THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Protocol Page

Clofarabine, Gemcitabine and Busulfan Followed by Allogeneic Stem Cell Transplantation for Chronic Lymphocytic Leukemia (CLL)
2012-0249

Core Protocol Information

Short Title	Allogeneic Stem Cell Transplant for CLL
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Which Committee will review this protocol?

- (●) The Clinical Research Committee - (CRC)

Protocol Body

1.0 Objectives

Primary:

1. To determine the maximum tolerated dose (MTD) of Gemcitabine when administered with Busulfan and Clofarbine)

2. To estimate the day 100 treatment-related mortality (TRM) for the preparative regimen busulfan, clofarabine, and gemcitabine followed by allogeneic hematopoietic cell transplantation (HCT) for patients with chronic lymphocytic leukemia (CLL).

Secondary:

To determine the rate of progression-free survival (PFS), graft versus host disease (GVHD), engraftment, and overall survival (OS) for this treatment regimen at one year post treatment completion.

2.0 Background

Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in the United States. It has been estimated that there are approximately 150,000 individuals living with CLL in the United States. The use of combination of chemoimmunotherapy as initial therapy is associated with a high response rates. Unfortunately, once disease relapse has been observed second line treatment is less effective and responses obtained tend to be shorter[1]. Second line treatments include the use of the monoclonal antibody (mAb) alemtuzumab or combination purine-analogue based treatment regimens.[2-4] Alemtuzumab is able to induce a response in up to 30% of the patients, but is associated with significant immunosuppression. Also, it lacks activity in patients that have developed bulk lymphadenopathies (larger than 5 centimeters).[4] Combination of purine-analogue based regimens have limited activity: the combination of fludarabine, cyclophosphamide, rituximab, alemtuzumab (CFAR) is associated with an overall response rate of 65% in patients that have received prior therapy and once patients are fludarabine refractory their median survival is less than twelve months based on published experience.[3]

2.1 Allogeneic Transplantation for chronic lymphocytic leukemia (CLL).

Allogeneic stem-cell transplantation after myeloablative conditioning is an effective therapy for relapsed/refractory CLL. However, the treatment -related mortality has been significant with a

30-40% risk of death within 100 days of the transplant. Allogeneic transplantation can confer an immune mediated graft-versus-malignancy effect. This observation led to the development of reduced dose, non-myeloablative conditioning regimens, which support allogeneic donor cell engraftment and allow graft-versus-leukemia (GVL) effect to occur.[5-6] A major advance in reducing the short-term morbidity and mortality of allogeneic SCT has been the introduction of non-myeloablative or reduced intensity conditioning (RIC) regimens to allow engraftment of allogeneic stem cells. Most patients to date have been treated on experimental treatment protocols which allowed enrolment of many patients with chemo-refractory end-stage disease. RIC regimens allow transplantation in older patients, making this approach more applicable to increased numbers of patients. In most series patients were heavily pre-treated and many were already refractory to therapy, but despite this the majority demonstrated donor engraftment and there is a high complete remission rate. The ability of such approaches to eradicate minimal residual disease in patients with advanced CLL[7] and the observation of late remissions in patients treated with low doses of chemotherapy provide a strong direct evidence for a powerful GVL in CLL. Results of some of the recently published studies of allogeneic stem cell transplantation in CLL are summarized in table.[1]

The outcome from the Fred Hutchinson Cancer Research Center multi-institutional protocol after RIC allogeneic SCT for 82 patients with advanced fludarabine refractory CLL using related (n=52) or unrelated donors (n=30) median age 56 (range 42-72) years demonstrated TRM of 23% at 5 years, with significant graft versus host disease (GVHD) remaining a problem. Five-year OS was 50% and DFS was 39%. Although complications were higher in the patients with unrelated donors, there were higher CR and lower relapse rates, suggesting more effective graft versus leukemia (GVL) activity with unrelated donors.[5]
In another study from Dana-Farber Cancer Institute, forty-six patients underwent RIC transplantation at 67% using unrelated donors. Factors associated with increased risk of relapse included low levels of donor chimerism at day 30, chemo-refractory disease, increased number of previous therapies and adverse cytogenetics.[8]

A study from the European Bone Marrow Transplant registry data base the outcome of 73 patients who had undergone RIC was compared with that of 82 matched patients who had undergone standard myeloablative allogeneic stem cell transplant for CLL during the same time period. Patients undergoing RIC-SCT had significantly reduced transplant related mortality

(TRM), but higher relapse incidence and there was no significant difference in OS or PFS between these two groups.[9] Of particular interest is the group of CLL patients with deletion of 17p and loss of p53. A recent report from EBMT of 44 such patients suggests that allogeneic SCT has the potential to induce long term remission in these very high risk patients.[10]

A recent analysis of the multicenter phase 2 trial from the German CLL study group (CLL3X trial) investigated the long-term outcome of RIC in patients with poor-risk CLL. They used fludarabine/cyclophosphamide-based conditioning regimen. A total of 90 patients out of 100 enrolled patients proceeded to allo SCT. With a median follow-up of 46 months (7-102 months), the 4-year NRM, EFS and OS were 23%, 42%, and 65%, respectively. EFS was similar for all genetic subsets, including 17p deletion (17p-). In multivariate analyses, uncontrolled disease at transplant and in vivo T-cell depletion with alemtuzumab, but not 17p-, previous purine analogue refractoriness, or donor source had an adverse impact on EFS and OS.[11]

