THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Protocol Page

Allogeneic stem cell transplantation for myelofibrosis and myelodysplastic syndrome using reduced intensity busulfan and fludarabine conditioning.
2005-0726

Core Protocol Information

Short Title	Allogeneic stem cell transplantation for myelofibrosis
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Which Committee will review this protocol?

(•) The Clinical Research Committee - (CRC)

Protocol Body

1.0 Objectives

Primary Objective
To assess the safety of fludarabine and busulfan as a preparative regimen for allogeneic stem cell transplantation in patients with myelofibrosis as determined by non-relapse mortality.

Secondary Objective
To evaluate efficacy of this therapy as determined by:
1.1 Engraftment
1.2 Complete clinical/hematological remission
1.3 Progression free survival
1.4 Overall survival

2.0 Background

2.1. Overview of the myeloproliferative disorders:

The myeloproliferative disorders (MPD) are chronic hematological malignancies originating at the level of pluripotent hematopoietic stem cells (1,2). The Philadelphia chromosome negative MPDs classified on the basis of the predominant feature include:

a) Polycythemia vera (PV): overproduction of red cells
b) Essential thrombocythemia (ET): overproduction of platelets
c) Idiopathic Myelofibrosis (IM): bone marrow fibrosis

The concepts common to these disorders are:

1) Clonal hematopoiesis
2) Transformation to acute leukemia (AL): Up to 10% of PV & ET patients develop AL.
3) Progression to myelofibrosis: Up to 10% of patients with ET and PV progress to myelofibrosis with development of massive hepatosplenomegaly, cytopenias and transfusion dependency. Typically, AL occurs following progression to myelofibrosis, although de novo AL is seen. These patients who have progressed to myelofibrosis and/or to acute leukemia have a poor prognosis.

2.2. Idiopathic Myelofibrosis:

Idiopathic myelofibrosis (IM) (agnogenic myeloid metaplasia, myelofibrosis with myeloid metaplasia) is a clonal myeloproliferative disorder due to an acquired somatic mutation of a hematopoietic progenitor cell, resulting in clonal erythrocytes, platelets, granulocytes, monocytes and their precursors. These malignant megakaryocytes and monocytes secrete fibrogenic cytokines – TGF , PDGF, FGF, which cause polyclonal fibroblast proliferation, collagen and reticulin deposition, and eventual bone marrow

fibrosis, the hallmark of the disease. This process progresses to bone marrow failure and extramedullary hematopoiesis (1,3,4).

The annual incidence of IM ranges from 0.5 –1.5 per 100,000, while the median age at diagnosis is 55-65 years. Most patients with IF present with anemia, marked splenomegaly, early satiety and constitutional symptoms. During the clinical course, most experience progressive anemia requiring frequent blood transfusions, and death occurs eventually due to bone marrow failure or leukemic transformation. Current treatment of IM is palliative, consisting of supportive care with blood transfusions, splenectomy (5), splenic radiation, hydroxyurea, and more recently thalidomide (6-9). None of these therapies alter the natural history of this disease.

The median survival is 5 years from diagnosis, but varies from 2 years to greater than 10 years depending on presence or absence of well-defined prognostic indicators. These include anemia (Hb<10gm/dl), advanced age (>64), constitutional symptoms (fever, night sweats, weight loss), leukocytosis (>30,000/l) or leucopenia (<4,000/l), circulating blasts (>1%), and high-risk cytogenetic abnormalities (+8, 12p-). A simple but widely accepted scoring system using two of these factors, namely Hb<10gm/dl and WBC >30,000/l or <4,000/l separates patients into three groups with low (0 factor), intermediate (1 factor), and high (2 factors) risks, associated with a median survival of 93, 26, and 13 months (4,10,11). **We propose to treat patients with intermediate and high-risk disease in this study of allogeneic stem cell transplantation.**

2.3. Allogeneic stem cell transplantation:

Allogeneic hematopoietic cell transplantation by eradicating the malignant clone and restoring normal hematopoiesis would be expected to alters the natural history of IM.

Table 1: Allogeneic transplantation for myelofibrosis.

