volunteers, and previously banked placental blood. Recipients of unrelated donor transplantation will be included in this study as they have been in all our previous studies. As in our previous studies, we will use molecular typing (DNA sequencing) of 10 relevant HLA alleles (A, B, C, DR, DQ) for determining donor/recipient matching.

5.5 Rationale for the current study

In a previous study, we utilized fludarabine-melphalan-campath, followed by transplantation of allogeneic matched sibling or matched unrelated donor stem cells. Engraftment was excellent and treatment related mortality much reduced compared with previous experience.[42] One year survival was improved compared with historical controls and most encouragingly, the quality of life of transplant recipients was excellent because of the near complete absence of chronic GVHD. Unfortunately the recurrence rate in patients with advanced hematologic malignancies was high. In the current study we continue to use campath GVHD prophylaxis because of its excellent tolerance and efficacy. We propose to combine it with a f ludarabine busulfan conditioning regimen and individualized pharmacokinetics based dosing. We hypothesize that intensification of the

conditioning regimen will result in improved disease control. We hypothesize that AUC based dosing will limit toxicity and that further dose escalation of this regimen will be possible. The phase I portion of this study will enroll patients with a variety of hematologic malignancies. However, we anticipate that, as in previous studies, approximately 50% of our patients will have AML and MDS. The phase II portion of this study will be limited to patients with advanced AML and MDS, a patient population where we have previously observed a poor outcome. Patients with other disease stages or histologies will then be entered on other protocols.

5.6 Pharmacogenomic determinants of hepatic toxicity

The major metabolic pathway of busulfan is through sulfonation by transfer of a sulfur group from Glutathione through Glutathione S-transferases (GST), a process that occurs mainly in the liver. Thereafter, the liphophilic intermediary undergoes oxidation by cytochrome P450 enzymes before excretion. [31] Two families of GSTs are distinguished, soluble and microsomal GST's. The soluble GST's in humans comprise at least 16 genes grouped in 8 classes and are the GST's involved in busulfan metabolism.[43] The microsomal GST's are structurally unrelated to the soluble GST's and of no relevance to busulfan metabolism.

At least eight subtypes of soluble GST have been described, each with specific affinities and more or less tissue restricted expression. The pivotal GST for busulfan metabolism is GST A1.[44] GSTM1 accounts for approximately 48% of busulfan metabolism and a third GST, GSTP1 accounts for approximately 18%. [45;46] GST A1 is expressed mainly in the liver, but can also be detected in the blood. In-vitro experiments indicate considerable inter-individual variation in hepatic expression of GST A1 which correlates closely with busulfan metabolizing potential.[44;47] Surprisingly GSTA1 level in the bloodstream as measured with a commercial kit also correlates with busulfan metabolizing activity and may represent a surrogate of GSTA1 activity in the liver.

SNP analysis demonstrates the existence of at least six polymorphic sites in the GSTA1 promoter, and the existence of five different alleles. [44;48] Based on the relations between the haplotypes, two subclasses of GSTA1 can be distinguished, GSTA1A and GSTAA1B with respectively two and three variants. According to Coles, allelic variation in GSTA1 genotype accounts to a large extent for the highly variable activity of GSTA1. But the in-vivo relation between GSTA1 genotypes and busulfan pharmacokinetics, remains to be studied.

In vitro data suggest that sulfonated busulfan metabolites as well as GSH depletion contribute to toxicity of hepatocytes.[49] Others have shown that overexpression of GSTA1 in cell lines, results in protection against busulfan induced cell cycle arrest and induction of tissue factor expression, thus suggesting a potential link between GST A1 expression and busulfan induced toxicity. [50] It is therefore conceivable that differences in GSTA1 activity caused by GSTA1 polymorphisms affect busulfan toxicity independent of their postulated effects of busulfan pharmacokinetics.

GST-M1 is another hepatic GST that is responsible for a substantial proportion of busulfan metabolism. The GST M1 gene is known to be highly polymorphic with deletion of either or both genes at varying but significant frequencies in different ethnic groups. In a recent study of children with thalassemia receiving busulfan cyclophosphamide conditioning, GSTM1 null genotype was the most important risk factor for VOD. [51]

Busulfan clearance was higher in patients with null genotype and first dose steady state concentration was lower. The mechanism by which GSTM1 null genotype causes increased risk for VOD is probably complex and may be related to direct toxicity of busulfan metabolites (perhaps due to reciprocally increased GSTA1 activity),[52] intrahepatic GSH depletion or interaction of GSH depletion and cyclophosphamide toxicity. Confirmation of these results after conditioning with busulfan-fludarabine, is likely to provide important new information on this issue.

5.7 Pharmacogenomic determinants of drug efficacy:

In addition to studies relating GST polymorphism to toxicity, we will, in patients with leukemia, study leukemia blasts to determine the relation between polymorphisms relating to busulfan and/or fludarabine metabolism and efficacy. For this purpose, we will study a sample of leukemia cells obtained from the bone marrow. Such samples will be obtained from AML patients participating in the phase I and or phase II portion of the protocol.

Although resistance to fludarabine may occur by several means, one proposed mechanism of cellular and clinical resistance is reduced intracellular drug accumulation[53]. The early steps of plasma membrane transport, nucleoside phosphorylation, and nucleotide dephosphorylation determine intracellular accumulation of fludarabine and its metabolites. Fludarabine is administered as the 5'-nucleotide monophosphate and is converted to the nucleoside by the activity of serum phosphatase and ecto-5'nucleotidase (CD73). As fludarabine is a hydrophilic compound and does not readily cross plasma membranes by diffusion, the presence of functional nucleoside transporters that accept fludarabine as a permeant is required for cellular entry at rates sufficient to achieve cytotoxic levels of intracellular metabolites. Three human (h) nucleoside transporter proteins, equilibrative nucleoside transporter 1 (hENT1), equilibrative nucleoside transporter 2 (hENT2), and concentrative nucleoside transporter 3 (hCNT3), have been shown to mediate cellular entry of fludarabine[54-56].