GVHD remains the major concern after RIC SCT and attempts have been made to utilize monoclonal antibodies to reduce the incidence of GVHD without increasing the subsequent risk of relapse. Excellent results have been obtained using RIC based on a combination of fludarabine, cyclophosphamide with the addition of rituximab at the MD Anderson Cancer Center, an approach designed to maximize GVL by early tapering of immune suppression with use of rituximab and DLI. Among 39 patients treated, median age was 57 (range 34–70) years, median time from diagnosis to transplantation was 4.5 years. All patients had recurrent advanced disease, were heavily pretreated with a median of 3 (range 2-8) chemotherapy regimens and all had been previously treated with fludarabine-rituximab-based regimens. At transplant, 34 patients (87%) had active disease, including 9 (23%) with evidence of Richter's transformation. In this series only four of the donors were unrelated. However, 14 of 39 patients required immunomodulation with rituximab and DLI for persistent disease after SCT. Only one patient died early and among the 38 evaluable patients, 27 (71%) achieved CR, with estimated OS at 4 years was 48% with current PFS was 44%. Acute grade II–IV GVHD was observed in 45%, but chronic extensive GVHD was reduced without concomitant increased risk of relapse.[12]

GVHD can also be decreased using alemtuzumab in the conditioning regimen, but this delays post-SCT immune reconstitution, increases the risk of infective complications and does appear to impair GVL. In 41 consecutive CLL patients treated (24 HLA-matched sibling donors and 17 unrelated volunteer donors, including 4 mismatched) the conditioning regimen alemtuzumab with fludarabine and melphalan had significant anti-tumor effects with 100% of patients with chemosensitive disease and 86% with chemorefractory disease responding. The TRM rate was

26%, overall survival 51% and relapse risk 29% at 2 years. GVHD rates were relatively low with acute GVHD occurring in 17 (41%) and chronic GVHD in 13 (33%). The unexpectedly high TRM rate was due to a high incidence of fungal and viral infections.[13]

Thus, RIC allogeneic transplantation is an effective therapy for patients with CLL who have failed conventional chemotherapy, and or who have high risk features or had Richter's transformation. However, disease progression after transplant occurs in a substantial number of patients and in some series about 50% of patients with refractory disease at study entry require early immunosuppression withdrawal after transplantation and donor lymphocyte infusion, due to either persistent or progressive disease. This results in significant mortality and morbidity in the form of graft versus host disease. The optimal conditioning regimen is unclear. Thus better conditioning regimens are needed.

2.2. Clofarabine in CLL.

Clinical studies of fludarabine and cytosine arabinoside have shown that when patients resistant to these agents they were still sensitive to a new nucleoside analog, Chloro-Fluoro-Ara-A (Clofarabine). Clofarabine is resistant to deamination and in addition to inhibiting DNA polymerase it also acts as an inhibitor of cellular ribonucleotide reductase.[14] Gandhi et. al. tested clofarabine in 13 patients with refractory CLL. Although none of the 13 patients achieved an objective response, cytoreduction was observed in these patients, who were heavily pretreated and mostly refractory to fludarabine phosphate or alkylators. In addition, it was noted that the log reduction in two patients at 15 mg/m2/d × 5 days were 1.1 and 1.5, which is much greater than that in the 10 patients who received 3 or 4 mg/m2/d × 5 days. The DLT with clofarabine in these patients was hematologic making it an attractive drug to be used in transplant regimens.[15]

2.3. Gemcitabine in CLL.

The present trial proposes to substitute the cytidine analog Gem for Flu. Both drugs are known to be incorporated into DNA as their nucleoside triphosphates and inhibit DNA repair and resynthesis.[16] We wish to study Gem because all patients with CLL who are candidates for allogeneic transplantation are fludarabine refractory or have Richter's transformation. Gemcitabine has shown modest activity as a single agent in patients with CLL and low grade lymphomas. Several unique features of Gem may explain its level of activity, such as the "masked chain termination" effect, which consists of the addition of one deoxynucleotide to the

end of the elongating DNA strand right after the Gem-nucleotide position, preventing its removal by exonucleases and ultimately locking the drug into DNA. In addition, through several self-potentiation metabolic mechanisms, Gem increases the formation and decreases the elimination of its active metabolites.[17] A multicenter phase II trial was conducted to evaluate the efficacy and toxicity of gemcitabine in patients with refractory or relapsed indolent non-Hodgkin's lymphoma. Thirty-six patients were enrolled onto the study, including 11 cases of mantle cell lymphoma (MCL), 10 cases of chronic lymphocytic leukaemia (CLL)/lymphocytic lymphoma, nine cases of follicular lymphoma, four cases of lymphoplasmacytic lymphoma and two cases of T-cell lymphoma. Gemcitabine 1 g/m(2) was administered as a 30-min infusion on d 1, 8 and 15 of a 28-d schedule, up to a maximum of six cycles. Complete responses were observed in two patients with MCL, and partial responses were observed in seven patients, including three patients with CLL/lymphocytic lymphoma, two patients with T-cell lymphoma, one patient with MCL and one patient with follicular lymphoma. Minor responses were observed in three patients, including two patients with MCL and one patient with CLL. . Haematological toxicity was observed as grade 3-4 leucopenia in 12 patients (33%) and grade 3-4 thrombocytopenia in 18 patients (50%). Severe non-haematological toxicity included one case of fatal veno-occlusive disease, one case of thrombotic microangiopathy leading to terminal renal failure, one case of capillary leak syndrome, one case of myocardial infarction and drug-induced fever in two patients. These data suggest that gemcitabine displays activity in patients with MCL and CLL/lymphocytic lymphoma. Haematological toxicity was frequent in these heavily treated patients. Severe non-haematological toxicity was noted however the doses we plan to use are much lower.[18]

2.4. Rationale for using busulfan, clofarabine, and gemcitabine as the preparative regimen in allogeneic transplantation.