Study	N	Median Age	NRM	Median Follow up	OS
Guardiola et al (12)	55	42	27%	36 months	47%
Daly et al (13)	25	48	48%	35 months	41%
Deeg et al (14)	56	43	32%	34 months	58%
Rondelli et al (15)*	21	54	10%	31 months	85%
Kroger et al (16)*	21	53	16%	22 months	84%

* Retrospective multicenter studies using multiple reduced intensity regimens

Due to the uncommon occurrence of this disease and older age at disease onset, only five studies with more than 20 patients have been reported in the literature, and all but one are multicenter/registry studies (12-16). In the largest study from the Fred Hutchinson Cancer Center in Seattle, 56 patients with a median age of 43 years were transplanted from HLA-matched donors for myelofibrosis. At the median follow up of 34 months, five-year survival was 58% with continuing resolution of fibrosis, reduction in spleen size, transfusion independence, and improvement in blood counts. Six patients rejected the graft (n=3) or were mixed chimera (n=3). The major reason for treatment failure was transplant related mortality of 32% (14).

A special problem area is the treatment of older or debilitated patients. Guardiola published a series of 55 patients demonstrating inferior results for older patients: Five year survival of 14% in patients older that 45 years compared to the 5 year survival of 62% in younger patients (12). These inferior results in older patients and patients with comorbidity who comprise majority of patients with this disease make myeloablative transplantation a promising but not suitable therapy most patients with myelofibrosis.

Another approach would be to use a reduced intensity or a nonmyeloablative conditioning regimen, which have been reported to extend allogeneic transplant to older patients and patients with other co-morbid medical conditions. Others and we have shown that reduced intensity stem cell transplantation can be safely utilized in older patients and those with comorbidities in other hematological malignancies (17-22). Rondelli et al and Kroger et al have reported encouraging data in multi institutional studies of reduced intensity transplantation in older patients with myelofibrosis as detailed in the table 1(15,16). In these retrospective studies with a median age of 54 years, a non-relapse mortality of 10%-16% and 3 year overall survival of 84%-85% was reported. We wish to prospectively evaluate the role of allogeneic stem cell transplantation using reduced intensity conditioning regimen of fludarabine and busulfan.

2.4 Busulfan and Fludarabine Conditioning

Several reduced intensity regimens have been studied in patients with hematological malignancies (17-22), but It is not clear if one is superior to the other. In this study we

would like to use fludarabine and busulfan as we have studied these agents extensively in myeloid malignancies. Kroger et all used this regimen in a retrospective study in patients with myelofibrosis with 16% non relapse mortality and 84% overall survival (16). In patients with CML, this regimen has been reported to be well tolerated with no treatment related mortality within first 100 days and 85% 5 year disease free survival (23).

3.0 Patient Eligibility

Inclusion Criteria

1. Patients with Idiopathic Myelofibrosis or Myelofibrosis secondary to Polycythemia Vera or Essential Thrombocythemia or Myelodysplastic syndrome with or without fibrosis.
2. Patients 75 years or younger
3. Patients must have an HLA matched or at least a 9/10 antigen (A, B, C, DQ or DR) matched related or unrelated donor.
4. Patients must have a Zubrod PS $\leq$2.
5. Creatinine < 1.6 mg/dl
6. Ejection fraction $\geq$ 40%, unless cleared by cardiology
7. Serum direct bilirubin < 2 mg/dl (unless due to Gilbert's syndrome), SGPT $\leq$ 4 x normal values.
8. FEV1, FVC, or DLCO $\geq$ 40% of expected.
9. Negative Beta HCG test in a woman with child bearing potential defined as not post-menopausal for 12 months or no previous surgical sterilization)

Exclusion Criteria

1. Uncontrolled life-threatening infections
2. HIV positive
3. Patients with active viral hepatitis

4.0 Pretreatment evaluation

Patient :

The following evaluation includes standard test performed to determine patient eligibility and should not be repeated prior to starting treatment unless treatment is delayed for more than 30 days after registration.

a) Patient medical history and physical examination.
b) CBC, differential and platelet count
c) Bone marrow aspiration and biopsy
d) Chemistry panel
e) Serology for CMV, HBV, HCV and HIV
f) B-HCG in women of child bearing potential
g) Sample to store pre-transplant DNA for future analysis of chimerism

h) Cardiac ejection fraction
i) Pulmonary function tests with DLCO
j) Chest x Ray
i) Urine analysis