Within the cell, fludarabine requires anabolism to the active 5'-triphosphate (fludarabine triphosphate) to exert its cytotoxic effects. The rate-limiting step in this process is the conversion by deoxycytidine kinase (dCK) of free nucleoside to the 5'-nucleotide monophosphate (Plunkett 91). Directly opposing dCK activity are the intracellular 5'-nucleotidases, including the high-K_m 5'-nucleotidase (CN-II) and deoxynucleotidase-1 (dNT-1) (Galmarini 01).

In order to identify molecular markers of potential predictive value in AML patients, we will evaluate AML patients who had not previously been treated with fludarabine to determine 1) the cellular expression of genes encoding nucleoside transporter proteins and enzymes mediating fludarabine metabolism, and 2) the role of polymorphisms in these genes.

RNA will be extracted from leukemia cells in bone marrow specimens by quantitative real-time PCR. We will then analyze the relationships between RNA

expression, germ line and somatic polymorphisms in the gene described above, and time to disease progression following fludarabine therapy.

Similarly, due to the role of GSTs in the inactivation of busulfan, we will evaluate 1) the expression of GST genes in leukemia cells, 2) its correlation with germ line and somatic GST polymorphisms, and 3) the correlation among GST expression, polymorphisms and time to disease progression.

6.0 BACKGROUND DRUG INFORMATION

6.1 Tacrolimus (Prograf)

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.
HUMAN PHARMACOLOGY: The oral absorption of TACROLIMUS iserratic 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 areheadache, 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.

6.2 Busulfan

BUSULFAN is an alkylating agent that interferes with DNA replication and transcription of RNA and ultimately results in the disruption of nucleic acid function.
DOSING INFORMATION: In transplantation, doses of 1 mg/kg PO are repeated to a usual cumulative dose of 16 mg/kg. The usual dose for IV Busulfan is 80% of the equivalent PO dose.

HUMAN PHARMACOLOGY: Busulfan is rapidly absorbed from the gastrointestinal tract, and measurable blood levels are obtained within 0.5-2 hours after oral administration. Within 3 minutes after IV administration in rats, 90% of the drug disappears from the blood; similar rapid decreases in blood concentrations have been reported in man. Busulfan is reported to be extensively metabolized; 12 metabolites have been isolated, but most have not been identified. The drug is slowly excreted in the urine, chiefly as methanesulfonic acid. Ten to 50% of a dose is excreted as metabolites within 24 hours.
CAUTIONS : Dose limiting toxicity is hematological. Long term therapy has been associated with pulmonary fibrosis and an Addison's like syndrome. Seizures have been reported after high dose Busulfan used for transplantation. Dilantin is often administered preventively.

6.3 Fludarabine

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

DOSING INFORMATION: Doses of 25 mg/m(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.

6.4 CAMPATH- 1H (Alemtuzumab)

CAMPATH-1H is a humanized 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.

DOSING INFORMATION: In transplantation, daily doses of 20 mg are repeated for up to five times.

HUMAN PHARMACOLOGY: Campath is extensively bound to circulating CD52 in the serum as well as present in unbound form. The half life of Campath is prolonged and free Campath can be detected for several weeks after administration. Campath is usually administered intravenously, but recent data indicate that subcutaneous administration is associated with a decreased incidence of severe reactions. In some studies Campath has been admixed with the stem cell infusate.

CAUTIONS: The infusion of Campath H1 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.

7.0 P ATIENT ELIGIBILITY

7.1 Patients with the following diseases:

Diagnosis:
Phase I portion:

- Relapsed or refractory acute myelogenous or lymphoid leukemia.
- Chronic myelogenous leukemia in accelerated phase or blast-crisis.
- Recurrent or refractory malignant lymphoma or Hodgkin's disease
- Recurrent or refractory multiple myeloma.
- Chronic lymphocytic leukemia, relapsed or with poor prognostic features.
- Myeloproliferative disorder (polycythemia vera, myelofibrosis) with transformation
- Myelodysplastic syndromes with more than 5% blasts.

Phase II portion:

- AML with active disease or beyond CR2
- MDS with more than 5% blasts

7.2 Zubrod performance status ≤ 2

7.3 Life expectancy is not severely limited by concomitant illness.

7.4 Adequate cardiac and pulmonary function. Patients with decreased LVEF or PFTS will be evaluated by cardiology or pulmonary prior to enrollment on this protocol.

7.5 Calculated creatinine clearance >50 ml/min .

7.6 Serum bilirubin ≤ 2.0 mg/dl, SGPT <3 x upper limit of normal

7.7 No evidence of chronic active hepatitis or cirrhosis.

7.8 HIV-negative

7.9 Patient is not pregnant

7.10 Patient or guardian able to sign informed consent.

8.0 DONOR SELECTION, STEM CELL SOURCE AND TREATMENT PLAN.

8.1 DONOR SELECTION

When possible, an HLA compatible sibling will be used as a donor. For patients who do not have an HLA-compatible sibling, an unrelated donor will be identified.