We have studied the principle of DNA damage/repair inhibition at M.D. Anderson using the purine nucleoside analog fludarabine as preparative regimen for allogeneic transplantation. The most extensively studied regimen is the Bu/Flu (busulfan/fludarabine) combination, designed for treatment of AML/MDS. Fludarabine is significantly immunosuppressive and thus facilitates engraftment in allotransplantation, and is a potent DNA repair inhibitor. The dose range used in transplant studies is 100-150 mg/m^2. These doses are well tolerated without any significant visceral organ toxicity. De Lima et al. reported the results of 74 patients treated with Bu/Flu suggesting an antileukemic activity at least equal to standard regimens and toxicity less than

that generally reported.[19] The i.v. busulfan dose administered in this study was derived from the PK data obtained from our previous Phase II –III studies of IV Bu with Flu as pretransplant conditioning therapy. The previously used Bu dose of 0.8 mg/kg has been demonstrated to be pharmacokinetically (PK) similar to what was achieved with an oral dose of 1.0 mg/kg, and the total daily dose of 3.2 mg/kg is equivalent to a dose of approximately 130 mg/m^2. Thus our current standard to dose Bu based on PK parameters derived from a test dose given a few days before starting the actual pretransplant conditioning regimen rather than the calculated BSA. This strategy yields better control of actual systemic drug exposure, reduces post-transplant complications and/or possibly improves long-term disease control based on more precise dose delivery of the alkylating agent component. The combination of once daily IV Bu and Flu has already been used successfully in our program, in myeloablative doses for AML/MDS (MDACC protocol ID01-011 and ID 2005-0366), in a reduced intensity regimen in AML (DM99-251) and for CML patients (MDACC protocol ID02-901). In the ID01-011 study of the IV BuFlu regimen, we examined the pharmacokinetics (PK) of IV Bu delivered at a daily dose of 130 mg/m^2; the median daily area under the plasma concentration vs time (AUC) curve was about 5,000 mcMol-min with a range of 2,900-8,300 mcMol-min.[20] The regimen was well tolerated, only 1 of the first 96 patient died of complications within 100 days. An intensive IV Bu-Flu regimen was then tested in a randomized study (randomization between fixed dose busulfan versus PK guided dosing in protocol 2005-0366). In this study a PK directed IV Bu-Flu regimen was used, in which a test dose of IV Bu was used to determine the dose needed to yield an average daily AUC of 6,000-6,500 µMol-min over the course of a 4-day regimen. A total of 225 high-risk patients with AML/MDS have been treated using this approach (139 received the PK guided dose). The regimen was well tolerated The patients receiving PK guided busulfan dosing had significantly better progression free survival at 3 years than the fixed dose group, 56% (45-66%) vs. 42% (32-52%) P=0.03, as well as lower cumulative incidence of progression 23% (16-34%) vs. 35% (27-46%) p=0.03. Overall survival at 3 years for the two groups was 57% (46-68%) vs 47% (36-57%) p=0.2. There was no significant difference in toxicity pattern, incidence of acute GVHD or treatment related mortality between the PK-guided/adjusted and fixed- dose groups. The antileukemia effect was improved in the PK-guided dose group. The PK-directed individualized dose adjustments allowed all patients to achieve a targeted AUC of 6500 mcMol-min (±400 mcMol-min).[21]

Brown et. al. studied the non-myeloablative conditioning regimen of busulfan and fludarabine to transplant in 46 patients with advanced CLL. The donors were HLA-matched unrelated (67%) or related (33%) donors. Fludarabine (30 mg/m2 x 4) and low-dose intravenous busulfan (0.8 mg/kg/day x 4) were used for conditioning. The 2-year OS and PFS rates in this refractory patient population were 54% and 34%, respectively, with a median follow-up of 20 months. The 2-year cumulative incidence of relapse was 48%. High hematopoietic donor chimerism · 75% at day +30 was a significant predictor of 2-year PFS (47% vs 11%; P = .03). In multivariate analysis, chemotherapy-refractory disease at transplantation was associated with a 3.2-fold risk of progression (P = .01) and a 4.6-fold risk of death (P = .02). Increasing number of previous therapies and increasing bone marrow involvement were also associated with decreased PFS and OS. Thus RIC using fludarabine and low-dose intravenous busulfan is a reasonable treatment option for patients with advanced CLL, but relapse remains a major cause for treatment failure.[8]