Donor:

Acceptability of the allogeneic stem cell donor (bone marrow or peripheral blood progenitor cell) will be determined according to MDACC Apheresis Unit (for related donors) or National Marrow Donor Program (for unrelated donors) standard operating procedures

5.0 Treatment Plan

5.1 Preparative Regimen

Treatment will start on D-7 as per the schema shown below:

-7	-6	-5	-4	-3	-2	-1	0
		Flu	Flu	Flu	Flu		
Bu test dose 32 mg/m^2	Rest	Bu	Bu	Bu	Bu		
				ATG*	ATG*	ATG*	
							HSCT

Fludarabine 40mg/m^2/day IV over 60 mins x 4 days (days -5,-4,-3,and-2)
Busulfan ~~(~~days -5,-4,-3,and-2~~)~~
Rabbit ATG (Thymoglobulin) 2.5 mg/kg (infused over 6 hours) X 3 days (day -3,-2 and –1)*
*Patients with HLA-identical sibling donors do not receive Thymoglobulin.

Donor bone marrow or blood stem cells to be infused on day 0.

BUSULFAN is administered at the dose of 100 mg/m^2 in normal saline over three (3) hours i.v. every twenty-four (24) hours for four (4) consecutive days (days -5 to -2). The busulfan dose on day -5 to –2 will be altered on the basis of the pharmacokinetic studies to target an AUC of 4000 mMol-min ± 12%. This altered dose will be administered in an identical fashion to the 100 mg/m^2 dose. If the patients cannot undergo the pharmacokinetic studies, the patient will not receive the 32 mg busufan “test dose” and will receive the 100 mg/m^2 dose day –5 to –2. Patients may also undergo the busulfan test dose as an outpatient, with inpatient admission on day -6.

Busulfan pharmacokinetic studies: Patients will receive a test dose of busulfan at 32mg/m^2 i.v. over on day – 7 for measurement of pharmacokinetics to determine the dose that will give a daily AUC of 4000 µMol-min ± 12% on days –5 through day –2. Blood samples (5ml each) will be drawn on day –7 at 30 min after the start of infusion and 5 min. before the end of infusion, then after the end of infusion at 15, 30, 60 min. and at 2, 4, 6 and 8 hours, and one sample approximately 10-11 hours.

Pharmacokinetic analysis and individualized IV busulfan dose will be calculated and ordered on day –6. Follow-up PK samples (quality control) will be drawn on days –5 and if necessary, for fine-tuning of the busulfan dose, and if necessary on day –3. Patients receiving the fixed-dose treatment arm will also have PK studies performed on day(s) –5, and if necessary on day –3 to allow for monitoring and if necessary, adjustment of busulfan dosing per above. This complete sampling schedule will be replaced with a limited sampling schedule when a validated limited sampling assay becomes available.

5.2 Allogeneic Stem Cell Collection

Stem cells will be harvested following MDACC Apheresis Unit or National Marrow Donor Program standard operating procedures. The choice between bone Marrow stem cells or blood stem cells will be made as per department of blood and marrow transplantation standard guidelines.

5.3 Post-transplant care

Post transplant care outlined below is considered standard clinical practice in BMT

GVHD prophylaxis: GVHD Prophylaxis will consist of tacrolimus and methotrexate, starting from day-2. Tacrolimus blood levels will be monitored and dose adjusted as clinically indicated. Patients will also receive methotrexate 5 mg/m2 on days 1,3,6, and 11 post BMT.

Donor leucocyte infusion (DLI): Patients with evidence of residual disease or progressive disease but without GVHD one month after stopping immunosuppression, may receive DLI as per departmental guidelines for dose and schedule.

Supportive Care: G CSF, prophylactic antibiotics, antivirals, antifungal and therapeutic antibiotics, transfusions as clinical indicated by the patients condition, will follow BMT standard practice. Due to the possibility of drug interaction with busulfan, Tylenol is contraindicated from day –5 to day 0, and alternative medications should be used.