In case of unrelated donor transplantation, high resolution DNA-based HLA typing will always be performed for HLA A, B, C and DR antigens

8.2 HEMATOPOIETIC STEM CELL SOURCE

Donor stem cells will be the preferred source of HSC in all cases. Based on donor's preference, bone marrow stem cells may be utilized in some recipients.

9.0 TREA TMENT PLAN

9.1.1 All patients shall be registered with the Data Management Office.

Complete all sections of the Registration Form located on the University of Chicago Cancer Research Center web site at http://www-uccrc.uchicago.edu/ and send the completed form by facsimile to 773-702-8855.

9.1.2 Conditioning regimen

Day	Clonazepam	Fludarabine	Busulfan	Bu pharmacokinetics	Campath
Test Dose					
-	PO				
-	PO		0.5 mg/kg	x 6	
-	PO				
Treatment Doses					
-7 PO		25 mg/m^2			20 mg
-6	PO	25 mg/m^2	AUC based	x 5	20 mg
-5	PO	25 mg/m^2	AUC based		20 mg
-4	PO	25 mg/m^2	AUC based	(x 5*)	20 mg
-3	PO	25 mg/m^2	AUC based	x 5*	20 mg
-2	PO				
-1					
0					

Comments:
Busulfan administration has been associated with a low incidence of seizures and dilantin is routinely used for seizure prophylaxis. Dilantin use is discouraged in this protocol because exposure to dilantin can affect Busulfan metabolism.[57]
We recommend benzodiazepines e.g clonazepam (klonopin ®) 1 mg PO TID
Scheduling:
Busulfan test dose:
The test dose can be scheduled any day within 8 days prior to admission. Clonazepam will be started with an evening dose, the day prior to the test dose and two doses one day after test dose. Busulfan test-dose will be based on actual body weight and will be accompanied by extensive pharmacokinetic studies. This dose will be given as an outpatient.
Patients will be admitted in the evening of day –8, to start fludarabine on day -7

Fludarabine:
Fludarabine dosing will be based on actual body weight. Fludarabine will be infused over 30 minutes before busulfan treatment dose.

Busulfan treatment doses:

On each day of treatment, busulfan will be administered through a fresh infusion line at a steady rate over 3 hours. Infusion will start in the morning around 5 AM and will be completed around 8 AM, allowing ample time for pharmacokinetic sampling in the afternoon.
*Pharmacokinetic studies will be performed on the day 1 dose and again on the day 4 dose. For logistical reasons (week-end, holidays), day 3 pharmacokinetics may be performed instead of day 4. Busulfan doses may be further adjusted based on levels obtained with treatment dose 1.

Campath
All patients will receive premedication for Campath as per current standards at the UC transplant unit.

9.1.3 Dose schedule (see also statistical plan)

The targeted daily AUC of busulfan will be determing using the modified continual reassessment method.

Target AUC's are the following:
Dose level 0**:** 4800 mcgr.min/L.dose
Dose level 1**:** 5800 mcgr.min/L.dose
Dose level 2**:** 6800 mcgr.min/L.dose
Dose level 3**:** 7800 mcgr.min/L.dose
Dose level 4**:** 8800 mcgr.min/L.dose
Dose level 5**:** 9800 mcgr.min/L.dose

We do not anticipate further dose-escalation beyond dose level 5. If such dose-escalation were to be considered, we would submit an amended protocol to the IRB.

9.1.4 Bone marrow/stem cel infusion

On day 0 the stem cell product will be infused according to BMT unit policy.
Bone marrow, PBSC will not be further processed except in the case of ABO-incompatibility. In such instances, red blood cells will be removed from bone marrow products or from PBSC products containing excessive amounts of RBC (as per transplant policy).
All stem cell products procured through the NMDP will be done so in strict compliance with the protocols, policies, and procedures established by the NMDP.

9.1.5 GVHD 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 unless the patient has developed GVHD. In recipients of mismatched or unrelated donor transplants, tacrolimus will be continued until day 180. Thereafter tacrolimus will be tapered by 20% every week unless the patient has developed GVHD.

9.1.6 Supportive care:

Infection prophylaxis and supportive care will be as BMT unit policy. No routine growt factor support will be administered. Growth factor support will be considered in case of delayed engraftment (ANC < $0.5x10^9$/L on day 12)

9.1.7 Back-up bone marrow harvest (only for recipients of mismatched or unrelated donor transplants)

For patients transplanted in remission, a backup bone marrow or peripheral blood stem cell collection will be considered within two weeks prior to hospital admission as long as the overall marrow cellularity is greater than 30%. All backup harvests will be cryopreserved after processing for buffy coat without any further manipulation. The backup stem cells will be given if deemed clinically necessary in the event of failure to engraft by day +35, graft rejection after day +35, or graft failure after day +35.

10.0 PHARMACOKINETIC STUDIES

Busulfan will be infused with a fresh infusion set whenever pharmacokinetic assessments will be performed. The tubing will be primed with the busulfan, and the entire volumeplus a volume of normal saline flush equal to the priming volume will be infused at a constant rate into a central venous catheter over the 3-hour infusion time. Sampling will be done with the test dose, and with the first and fourth treatment doses. If, for logistical reasons, sampling on day 4 is not possible, then day 3 may be used as the time point for the second sample. Samples will be taken from a peripheral vein (through the same line after additional flushing) into prechilled heparin tubes with plasma separation within 30 minutes.

The actual start and stop times of busulfan will be recorded, as will the exact times when the blood levels are drawn. Plasma will be separated by centrifugation at 2500 rpm for 10 minutes at 4°C within 1 hour, placed in cryogenic vials, and analyzed or stored at -40°C until analyzed by a validated high-pressure chromatography in the Pharmacokinetics core lab at the University of Chicago Cancer Center.