As most patients with CLL who are referred for allogeneic transplantation are fludarabine refractory, we propose to replace fludarabine with clofarabine. There are several clofarabine-based transplant conditioning regimen studies on-going at MDACC and elsewhere.[22-24] We have published the results of our clinical trial adding clofarabine to our established BuFlu regimen for patients with advanced AML and CML (protocol 2006-0200). Patients were randomized in an adaptive fashion to four treatment groups: Clo:Flu 10:30 mg/m^2, Clo:Flu 20:20 mg/m^2; Clo:Flu 30:10 mg/m^2, Flu 40 mg/m^2. The nucleoside analogues were infused daily for 4 days, followed by busulfan daily for 4 days, targeted to an AUC of 6000 umol-min. All patients engrafted with grade 2-3 mucositis as most common toxicity seen in 50% of patients; no significant hepatic or neurologic toxicity was noted. The conclusion of that study was that Clo ± Flu with i.v. Bu as pretransplant conditioning is safe in high-risk myeloid leukemia patients and that clofarabine is sufficiently immunosuppressive to support allo-SCT in myeloid leukemia. The median OS of 23 months in this high-risk patient population was encouraging.[24] A combination of clofarabine and busulfan is currently been tested for allogeneic transplantation in ALL (2009-0209). Valdez et. al. have demonstrated that a combination of busulfan with two nucleoside analogues (NAs) - clofarabine, fludarabine, or gemcitabine - has synergistic cytotoxicity in B-cell lymphoma cell lines (Figure 1). This combination [2 NAs+Bu] combination activates DNA damage response through the ATM-CHK2 and ATM-CHK1

pathways, leading to cell cycle checkpoint activation and apoptosis. Histone modifications and KAP1 phosphorylation are indicative of chromatin relaxation mediated by the nucleoside analogs which sequentially increase Bu alkylation. Addition of suberoylanilide hydroxamic acid (SAHA) enhanced chromatin relaxation through increased histone acetylation and further augmented the cytotoxicity of [2 NAs+Bu].[25] This concept is now being tested in autologous setting (protocol 2011-0407). We hypothesize that a similar combinatory approach using busulfan with 2 NAs (clofarabine and gemcitabine) can be used for allogeneic transplantation in CLL and that this novel regimen will improve disease control and lead to successful engraftment without additional toxicity. The addition of SAHA to the conditioning regimen may further improve the results and is a consideration for future studies. As majority of the CLL patients are expected to be older than 55 years at the time of their transplant in the current study we propose to administer IV busulfan at a PK-guided dose that yields an AUC of 4,000 mcMol-min (±12%), such that all patients will fall within the optimum therapeutic exposure window for busulfan. This lower dose has been shown to be well tolerated in patients older than 60 years of age with acceptable engraftment rates when combined with nucleoside analogue (Protocol 2005-0726). The pharmacological parameters will be calculated using a pharmacokinetic model, derived from our previous data base on the pharmacokinetics of IV busulfan analyzed with the ADAPT II program for clinical pharmacokinetics.

Figure 1

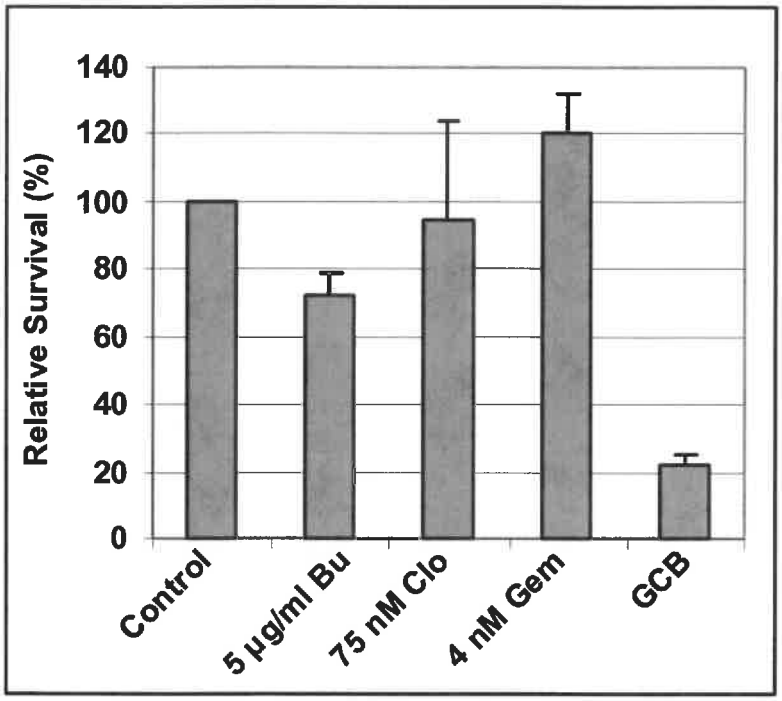

Figure 1. Cytotoxicity of Busulfan and nucleoside analogs (gemcitabine and clofarabine) toward Daudi B-cell lymphoma cells.
Cells were continuously exposed to drugs alone, or in combination, for 96 h and analyzed by MTT assay. Bu or B, busulfan; Clo or C, clofarabine; Gem or G, gemcitabine

Table 1. RIC allogeneic SCT for CLL.

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N	90	86	136	84
Regimen	FC±ATG	FCR	F+ 2 GyTBI	FB
PFS	42% @ 4 years EFS	36% @ 5 years	32% @ 5 years	51% @ 4 years
OS	65% @ 4 years	51% @ 5 years	41% @ 5 years	65% @ 4 years
NRM	23% @ 4 years	17% @ 5 years	32% @ 5 years	16% @ 4 years
Relapse	40% @ 4 years	39% @ 3 years	36% @ 5 years	33% @ 4 years
cGVHD extensive	55% @ 2 years	56% @ 5 years	51%	64% @ 2 years
Median follow up	46 months	37 months	NR	57 months

3.0 Background Drug Information

3.1 Busulfan (IV Busulfex®)

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. DO NOT use if the solution is cloudy or if particulates are present.