6.0 Evaluation During Study

Evaluations for this study (6.1-6.5) are considered standard practice in BMT and should be done around 1, 3, 6, 9, 12, 18 and 24 months post transplantation. If clinically indicated these studies may be done at other time points to evaluate for potential relapse of the underlying malignancy, which can replace the nearest planned time points. After two years patient will be seen annually or information about patient's condition will be obtained by a telephone call or written communication.

6.1 Bone marrow Bx and aspirate with cytogenetics, and chimerism studies.
6.2 History and physical examination with evaluation of adverse events including GVHD
6.3 CBC, differential, platelets
6.4 SGPT, calcium, glucose, uric acid, magnesium, LFT, serum bilirubin, BUN and creatinine, serum protein, albumin, alkaline phosphatase, electrolytes.
6.5 Peripheral blood for chimerism studies.(the first chimerism study will be done 3-4 weeks post transplant).

7.0 Criteria for Study Evaluation

7.1 Primary end point:

Deaths not attributable to the disease recurrence will be considered as an event for assessment of non-relapse mortality.

7.2 Secondary end points:

Engraftment is defined as a sustained ANC $\geq$ 0.5 x 10^9/L for for 3 consecutive days and evidence of donor chimerism by D+28 (the first chimerism study will be done 3-4 weeks post transplant).

Primary Graft Failure is defined as failure to achieve an ANC $\geq$ 0.5 x 10^9/L for 3 consecutive days by day 28, with <10% cellularity on bone marrow biopsy and no evidence of donor chimerism.

Secondary graft failure is defined as a sustained declined of ANC <0.5 x 10^9/L for 3 consecutive days after initial documented engraftment with no evidence of disease progression.

Complete remission is defined as per response criteria defined by European Myelofibrosis network. (appendix)

Relapse will be recorded by the day of detection.

Progression free survival is defined as the interval between day of transplant and day of death or disease progression.

Overall Survival is defined as the interval between day of transplant and day of death.

8.0 Statistical Considerations

This is a phase II study with a primary objective of assessing the safety of Fludarabine, and Busulfan as preparative regimens for allogeneic stem cell transplantation in patients with Myelofibrosis and MDS. Patients enrolled in the study will be younger than 75 years of age and must have an HLA matched or at least a 9/10 antigen matched related or unrelated donor. The major reason for treatment failure in these patients is transplant related mortality. Therefore, in order to assess the primary objective of safety, the primary outcome measure will be the rate of non-relapse mortality (NRM) at 100 days. We will define non-relapse mortality as any death not attributable to disease recurrence. The cumulative incidence rates of NRM ranged from 27%-48% in three studies of younger patients with this disease. The NRM rate is expected to be much higher in this mostly older group of patients with advance myelofibrosis we are proposing to treat because NRM increases with age and coexisting medical illnesses. Therefore, the goal of the trial is to achieve better than a 30% NRM rate at 100 days post transplant with this treatment regimen. The proposed trial will evaluate the role of allogeneic stem cell transplantation a using reduced-intensity conditioning regimen of fludarabine and busulfan. These patients will receive 4 days of the Fludarabine regimen and 2 days of the Busulfan regimen. The maximum total sample size for this trial was originally 30 patients. It was increased in July 2009 to 60 patients. As of September 2011, 49 patients have been enrolled, and we now plan to increase enrollment to 110 patients. We expect to accrue 20 patients a year for a total of 3 years. The goal of the trial is to achieve better than a 30% NRM rate at 100 days post transplant. We will stop the trial if we have evidence that the NRM rate is higher than targeted.

The method of Thall and Simon will be employed to perform interim safety monitoring. As of September 2011, a total of 49 patients have been enrolled, of which 45 have been evaluated for NRM at day 100. Monitoring has proceeded according to the original stopping rule with no need for early study termination. For the remaining 65 patients, we will employ the following monitoring rule. The trial will be stopped early if:

$$Pr[\ \theta E,\ D > 0.30 \mid data\ ] > 0.90$$

That is, given the outcomes from the patients who have already been evaluated, if we determine that there is greater than a 90% chance that the NRM rate is 30% or more, we will stop the trial. We assume a Beta(0.6, 1.4) prior distribution for the rate of non-relapse mortality. Because 45 patients have been evaluated to date, stopping boundaries corresponding to the above probability criterion start at the 45th patient. We will
terminate the trial if:

(# of non-relapse mortalities) / (# patients evaluated)

>= 18/45, 19/47, 19/48, 20/50, 20/51, 21/53, 21/54, 22/56, 22/57, 23/59, 23/60, 24/62, 24/63, 25/65, 26/67, 26/68, 27/70, 27/71, 28/73, 28/74, 29/76, 29/77, 30/79, 30/80, 31/82, 31/83, 32/85, 32/86, 33/88, 33/89, 34/91, 34/92, 35/94, 35/95, 36/97, 36/98, 37/100, 37/101, 38/103, 38/104, 39/106, 39/107, or 40/109

It should be noted that these boundaries are unconditional on the fact that 43/45 evaluable patients to date did not experience NRM. Thus, the first several stopping boundaries are impossible to meet, but they are listed for completeness. If each of the subsequent patients experience NRM, the first stopping boundary that could potentially stop the study is if 27/70 evaluable patients experience NRM.

Accrual will not be suspended while waiting to evaluate the 100-day NRM rate.

The operating characteristics of this study design are shown in the following table. As was the case above, it should again be noted that the listed stopping probabilities and sample size quantiles are unconditional upon the observed data.

Table 2. Operating Characteristics for Stopping Rule

Clinical Scenario	**Early Stopping Probability**	**Sample Size: 25th, 50th, 75th percentiles**		
Non-relapse mortality rate is 0.20	0.002	110	110	110
Non-relapse mortality rate is 0.25	0.04	110	110	110
Non-relapse mortality rate is 0.30	0.22	110	110	110
Non-relapse mortality rate is 0.35	0.59	45	80	110
Non-relapse mortality rate is 0.40	0.89	45	45	68
Non-relapse mortality rate is 0.45	1.00	45	45	45

Secondary Endpoints

Efficacy endpoints such as time to engraftment, complete remission (CR), progression-free survival (PFS), and overall survival (OS) will be evaluated after 3-years of follow-up. We will estimate each of these efficacy endpoints with the Kaplan-Meier estimators. In addition, we will report each of the estimates along with their corresponding two-sided 95% confidence intervals. We will use Cox proportional hazards regression analysis to assess the association between the above survival parameters and clinical and demographic covariates of interest. We will use logistic regression to assess the association between clinical response (CR/PR vs. SD/PD) and the same covariates.

9.0 Criteria for Removal from the Study

1. Any patient can be removed from study if, in the judgment of the Principal Investigator, further treatment is not in the best interest of the patient for whatever reason.
2. Disease progression after DLI.
3. Death.
4. Patient withdraws informed consent.
5. First bone marrow aspiration performed after transplantation showing primary engraftment failure.
6. Inability or unwillingness to have follow-up visits and/or laboratory tests required by this protocol.

10.0 Data and Protocol Management

Data necessary to assess and analyze primary and secondary objectives of this study will be collected in the departmental research database at different time points as mandated by the study plan.

Adverse event (toxicity) definition and data collection:
An AE is defined as any untoward medical event, including worsening of increased frequency of an event present at baseline, in a subject registered in this study and receiving Fludarabine/Busulfan//ATG plus allogeneic stem cell transplant or unanticipated benefit, without regard to the possibility of a casual relationship.
In this study, patients are expected to experience changes in laboratory parameters due to the underlying disease and the nature of the treatment for allogeneic stem cell transplant. These expected changes will not be considered AE(s) and will not be recorded in the database unless in the view of the investigator they are judged to be clinically significant.
Transplant related adverse events:
For the purpose of this study, common transplant related AE are those known to be related to the preparative regimen and stem cell infusion occurring up to 30 days post transplant. The most common are listed below.
Neutropenic Fever without infection, non-Neutropenic fever, infections associated with grade 3 or 4 neutropenia, nausea and vomiting, readmission during the active treatment period (D+30 post stem cell infusion) transfusions of platelets and RBCs,

low blood pressure due to dehydration requiring fluid replacement, mucositis, engraftment syndrome, These events will be monitored and captured in the database during the first 30 days post transplant.
The following common transplant related events will be captured in the database any time when observed: cytopenias post-transplant, including secondary graft failure whether or not leading to death, hemorrhagic cystitis, liver function test abnormalities associated with VOD, TTP, significant infections and graft vs. host disease.