10.1 Timing of samples:

10.1.1 Test dose:

Blood samples (5 mL) will be taken before Bu infusion, at 175 minutes (five minutes before completion), 240 minutes (1 hour after completion) 300 minutes (2 hours after completion), 360 minutes (3 hours after completion) and 420 minutes (4 hours after completion) of the test dose

10.1.2 Treatment doses 1 and 3 (or 4):

Blood samples (5 mL) will be taken before Bu infusion, at 175 minutes (five minutes before completion), 240 minutes (1 hour after completion) 300 minutes (2 hours after completion), 360 minutes (3 hours after completion of the treatment dose 1 (day -6) and treatment dose 4 (day -3) (or in selected patients, treatment dose 3, day –2)

The Software program WinNonLin Pro ® will be used for calculating AUC.

11.0 PRETREA TMENT EVALUATION

11.1 RECOMMENDED EVALUATION OF THE PATIENT

The pre-transplant evaluation of the patient will follow recommendations as per transplant policies (on website). The following list can be used for guidance.

- Complete history and physical examination
- Bone marrow biopsy and aspirate with leukemia markers,
- cytogeneticsCBC, platelets, differential, reticulocyte count, PT and PTT
- Chest X-ray and PFTs with DLCO
- Baseline EKG,
- MUGA with measurement of LVEF
- Chemistry profile with complete liver function panel.
- Complete urinalysis
- HLA Class I and class II (molecular typing in case of unrelated donor transplantation)
- ABO and Rh typing
- Serum titers for CMV, HSV,HIV antibody, hepatitis screen and any other assays required for donor work-up by transplant unit policy (usually based on FDA recommendations and/or recommendations from federation for accredition of cell therapy, FACT)
- Peripheral blood for chimerism studies.
- Lumbar puncture for cell count, protein, glucose, and cytology in patients with ALL and high grade NHL, or if prior history of CNS involvement.
- CT scan of the chest (with IV contrast) for all patients.
- CT of abdomen and pelvis for lymphoma/Hodgkin's disease patients only.
- Quantitative immunoglobulin, and bone survey for multiple myeloma patients only.
- Urine pregnancy test if female
- Any additional tests that may be required for clinical care and described in the BMT unit policies.
- 20 cc of blood and 5 cc of bone marrow will be stored on all recipients to be used for research purposes. **(including pharmacogenomic assays)**

11.2 RECOMMENDED EVALUATION OF THE DONOR

The pre-transplant evaluation of the donor will follow recommendations as per transplant policies (on website). The following list can be used for guidance.

- Complete History and Physical Examination
- CBC, platelets, differential, reticulocyte count, PT and PTT
- Chest X-ray
- EKG
- Electrolytes, BUN, Creatinine, SMAC, magnesium
- Complete Urinalysis
- HLA Class I and Class II typing (molecular typing in case of unrelated donor transplantation)
- ABO and Rhesus typing
- Serum titers for CMV, HSV, EBV
- HIV serology, Hepatitis screen and any other assays required for donor work-up by transplant unit policy (usually based on FDA recommendations and/or recommendations from federation for accredition of cell therapy, FACT)
- Peripheral blood for chimerism studies.
- 20 cc of blood will be stored on all donors to be used for research purposes.
- 20 cc of blood will also be stored on each day of stem cell collection

12.0 EVALUATION DURING STUDY

12.1.1 EVALUATION DURING THE FIRST 100 DAYS

Evaluation during the first 100 days will be done as per routine for the allogeneic transplant patient (see transplant policies).

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Restaging of disease and engraftment studies will be performed between day 25 and day 35.

12.1.2 DAY 100 EVALUATION (to be completed day 84-100)

The day 100 evaluation of the patient will follow recommendations as per transplant policies (on website). The following list can be used for guidance.

12.1.2.1 Review of Systems and Physical examination
12.1.2.2 CBC, differential, platelets, electrolytes, BUN, Creatinine, Magnesium, glucose, SMAC,
12.1.2.3 Bone marrow biopsy and aspirate with cytogenetics
12.1.2.4 Peripheral blood chimerism studies.
12.1.2.5 Bone marrow aspirate and biopsy
12.1.2.6 restaging of disease as indicated by disease specific testing.

12.1.3 EVALUATION DAYS 100-365 (patient without chronic GVHD)

The one year evaluation of the patient will follow recommendations as per transplant policies (on website). The following list can be used for guidance.

12.1.3.1 PE and screening labs at least monthly through day 365
12.1.3.2 Restaging of disease approximately 3 months, 6 months, 9 months and one year after BMT.
12.1.3.3 Follow-up for patients with chronic GVHD as per the chronic GVHD protocol.

12.1.4 ANNUAL EVALUATION

The yearly evaluation of the donor will follow recommendations as per transplant policies (on website) . The following list can be used for guidance**.**

12.1.4.1 Review of systems and physical examination
12.1.4.2 Schirmer's test
12.1.4.3 Chest X-ray
12.1.4.4 Pulmonary function tests
12.1.4.5 CBC, differential, platelets, electrolytes, BUN, Creatinine, glucose, SMAC, magnesium.
12.1.4.6 Bone marrow biopsy and aspirate with cytogenetics and chimerism studies.
12.1.4.7 Thyroid function tests
12.1.4.8 Restaging of leukemia by bone marrow aspirate and biopsy with cytogenetics and chimerism studies.
12.1.4.9 restaging of disease.