Solution Preparation: mix into normal saline to a final concentration of 0.5 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 when using the controlled rate infusion pump).

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 high-dose 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. 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.

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: veno-occlusive disease (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. Human Pharmacology: Limited pharmacology data are available for the parenteral formulation to be used in this study and is detailed in the evaluation of IV Bu in a Phase II Trial using IV Bu at 0.8 mg/kg BW given over 2 hr every 6 hr for a total of 16 doses and when administered once daily for 4 days at a dose of 130 mg/m2 in combination with Fludarabine. The pharmacokinetic data suggests that the plasma decay of the formulation fits an open one-compartment model with linear pharmacokinetics in the dose range of 12 mg-130 mg/m2. Based on studies of oral Bu, the drug is reported to be extensively metabolized; twelve 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.

3.2 Clofarabine (Clolar®)

Clofarabine is supplied in a 20mL flint vial containing 20mg. The pH range of the solution is 4.0 to 7.0. The solution is clear with color ranging from colorless to yellow and is free from visible particulate matter.The concentration used is 0.4 mg/ml.

Expected toxicities: myelosuppression, nausea/vomiting, diarrhea, mucositis, skin rash (particularly hand-foot syndrome), fatigue, mental status changes/coma, allergic reactions (including fever, muscle aches, edema, dyspnea), congestive heart failure, conjunctivitis, anorexia, febrile neutropenia, pruritus, headache, flushing and pyrexia, liver failure.

Clofarabine Formulation and Stability: Clofarabine vials containing undiluted clofarabine for injection should be stored at 25°C or 77°F with temperature excursion permitted to 15 to 30°C or 59 to 86°F. Ongoing self-life stability indicate that clofarabine is stable for 36 months at 25°C (±2°C) and 60% (±5%) relative humidity and for 6 months at 40°C (±2°C) and 75% (±5%) relative humidity.

3.3 Thymoglobulin (Thymoglobulin®)

Thymoglobulin® (Rabbit antithymocyte globulin, Genzyme Corporation) will be used as an in vivo immunosuppression.

Therapeutic classification: Polyclonal anti-lymphocyte preparation.

Stability and storage requirements: The lyophilized powder should be stored in a refrigerator at 2 to 8°C (36 to 46°F). For vials containing the unreconstituted lyophilized powder, the product is stable for 36 months at 5-3°C and 12 months at 37°C. Reconstituted product is stable for 24 hours at room temperature 20 to 25°C and should not be stored under refrigeration..

Preparation: Immediately before intravenous administration, dilute reconstituted Thymoglobulin in isotonic saline or dextrose solution to a total infusion volume of 50 to 500 mL (usually 50 mL

of IV admixture solution per 25 mg vial).

Usual dosage range: 4.5-7.5 mg/kg over 3 days.

Administration: The recommended route of administration is intravenous infusion through an in-line 0.22 micron filter into a high-flow vein. Thymoglobulin should be infused over a minimum of 6 hours for the first infusion and over at least 4 hours on subsequent days of therapy.

Known side effects and toxicities: The most common adverse reactions are fever, chills, leukopenia, thrombocytopenia, rashes, systemic infections, abnormal renal function tests, and serum sickness-like symptoms. Other reported side effects are arthralgia, chest and/or back pain, diarrhea, dyspnea/apnea, nausea and vomiting.

Mechanism of action: Possible mechanisms by which Thymoglobulin may induce immunosuppression *in vivo* include: T-cell clearance from the circulation and modulation of T-cell activation, homing, and cytotoxic activities. Thymoglobulin is thought to induce T-cell depletion and modulation by a variety of methods, including Fc receptor-mediated complement-dependent lysis, opsonization and phagocytosis by macrophages, and immunomodulation leading to long term depletion via antibody dependent cell-mediated cytotoxicity and activation induced cell death, commonly referred to as apoptosis.

3.4. Gemcitabine (Gemzar®) SYNONYM(S):

Gemcitabine hydrochloride, difluorodeoxycytidine, 2',2'-difluorodeoxycytidine, dFdC, LY 188011

CLASSIFICATION: Antimetabolite, cytotoxic

MECHANISM OF ACTION: Gemcitabine, a pyrimidine analog, is structurally similar to cytarabine, but has a wider spectrum of antitumour activity due to its different cellular pharmacology and mechanism of action. Gemcitabine is metabolized intracellularly to two active metabolites, Gemcitabine diphosphate (dFdCDP) and Gemcitabine triphosphate (dFdCTP). The cytotoxic effects of Gemcitabine are exerted through incorporation of dFdCTP into DNA with the assistance of dFdCDP, resulting in inhibition of DNA synthesis and induction of apoptosis. Gemcitabine is a radiation-sensitizing agent.5 It is cell-cycle phase specific (S and G1/S-phases).