AE Assessment

AE(s) will be assessed according to the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0

Reporting Adverse Events:

Serious and unexpected adverse events will be reported accordingly to MDACC guidelines and the BMT reporting requirements. The end of active treatment is the day of the allogeneic stem cell infusion. These events will be reported to the study chairman, who in turn will notify the MDACC IRB.

11.0 Background Drug Information

11.1 Busulfan Injection

Therapeutic Classification: Antineoplastic Alkylating agent

Pharmaceutical data: Busulfan injection is a sterile, pyrogen-free solution provided in a mixture of dimethylacetamide (DMA) and polyethyleneglycol 400 (PEG400). It is supplied in 10 ml single use ampoules at a concentration of six (6) mg busulfan per ml. Each ampoule contains 60 mg of busulfan in 3.3 ml of DMA and 6.7 ml of PEG400. When diluted in normal saline or D5W to a concentration of 0.5 mg/ml, the resulting solution must be administered within eight (8) hours of preparation including the three (3) hour infusion of the drug.

Stability and storage: Ampoules should be stored refrigerated at 2-8°C (35-46°F). Stable at 4°C for at least twelve (12) months. Additional stability studies are in progress. DO NOT use beyond the expiration date. DO NOT use if the solution is cloudy or if particulates are present.

Solution Preparation: Prepare the busulfan solution as follows (The patient is to receive a dose of 130 mg/m^2 of busulfan):

Use sterile, non-pyrogenic, disposable containers, syringes, needles, filters, stopcocks, and transfer tubing, etc.

All transfer procedures require strict adherence to aseptic techniques. Preferably, these transfer procedures will be carried out in a laminar flow hood.

Calculate the amount of drug to be administered based on the dosage of 130 mg/m^2 and the patient's body surface. For example: A 70 kg patient with a height of 170 cm, (BSA of 1.8 m^2) who receives a dose of 130 mg/m^2 of busulfan (6mg/ml) would receive 39 ml of busulfan for injection:

(1.8 m^2)(130 mg /m^2)·(6mg/ml)= 39 ml busulfan.

Prepare a solution of 0.9% Sodium Chloride Injection USP (normal saline), that is 10 times the volume of the calculated busulfan dose in ml from the step above (39 ml busulfan). This preparation will insure a final concentration of at least 0.5 mg busulfan/ml. For example, (39 mL dose) x (10) = 390 mL of normal saline. The final volume of solution will be 390 + 39 = 429 mL

(390 mL+39 mL = 429mL)

The resulting busulfan concentration will be 0.545mg/ml
(234 mg ÷ 429 mL = 0.545 mg/mL).

In each bag 6.0 mg busulfan (1.0 ml at 6 mg/ml and 11 ml saline) should be added to compensate for drug lost in the tubing with each infusion (approximately 12 ml at 0.5 mg/ml is lost in the tubing).

Break off the top of the ampoule and use a syringe needle to remove the calculated volume of busulfan from the primary container. Remove the needle, replace with a new needle which has been fitted with a 5.0 micron nylon filter (provided with packaged drug) and transfer the contents of the syringe into the calculated amount of either normal saline or D5W making sure that the drug flows into and through the solution. Do not put the busulfan solution into a syringe or IV bag, which does not contain the normal saline or D5W. Mix by inverting the container numerous times to ensure a homogenous solution. Place an appropriate label on the container with an expiration time of eight (8) hours from the time of preparation with directions to store at room temperature. Do not use if solution contains visible particulates. Record the actual volume on the label (429 mL in the example).

Place a suitable (non-vented or universal) intravenous administration set (gravity flow) into the outflow port of the container of the infusion solution.

Route of Administration: It is to be noted, that a sufficient amount of diluted busulfan should be added to compensate for the amount needed to prime the IV tubing; when hanging the infusate, the tubing should be primed with the busulfan solution and connected as close to the patient as possible, i.e. by a 3-way connector at the level of the central venous catheter. After completed infusion, the tubing with remaining busulfan (approximately 12 mL) should be disconnected and discarded. All busulfan

infusions should be performed by programmable pump.