12.1.5 blood and bone marrow samples

Samples of blood and/or bone marrow will be obtained at the following time points:

- Prior to admission (BM and blood)
- On day 0 (Blood)
- On day 7 (Blood)
- On day 14 (Blood)
- On day 28 (BM and blood)
- Day 50 (Blood)
- Day 75 (Blood)
- Day 100 (Blood)
- Day 150 (BM and blood)
- At relapse (BM and blood)
- One year and yearly thereafter (BM and blood).

These samples will be stored for our ongoing studies of MRD and for assessment of immune reconstitution as well as chimerism. Chimerism will be determined by molecular analysis of peripheral blood samples.

An initial blood sample will also be stored for pharmacogenomic studies.

As of February 2013, after IRB approval, cryopreserved pre-transplant and post-transplant recipient specimens and pre-transplant donor specimens collected under this protocol will be used to evaluate the effects of minor histocompatibility antigens and major histocompatibility antigens (HLA) mismatches on transplant outcomes. Genomic, proteomic, functional tests, and other tests will be performed to identify predictors of compatibility between patient and donors. These predictors may help future studies exploring identification of the most suitable donor(s) for patients planning allogeneic hematopoietic cell transplantation. This will be done for protocol IRB 11300A and IRB 12-0132.

DNA isolation will be performed from 1 ml of EDTA-blood sample from each patient sample using the Magnapure Compact system for DNA isolation (Roche Applied Science).

Sample shipment information
3-5 ml of patient blood sample should be drawn in a purple top EDTA tube. Samples should NOT be spun down. Samples should be shipped in appropriate packaging by overnight delivery at room temperature. If a sample cannot be sent the day it is drawn, it can be stored refrigerated at 4°C for 2-3 days prior to shipment. Samples may be shipped to the laboratory on Monday through Thursday. Samples should not be shipped on Friday as the laboratory cannot receive samples on the weekend. All samples need to be appropriately labeled with patient name/ID and protocol number. In addition, all samples should be accompanied by paperwork that specifies the sample ID, protocol number, physicians name and contact information (address, phone number and fax number) to whom the result needs to be reported to. Samples should be shipped to the University of Chicago Genetic Services Laboratory at the following address:

University of Chicago Genetic Services Laboratory
Department of Human Genetics
The University of Chicago
5841 S. Maryland avenue, Room L038
M/C 0077
Chicago, IL 60637

phone: 773-834-0555
fax: 773-834-5337

Protection of confidentiality
Patient samples will be processed and genotyped at the University of Chicago Genetic Services Laboratory which is a CAP and CLIA certified laboratory that routinely performs genetic testing of multiple disorders on a clinical basis. The laboratory

therefore has all necessary procedures related to protecting of patient confidentiality. All samples will be processed by trained technologists and results reported only to the patient's physician or other appropriate health care person. All patient samples will have a protected identification code that will be used.

Table I: Schedule of tests (The following table can be used for guidance. Some individualization may be necessary)

Tests & Observations	**Prior to Study**	**During conditioning**	**Day of Transplant**	**Day 28 (± 1 wk) Post Transplant**	**Day 100, (± 1 wk)**	**Day 180 (± 1 wk)**	**Post-Tx Follow-up****
History and Progress Notes	X		X	X	X X		X
Physical Examination	X	X	X	X	X X		X
Pulse, Blood Pressure	X					X	X
Height/Weight X		X				X	X
Performance Status	X		X	X	X X		X
Toxicity Assessment			X	X			
AGVHD Assessment			X	X	X X		
CGVHD Assessment					X		X
Lumbar puncture	E						
Surveillance Tests							
EKG	X						
PFTs	X						X
Thyroid function tests							X
Schirmer test							X
MUGA (or 2-D Echo)	X						
Staging							
Chest x-ray, PA & Lateral	X						X
CT/MRI scan chest/abd/pelvis	X				D	D	D
Bone Marrow Asp & Bx	X			X	X X		X
Laboratory Studies							
CBC, Differential, Platelets	X	X	X	X	X X		X
Serum Creatinine, BUN	X			X	X X		X
Serum Electrolytes	X			X	X X		X
AST, ALT, Bilirubin	X			X	X X		X
LDH	X			**X**	**X**	**X**	**X**
Urinalysis	X						
serum or u-HCG (for pre-menopausal females)	X						
Hepatitis Screen, CMV Ab, HIV, EBV, HSV-I	X						
Serologies & HLA Typing	X						
Pharmacokinetics		X					
Chimerism							
Peripheral blood (20 cc min.)**	X		X	X	X X		X
Donor peripheral blood (5 cc)	X						
Immune reconstitution							

Tests & Observations	Prior to Study	During conditioning	Day of Transplant	Day 28 (± 1 wk) Post Transplant	Day 100, (± 1 wk)	Day 180 (± 1 wk)	Post-Tx Follow-up**
Peripheral blood (20 cc min.)	X		X	X	X X		X
Optional Tests (i.e. dependent on patient approval)							
MRD (WT1 or IgG gene)							
Peripheral blood (20 cc min.)	X		X	X***	X X		X
Bone marrow aspirate (8 cc)	X			X	X X		X
Pharmcogenomics	X						
Blood (20 cc)	X						
Bone Marrow (5 cc)	X						

** At one-year post-transplant, and then yearly thereafter for a maximum of 5 years from study entry, and at relapse.
*** Additional samples on day 7, 14,
D For lymphoma patients. Other tests may be indicated in myeloma (e.g MRI or bone scan)
E: Only for pts with ALL, High grade lymphoma or history of CNS involvement.

13.0 CRITERIA FOR STUDY EVALUATION

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 molecular detection of MRD will not be taken into account for the definition of clinical recurrence. The diagnosis of disease recurrence will be based on clinical and pathological criteria.