PHARMACOKINETICS: 3- to 4-fold interpatient and intrapatient variability. widely distributed into tissues; also present in ascitic fluid. plasma protein binding< 10%. Metabolized intracellularly by nucleoside kinases to active metabolites dFdCDP and dFdCTP; also metabolized intracellularly and extracellularly by cytidine deaminase to inactive metabolite difluorodeoxyuridine (dFdU). active metabolite(s)dFdCDP, dFdCTP. inactive metabolite(s)dFdU. Urine92-98% over one week (89% as dFdU, < 10% as Gemcitabine) after a single dose of 1000 mg/m2 given over 30 minutes. Urine92-98% over one week (89% as dFdU, < 10% as Gemcitabine) after a single dose of 1000 mg/m2 given over 30 minutes. terminal half life IV infusion < 70 min: 0.7-1.6 h. IV infusion 70-285 min: 4.1-10.6 h

SPECIAL PRECAUTIONS:

Carcinogenicity: No information found.

Mutagenicity: Not mutagenic in Ames test but mutagenic in mammalian in vitro mutation test. Gemcitabine is clastogenic in mammalian in vitro and in vivo chromosome tests.

Fertility: Decreased spermatogenesis and fertility in male mice.

Pregnancy: FDA Pregnancy Category D. There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (e.g., if the drug is needed in a life-threatening situation or for a serious disease for which safer drugs cannot be used or are ineffective).

Breastfeeding is not recommended due to the potential secretion into breast milk.
SIDE EFFECTS: Allergic reaction (4%, severe 0.2%), Leucopenia (62%, severe 9%), neutropenia (63%, severe 25%) nadir 7-10 days, recovery within 7 days, thrombocytopenia (24%, severe 5%) nadir 7-10 days, recovery within 7 days, cardiac arrhythmia (2%, severe 0.2%), edema/peripheral edema (28%, severe 3%), hemolytic uremic syndrome (0.3%), asthenia (42%, severe 2%), fever (37%, severe < 1%), alopecia (14%), skin rash (25%, severe < 1%), constipation (8%, severe < 1%), diarrhea (12%, severe < 1%), emetogenic potential: low moderate, nausea and vomiting (64%, severe 18%), stomatitis (8%, severe < 1%), hematuria (31%, severe < 1%), elevated alkaline phosphatase (55%, severe 9%), elevated AST (67%, severe 9%), elevated ALT (68%, severe 10%), elevated bilirubin (13%, severe 2%), infection (9%, severe 1%), decreased level of consciousness (9%, severe < 1%), peripheral neuropathy (3%), dyspnea (8%, severe 1%), elevated BUN (16%, severe 0%), elevated creatinine (7%, severe < 1%), Proteinuria (36%, severe < 1%), flu-like symptoms (19%, severe 1%).

Hemolytic uremic syndrome has been infrequently reported and is characterized by microangiopathic hemolytic anemia, thrombocytopenia and renal failure. The syndrome can present either acutely with severe hemolysis, thrombocytopenia and rapidly progressive renal failure, or more insidiously with mild or no thrombocytopenia and slowly progressive renal failure. The etiology of hemolytic uremic syndrome is unknown.The onset of the syndrome has been reported to occur during and shortly after Gemcitabine therapy. If not treated promptly, the syndrome may result in irreversible renal failure requiring dialysis. Therefore, patients with impaired renal function should be monitored closely while being treated with Gemcitabine.

Elevated liver enzymes: Gemcitabine causes transient and reversible elevations of liver function enzymes in about two-thirds of patients. However, these increases are rarely of clinical significance and there is no evidence of increasing hepatic toxicity with either longer duration of Gemcitabine treatment or cumulative dose.

Fever/Flu-like symptoms: Fever of any severity was reported in 37% of patients. It is frequently associated with other flu-like symptoms such as headache, chills, cough, rhinitis, myalgia, fatigue, sweating and insomnia. These symptoms are usually mild and transient, and rarely dose-limiting. The use of acetaminophen may provide symptomatic relief.

Severe pulmonary toxicity: Acute dyspnea may sometimes occur with Gemcitabine therapy, but is usually self-limiting. However, severe pulmonary toxicities such as pulmonary edema, interstitial pneumonitis and adult respiratory distress syndrome have rarely been reported. The symptoms are manifested as progressive dyspnea, tachypnea, hypoxemia and pulmonary infiltrates on chest radiograph that are sometimes accompanied by fever and cough. Pulmonary toxicities usually occur after several cycles of Gemcitabine, but have also been seen as early as the first cycle. Risk factors for pulmonary toxicities include prior radiation to the mediastinum. Because of its structural similarities to cytarabine, Gemcitabine is thought to cause lung injury by the same mechanism by inducing pulmonary capillary leakage. Management of pulmonary toxicities consists of discontinuation of Gemcitabine and early supportive care with bronchodilators, corticosteroids, diuretics, and/or oxygen. Although pulmonary toxicities may be

reversible with treatment, fatal recurrence of severe pulmonary symptoms was reported in one patient upon rechallenge with Gemcitabine.

Skin rash: Typically mild to moderate in severity, with macular or finely granular maculopapular pruritic eruption on the trunk and extremities. It is not dose-limiting and usually responds to topical corticosteroids. If needed, antihistamines such as diphenhydramine can be used.

SOLUTION PREPARATION AND COMPATIBILITY:

Injection: 200 mg and 1000 mg vials (as the hydrochloride salt). Store at room temperature.

Reconstitute 200 mg vial with 5 mL of NS without preservative and 1000 mg vial with 25 mL of NS without preservative to yield a Gemcitabine concentration of 38 mg/mL. Reconstitution of concentrations greater than 40 mg/mL may result in incomplete dissolution and should be avoided.

Reconstituted solution is stable for 24 hours at room temperature and should not be stored under refrigeration. However, the manufacturer recommends that the admixture be used within 24 hours since the solution does not contain preservatives.