The busulfan will be given by slow intravenous infusion over three (3) hours into a central venous catheter.

CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.

An infusion pump will be used with the busulfan solutions as prepared above. A new infusion set must be used for administration of each dose. Prior to and following each infusion, flush the catheter line with normal saline or (approximately 5 ml). Start the three-hour infusion at the calculated flow rate (i.e. 429 mL/180 min 2.4 mL/min). DO NOT infuse concomitantly with another intravenous solution of unknown compatibility.

If a delay in administration occurs after the infusion solution is prepared, the properly identified container should be kept at room temperature (20-25°C), but administration must be completed within eight (8) hours of preparation including the three (3) hour drug infusion.

Known Side effects: Dose limiting toxicity is expected to be hematological when used without stem cell support. Other toxicities seen frequently following high-dose busulfan in preparative regimens for bone marrow transplantation include: VOD, nausea, vomiting, pulmonary fibrosis, seizures, rash, and an Addison's-like syndrome.

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

Animal Tumor Data: Busulfan has been shown to be active against a variety of animal neoplasm in vivo, including mouse sarcoma 180 and Ehrlich's mouse ascites tumor.

Animal Toxicology: Busulfan fed to rats in an amount equivalent to about 0.5 mg/kg of final body weight per day slowed weight gain and produced bone marrow depression, pancytopenia and cataracts after about 10 weeks. In rats, LD5O was found to be 34 mg/kg intraperitoneally. When the drug was administered on day 13, 14, or 15 of gestation at a dose of 10 mg/kg to rats, the progeny were prematurely sterile.

Human Pharmacology: Limited pharmacology data are available for the parenteral formulation to be used in this study and is detailed in Attachment II, Preliminary Pharmacokinetic Evaluation of Busulfan in a Phase II human Trial. The pharmacokinetic data suggests that the plasma decay of the formulation fits a two-compartment model. The oral formulation is absorbed from the gastrointestinal tract, and measurable blood levels are obtained within one-half to two (0.5-2.0) hours after ingestion. Within three (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; twelve (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 fifty percent (10-50%) of

a dose is excreted as metabolites within twenty-four (24) hours.

11.2 Fludarabine

Therapeutic Classification: fluorinated nucleoside analog

Pharmaceutical Data: Each vial contains 50 mg lyophilized drug, to be reconstituted with 2 ml sterile water to a solution that is 25 mg/ml for IV administration.

Solution Preparation: mix each vial with 2 ml sterile pyrogen-free water to a clear solution, which is 25 mg/ml for IV administration only. Reconstituted solution should be used within 8 hours.

Known Side Effects: pancytopenia, immunosuppression, autoimmune hemolytic anemia has (rarely) been reported, and recurred when patients were retreated with the drug. Nausea, vomiting, anorexia, weakness. From the CNS: agitation, visual disturbances, confusion, coma, peripheral neuropathies have been reported. With high dose use confusion, blindness, coma and death have been reported.

Special Precautions: As for other antineoplastic agents Fludarabine should be handled by trained personnel using procedures for proper handling. The use of gloves and protective glasses is recommended to avoid exposure upon accidental spillage.

Mechanism of Action: After phosphorylation to fluoro-ara-ATP the drug appears to incorporate into DNA and inhibit DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis.

Human Safety and Pharmacology: The half-life of the activated compound is approximately 10 hours, but the pharmacology is incompletely understood. Excretion is impaired in patients with impaired renal function.

11.3 Thymoglobulin

Thymoglobulin® (Rabbit anti-thymocyte globulin Sangstat Medical Corporation) will be used as an in vivo fortification of both the pretransplant immunosuppression and the post-transplant immunoprophylaxis against graft-vs-host disease (GVHD). The Thymoglobulin will be given during the 3 days preceding the graft infusion, thus it will both deplete circulating T-cells from the donor and, due to its long half-life it will deplete infused T-cells from the graft, contributing to both engraftment and decreasing the risk for developing clinically serious GVHD.

12.0 References

Reference List

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