Toxicity will be scored according to NCI/CTC version 3 (http://ctep.cancer.gov/reporting/ctc.html). Any grade 5 (fatal) toxicity will be considered a DLT. In addition grade 4 toxicities will be considered DLT with the exception of the following: Grade 4 hematologic toxicities will not be DLT. Grade 4 infections will not be DLT. Any grade 4 toxicity that can be attributed to infection will not be considered DLT.

To assess severity of GVHD we will use the Seattle criteria (Bearman et al, Blood 85, 2005, 1995). In this system, mild VOD is clinically obvious, but does not require treatment and resolves completely. Moderate VOD requires treatment such as diuretics or pain medications, but resolves completely. Severe VOD requires treatment and does not resolve completely. Severe VOD will be considered DLT.

Acute GVHD will be scored according to the criteria proposed by Przepiorka et al.[58] Chronic GVHD will be scored according to appendix C. 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).[59]

The diagnosis of Veno-occlusive disease will be based on Baltimore criteria as follows: development of hyperbilirubinemia with serum bilirubin >2mg/dl with any two of the following symptoms: ascites, painful hepatomegaly and unexplained weight gain >5% from baseline within 20 days of BMT. Whenever possible, a presumed diagnosis of VOD will be confirmed by liver biopsy.[60]

Engraftment will be defined as per IBMTR and NMDP guidelines. Cytogenetic and chimerism studies will be performed to confirm donor origin.

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.

14.0 ST ATISTICAL CONSIDERATIONS

Phase I

For the phase I portion of the study, we will em ploy the modified continual reas sessment method (CRM) to determ ine the m aximum tolerated dose (Faries, 1994; Goodm an et al., 1995) [61;62]. Dose-limiting tox icity (DLT) will b e as defined und er 13.0 and, occurring within the f irst 21 days after transplant. Dose cohorts of size th ree w ill be u tilized and we will targ et the 25^{th} percen tile o f the tolerance distribution, i.e., the dose level producing DLT in 25% of the patient population. Briefly, the modified-CRM design utilizes a one-parameter, logistic regression model for the dose-toxicity curve

$$p(x) = \frac{\exp(3 + \beta x)}{1 + \exp(3 + \beta x)}, \qquad (1)$$

where $p(x)$ is the probability of DLT at dose level x. A prior distribution is assumed for the parameter β and the first thre e patients are assigned to the lo west dose level. Af ter outcomes are observed for these patients, the posterior distribution for β is calculated and the estim ated toxicity probabilities are updated. T he next cohort of patients are assigned to the dose level cl osest to the targeted percentile, with the restriction that doses can not be esca lated m ore that one le vel at a tim e. Enrollm ent is continued u ntil a p re-determined total num ber of patients have been stu died, whereupon the MTD is derived based on the final, estimated dose-toxicity curve.

In our trial, the f irst cohort of three patien ts will be enrolled at dose lev el 0 and af ter all th ree of these patients hav e been f ollowed to day 60 the observed nu mber of DLTs will be used to up date the posterior d istribution an d determ ine the dose level fo r the succeeding cohort. Sin ce we anticip ate an accrual rate of approximately th ree patients per month, it is likely that all three patients from a given cohort will not have been followed to 60 days before the next eligible patient becom es available. In this c ase w e will assig n such patients (termed *excess* p atients) to th e curr ent d ose leve l; o nce the original th ree patients p ass the 60 -day landm ark, we will up date th e pre diction prob abilities using all available data. However, so as not to bias the procedure, if *excess* patients have not been followed for a full 60 days, we will not include their toxicity outcom es in the updated analysis , even if a D LT has been observed. A total of 30 patients will be enrolled in the phase I trial.

Because delayed VOD (day 30 and day 60) of moderate severity has been obser ved in two of the 12 initial patients on study, the observation perio d for su ccessive patients will be extended to 6 0 days before dose escalation. This will lead to accru al of a higher num ber of patients per dose level and therefore we anticipate up to 50 patients in the phase I trial.

Addendum June 2006: Pharm acokinetics for the initia l 20 patients was perform ed in the cancer center core facility at UC. For logistica l reasons and for more intensive monitoring another laboratory will be

used and technical modifications in sampling have been introduced. Therefore accrual will resume at dose level 0. This will result in accrual of an additional 20 patients to the protocol.

Phase II

The primary endpoint for the phase II portion of the trial is the disease-free survival rate (DFS) at one year defined as the proportion of patients alive and without evidence of recurrence one year after the initiation of therapy. All patients will be followed for a minimum of one year and we will test the null hypothesis that the DFS rate at twelve months is $\leq$ 25% versus the alternative that it is at least 40%. A Simon (1989), optimal two-stage design will be employed in which 29 patients are enrolled in the first stage.[63] (Patients treated at the MTD during the phase I portion of the study will be included in the phase II trial.) If the number alive and without evidence of recurrence at twelve months is 7 or fewer, the study will be terminated for lack of efficacy. Otherwise, subject to the monitoring described below for graft failure, liver toxicity, and GVHD, an additional 43 patients will be enrolled for a total of 72. If 22 or fewer patients are disease free at 12 months the regimen will be rejected, whereas if 23 or more of the total 72 patients ($\geq$ 32%) are disease free, the regimen will be considered worthy of further evaluation in phase III trials. This design has an α-level of 10% and a power of 90% under the alternative hypothesis that the true 12-month DFS rate is 40%. The probability of early termination when the 12-month DFS rate is 25% is 0.56. However, to avoid a long suspension of the trial, accrual will continue into the second stage while the one-year outcomes in the 29 first stage patients are being accumulated.