4.0 Patient Eligibility

4.1 Inclusion Criteria

Subjects must meet the following inclusion/exclusion criteria to be eligible for the study.

1) Age 18 to 70 years of age.

2) Patients with chronic lymphocytic leukemia, prolymphocytic leukemia, or Richter's transformation who are eligible for allogeneic transplantation and are not eligible for protocols of higher priority.

3) A 10/10 HLA matched (high resolution typing at A, B, C, DRB1, DQ1) sibling or unrelated donor.

4) Left ventricular EF > 40%.

5) FEV1, FVC and corrected DLCO > 40%.

6) Serum creatinine < 1.6 mg/dL. Serum bilirubin < 2X upper limit of normal.

7) SGPT < 2X upper limit of normal.

8) Voluntary signed, written IRB-approved informed consent.

9) Men and women of reproductive potential must agree to follow accepted birth control methods for the duration of the study. Female subject is either post-menopausal or surgically sterilized or willing to use an acceptable method of birth control (i.e., a hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study. Male subject agrees to use an acceptable method for contraception for the duration of the study.

4.2. Exclusion Criteria:

1) Patient with active CNS disease.

2) Pregnant (Positive Beta HCG test in a woman with child bearing potential defined as not post-menopausal for 12 months or no previous surgical sterilization) or currently breast-feeding. Pregnancy testing is not required for post-menopausal or surgically sterilized women.

3) Known infection with HIV, HTLV-I, Hepatitis B, or Hepatitis C.

4) Active uncontrolled bacterial, viral or fungal infections.

5) Patient has received other investigational drugs within 1 week before enrollment.

5.0 Pretreatment evaluation

The following will be performed within 30 days prior to start treatment.

5.1 Complete history and physical examination.

5.2. Baseline evaluations to include CXR, chemistry panel, hematology survey. Bone marrow biopsy with aspirate for morphology, immunophenotyping, cytogenetic analysis, and FISH when indicated.

5.3. Serum immunoglobulins, beta-2 microglobulin will be checked in the peripheral blood.

5.4. CT and/or PET-CT when indicated.

6.0 Treatment Plan

The transplant day is referred to as day zero (D0), treatment plan activities prior or after D0 are denominated as day minus (D-) or day plus (D+).

Preparative Regimen: Clofarabine/Gemcitabine/Busulfan.

Acetaminophen should not be used within 24 hours prior to Busulfan administration. Other drugs known to interfere with the metabolism of busulfan should not be concomitantly used during the chemotherapy administration. In particular, voriconazole, posaconazole, fluconazole, itraconazole and metronidazole should be stopped at least one week prior to start of busulfan as feasible, since these agents have well described interference with busulfan.

Between D-15 and D-8 Busulfan test dose.

Busulfan test dose can be administrated in the outpatient setting prior to admission for the first therapeutic Busulfan dose or given in the inpatient setting on D-8. The Busulfan test dose of 32 mg/m^2 will be based on actual body weight and will be given IV over 60 minutes by

controlled-rate infusion pump.

D-9 or -7 Hospital Admission.

For optimal efficacy the sequence of drug administration will be gemcitabine followed by clofarabine followed by busulfan. Patients admitted on D-9 for inpatient test dose should be preferentially admitted on Sunday or Monday for pharmacokinetic directed therapy. Patients admitted on D-7 after outpatient test dose should be preferentially admitted on Monday, Tuesday, or Wednesday for pharmacokinetic directed therapy.

D-6 and D-4 Gemcitabine Administration.

Gemcitabine dosing will begin at 275 mg/m^2/dose IV; dose level 3. With this dose, the infusion duration would be 20 min, preceded by a loading dose of 75 mg/m^2 administered as a bolus: 75 mg/m^2 + (10mg/m^2/ min × 20 min) = 275 mg/m^2. Gemcitabine will be dosed on adjusted BW if actual > 20% above IBW. Dose escalation of Gemcitabine will follow guidelines described in section 11.0. On days -6 and -4 gemcitabine will be administered first followed by clofarabine followed by busulfan.

Gemcitabine Dose Levels

Level	Gemcitabine mg/m2/d * (on Days -6 and -4)	Total Gemcitabine dose (mg/m2) on Days -6 and -4	Clofarabine (on Days -6 to -3) Mg/m2	Busulfan target AUC/day (or fixed dose in mg/m2/d) (on Days -6 to -3)
1	100 (10 mins)	**175**	30	4000/(100)
2	150 (15 mins)	**225**	30	4000/(100)
3	200 (20 mins)	**275**	30	4000/(100)
4	250 (25 mins)	**325**	30	4000/(100)

* each Gem infusion is preceded by 75 mg/m^2 Gemcitabine given as a one min bolus dose.

D-6 to D-3 Clofarabine administration.

Clofarabine infusion will be started after the completion of the gemcitabine infusion on days -6 and -4. Clofarabine is administered at a dose of 30 mg/m^2 diluted in NS to produce a final concentration of 0.4mg/mL, and infused on each of four (4) consecutive days (days -6 through -3). Clofarabine will be dosed per actual body weight/actual body surface area.

D-6 to D-3 Busulfan administration.

The pharmacokinetic- guided daily high-dose busulfan dose(s) will start after the daily Clofarabine doses. The Busulfan doses will be diluted in normal saline and administered over 3 hours daily by controlled rate infusion pump. Busulfan pharmacokinetics will be repeated with the Busulfan dose given on D -6.