In addition, the incidence of graft failure, liver toxicity, and high-risk, extensive chronic GVHD at 18 months will be closely monitored and evaluated. For the primary endpoint of DFS, we will employ a Simon (1989) two-stage design. Graft failure, liver toxicity, and GVHD will be monitored at specified intervals as described below. An independent Data and Safety Monitoring Committee will be established to review the data and make recommendations regarding the continuation or discontinuation of the trial. This committee is organized by the University of Chicago Cancer Center (High risk protocol monitoring committee) Since the assumptions regarding efficacy and toxicity are the same, the related donor and unrelated donor subgroups will be combined for all analyses.

Adverse Event Monitoring

Graft failure and liver toxicity will be monitored and early termination of the trial will be considered if there is evidence that the graft failure rate is >10% or the rate of liver toxicity is >15%. Specifically, for graft failure the data will be reviewed after successive cohorts of 10 patients have been treated and we will consider terminating the trial if 4 of the first 10, 6 of the first 20, 7 of 30, 9 of 40, 10 of 50, 12 of 60, or 13 of 70 have graft failure. For liver toxicity the trial will be stopped if 5 of the first 10, 7 of the first 20, 9 of 30, 11 of 40, 13 of 50, 15 of 60, or 17 of 70, develop liver toxicity. With regard to high-risk, extensive GVHD, we will review the data after the first 10, 20, and 30patients with successful engraftment and without evidence of relapse reach their 18-month landmark. (By the time 30 patients have reached this point, all 72 patients will have almost surely been accrued.) Evidence that the rate of high-risk, extensive GVHD is >40% will lead to consideration for early stopping. Specifically, if 8 of the first 10, 13 of the first 20, or 17 of 30 such patients develop GVHD at any time

prior to 18 months we will consider terminating the trial. Each of these three stopping guidelines approximates a Pocock (1977) [64] group sequential monitoring boundary for a one-sided, overall alpha level of 0.10.

Additional Statistical Analyses

At the conclusion of the phase II trial, 90% confidence intervals will be generated for the DFS rate at 12 months, as well as for the incidence of graft failure, and the incidence of active and extensive GVHD. Kaplan-Meier (1958) estimates of disease-free and overall survival rates will be calculated, [65] and the median disease-free and overall survival times and their associated 90% confidence intervals derived using the method given in Brookmeyer and Crowley (1982). [66] Descriptive statistics related to the frequency of adverse events, including liver toxicity, and of changes in laboratory values will be generated. Association between GSTA1 enzymatic activity and busulfan metabolic rate (i.e. AUC, $T_{1/2}$) will be determined using the Pearson correlation coefficient or, in case of a monotone but non-linear relationship, by Spearman's rank correlation coefficient. To account for other determinants of metabolism, multiple linear regression analysis will also be performed. The relation between enzymatic activity and incidence of VOD will be assessing using logistic regression analysis. Multiple logistic regression analysis may be attempted, depending on the frequency of VOD.

The relationship between GST polymorphisms and VOD will be analyzed using chi-square or Fisher's exact test. The association between polymorphisms and busulfan pharmacokinetics will be analyzed using a t-test or non-parametric Wilcoxon rank-sum test, as appropriate. Finally, the pharmacogenomic determinants of drug efficacy will be assess using Cox (1972) multivariable regression analysis[67] with time to disease progression or death as the outcome variable and genotype and busulfan pharmacokinetic parameters (AUC, etc.) as predictor variables.

Estimated duration of accrual

For the phase I trial, we estimate approximately 4-5 patients per month will be accrued, so that the target of 30 subjects should be reached in 6-7 months. For the phase II study, we expect to accrue about 30 such patients per year. Assuming about 6- 10 patients from the phase I study participate, a little over two years of recruitment and one year of additional follow-up will be required to complete the phase II trial.

15.0 DATA AND PROTOCOL MANAGEMENT

15.1 PROTOCOL COMPLIANCE Patients will be reviewed weekly during admission by the study investigators who will score the patient for standard endpoints. After discharge they will be reviewed at least once a month.

15.2 DATA ENTRY Data must be entered into the transplant database on a regular basis. Flowsheets will be kept for clinical and data collection purposes. A brief explanation for required but missing data should be recorded as a comment.

15.3 ACCURACY OF DATA COLLECTION The Study Chairman will be the final arbiter of toxicity should a difference of opinion exist.

15.4 REPORTING TO IBMTR AND NMDP

As is customary in most transplant centers we plan to provide data to the International Bone Marrow Transplant Registry. We will also provide outcome data on unrelated donor recipients to NMDP as requested by that organization. Appropriate language will be included in the consent forms for this purpose.

16.0 CRITERIA FOR REMOVAL FROM PROTOCOL

16.1 At patient request.

16.2 Clinical progression. Such patients may be treated on other treatment protocols or at the investigator's discretion. Such patients will continue to be monitored for survival and, may be asked to continue to provide specimens for studies of minimal residual disease and immune reconstitution as other treatments are recommended.

17.0 REPORTING REQUIREMENTS

Any unexpected life-threatening and serious (grade 3 or 4) toxicity will be reported immediately to the Study Chairman. The Chairman will be responsible for notifying the Surveillance Committee.

<u>Expected toxicities are those listed in the consent form</u> and include regimen-related toxicities, myelosuppression, opportunistic infections such as CMV reactivation, or GVHD. These will not be routinely reported to the IRB even if they require admission.

On the other hand, such toxicities will be monitored by the PI and the transplant team and reported regularly at the High Risk Protocol Committee of the cancer center.

All deaths that are not due to disease recurrence will be reported to the IRB.

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APPENDIX A: **Treatment plan**

Day	Clonazepam	Fludarabine	Busulfan	Bu pharmacokinetics	Campath
Test Dose					
-	PO				
-	PO		0.5 mg/kg	x 6	
-	PO				
Treatment Doses					
-7 PO		25 mg/m^2			20 mg
-6	PO	25 mg/m^2	AUC based	x 5	20 mg
-5	PO	25 mg/m^2	AUC based		20 mg
-4	PO	25 mg/m^2	AUC based	(x 5*)	20 mg
-3	PO	25 mg/m^2	AUC based	x 5*	20 mg
-2	PO				
-1					
0					

Comments:
Busulfan administration has been associated with a low incidence of seizures and dilantin is routinely used for seizure prophylaxis. Dilantin use is discouraged in this protocol because exposure to dilantin can affect Busulfan metabolism.[69]
We recommend benzodiazepines e.g clonazepam (klonopin ®) 1 mg PO TID
Scheduling:
Busulfan test dose:
The test dose can be scheduled any day within 8 days prior to admission. Clonazepam will be started with an evening dose, the day prior to the test dose and two doses one day after test dose. Busulfan test-dose will be based on actual body weight and will be accompanied by extensive pharmacokinetic studies. This dose will be given as an outpatient.
Patients will be admitted in the evening of day –8, to start fludarabine on day -7

Fludarabine:
Fludarabine dosing will be based on actual body weight. Fludarabine will be infused over 30 minutes before busulfan treatment dose.

Busulfan treatment doses:

On each day of treatment, busulfan will be administered through a fresh infusion line at a steady rate over 3 hours. Infusion will start in the morning around 8 AM and will be completed around 11 AM, allowing ample time for pharmacokinetic sampling in the afternoon.
*Pharmacokinetic studies will be performed on the day 1 dose and again on the day 4 dose. For logistical reasons (week-end, holidays), day 3 pharmacokinetics may be performed instead of day 4.

Campath
All patients will receive premedication for Campath as per current standards at the UC transplant unit.

18.1.1 Dose escalation schedule (see also statistical plan)

The targed AUC of busulfan will be escalated and de-escalated in an up and down design to achieve a dose where less than 4 of 12 patients have a DLT.

Target AUC's are the following:
Dose level 0**:** 4800 mcgr.min/L.dose
Dose level 1**:** 5800 mcgr.min/L.dose
Dose level 2**:** 6800 mcgr.min/L.dose
Dose level 3**:** 7800 mcgr.min/L.dose
Dose level 4**:** 8800 mcgr.min/L.dose
Dose level 5**:** 9800 mcgr.min/L.dose

We do not anticipate further dose-escalation beyond dose level 5. If such dose-escalation were to be considered, we would resubmit an amended protocol to the IRB.

18.1.2 Bone marrow/stem cel infusion

On day 0 the stem cell product will be infused according to BMT unit policy.
Bone marrow, PBSC will not be further processed except in the case of ABO-incompatibility. In such instances, red blood cells will be removed from bone marrow products or from PBSC products containing excessive amounts of RBC (as per transplant policy).
All stem cell products procured through the NMDP will be done so in strict compliance with the protocols, policies, and procedures established by the NMDP.

18.1.3 GVHD 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 unless the patient has developed GVHD.

18.1.4 Supportive care:

Infection prophylaxis and supportive care will be as BMT unit policy. No routine growth factor support will be administered.Growth factor support will be considered in case of delayed engraftment (ANC $< 0.5x10^9$/L on day 12)

18.1.5 Back-up bone marrow harvest (only for recipients of mismatched or unrelated donor transplants)

For patients transplanted in remission, a backup bone marrow or peripheral blood stem cell collection will be considered within two weeks prior to hospital admission as long as the overall marrow cellularity is greater than 30%. All backup harvests will be cryopreserved after processing for buffy coat without any further manipulation. The backup stem cells will be given if deemed clinically necessary in the event of failure to engraft by day +35, graft rejection after day +35, or graft failure after day +35.

APPENDIX B

GVHD GRADING CRITERIA

CLINICAL GRADING OF ACUTE GVHD
(Thomas et al., NEJM, 229:895, 1975)

Grade	Degree of Organ Involvement
I	+ to ++ skin rash; no gut involvement; no liver involvement; no decrease in clinical performance
II	+ top +++ skin rash; + gut involvement or + liver involvement (or both); mild decrease in clinical performance
III	++ to +++ skin rash; ++ to +++ gut involvement or ++ to ++++ liver involvement (or both); marked decrease in clinical performance
IV	Similar to grade III with ++ to ++++ organ involvement and extreme decrease in clinical performance

Stage	Skin	Liver	Intestinal Tract
+	Maculopapular rash <25% to body surface	Bilirubin 2-3 mg/100 ml	>500 ml diarrhea/day
++	Maculopapular rash 25-50% body surface	Bilirubin 3-6 mg/100 ml	>1000 ml diarrhea/day
+++	Generalized erythroderma	Bilirubin 6-15 mg/100 ml	>1500 ml diarrhea/day
++++	Generalized erythroderma with bullous formation and desquamation	Bilirubin >15 mg/100 ml	Severe abdominal pain, with or without ileus

CLINICAL GRADING OF CHRONIC GVHD

(Shulman et al. Am J Med 69:204, 1980)

Extensive - multiorgan involvement clinically
Limited - only skin involvement clinically
Subclinical - only histologic evidence

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