Busulfan is administered at the dose calculated to achieve a systemic exposure dose of 4000 µMol-min in normal saline over three (3) hours IV every twenty-four (24) hours for four (4) consecutive days (days -6 to -3), starting after the completion of Clofarabine. On days -6 and -4 Clofarabine infusion will be followed by Gemcitabine infusion followed by busulfan. The Busulfan dose will be based on the pharmacokinetic studies to target an AUC of 4000 µMol-min ± 12%.

The PK adjusted dose of Busulfan = Target AUC x Busulfan mol. wt (0.2463) x Busulfan gross clearance normalized to body surface area (L/min)+ the dose uninfused in the IV line.

Fixed dose Busulfan: If it is not feasible to perform pharmacokinetic monitoring, patients will receive a fixed Busulfan dose of 100 mg/m^2/day for 4 days, which is expected to yield a median daily AUC of 4,000 mcMol-min. Fixed dose will be based on adjusted BW if Actual body weight is > 20% above IBW.

D-3 to D-1 Anti-thymocyte globulin administration:

Patients receiving a graft from a matched unrelated donor will receive Thymoglobulin; 0.5 mg/kg on D–3, 1.5 mg/kg on D–2 and 2.0 mg/kg on D–1. The Thymoglobulin will be administered as per regular departmental guidelines and will be infused in the afternoon of respective days so not to interfere with the chemotherapy administration.

D0 Stem Cell infusion:

Fresh or cryopreserved bone marrow or peripheral blood progenitor cells will be infused on day 0. Premedication for the infusions will be per standard SCTCT department procedures.

Prophylaxis and Supportive Care as per standard practice in patients receiving allogeneic transplant and SCTCT Guidelines.

Filgrastim (G-CSF) administered at a dose of 5 mcg/kg/day (rounded up the nearest vial size) subcutaneously.

GVHD prophylaxis with Tacrolimus and Mini Methotrexate with dose adjustment as clinically indicated. Tacrolimus will be administered at starting dose of 0.015 mg/kg (ideal body weight) as a 24 hour continuous infusion daily adjusted to achieve a therapeutic level of 5-15 ng/ml. Tacrolimus is changed to oral dosing when tolerated and can be tapered off after day +90 if no GVHD is present. Methotrexate 5 mg/m^2 will be administered intravenously on days +1,+ 3, +6 and +11 post transplant. D+11 methotrexate may be held if the patient has symptomatic mucositis.

Antiseizure prophylaxis and other supportive care (allopurinol, menstrual suppression, prophylactic antibiotics, empiric antibiotics, IVIG, transfusions of blood products, hyperalimentation, etc.) as indicated

7.0 Evaluation During Study

A. Assessment of DNA damage analysis:

DNA damage will be assessed by quantitative analysis of the phosphorylation of histone 2 AX (ã-H2AX) using flow cytometry. Samples (10 mL of blood) will be drawn in a heparinized tube. The first sample (baseline) will be collected prior to the Busulfan test dose. Samples 2 and 3 will be drawn a day after the first and second dose of Gemcitabine, respectively. The ã-H2AX assay will be conducted in the laboratory of Dr. B. S. Andersson following a published procedure (Ewald B et. al.)[27] Briefly, mononuclear cells will be isolated and immunostained with a monoclonal antibody specific to ã-H2AX and an anti-mouse IgG antibody conjugated to Alexa Fluor 488. Fluorescence of at least 10,000 cells will be determined by flow cytometry.

B. To be performed around engraftment time:

1. Chimerism studies from peripheral blood performed on separated T-cells and myeloid cells.
2. Physical examination and adverse event documentation including GvHD assessment.

C. To be performed at approximately 3, 6 and 12 months post transplant.

These evaluations follow our standard practice and are done to monitor engraftment and disease status. If clinically indicated these studies may be done at other time points which can replace the nearest planned timepoint.

1. Chimerism studies from peripheral blood performed on separated T-cells and myeloid cells.
2. At each visit, a physical examination and adverse event documentation including GvHD assessment.
3. Disease specific assessment with bone marrow aspirate with cytogenetics, minimal residual disease using flow panel for CLL, serum immunoglobulins in peripheral blood and CT and/or PET-CT for lymphoma staging as indicated.

D. After the first year patients will be follow up for disease status, presence of GVHD and survival as per routine follow-up and standard of practice for patients receiving allogeneic stem cell transplantation.

The following lab tests are to be performed as frequently as clinically indicated:CBC, differential, platelets, SGPT, calcium, glucose, uric acid, magnesium, serum bilirubin, BUN and creatinine, serum protein, albumin, alkaline phosphatase, electrolytes, urinalysis,

tacrolimus levels and CMV antigenemia.

8.0 Study Definitions

Active treatment administration is defined from the first day of treatment administration as outlined in the treatment plan through D0.

Active treatment period is defined from the first day of treatment administration through Day +30.

Follow-up period is defined from BMT Day +31 until five years of treatment completion.

Engraftment is defined as the evidence of donor derived cells (more than 95%) by chimerism studies in the presence of neutrophil recovery by day 28 post stem cell infusion.

Other definitions used to assess engraftment:

Neutrophil recovery is defined as a sustained absolute neutrophil count (ANC) > 0.5 x 10^9/L for 3 consecutive days.

Engraftment date is the first day of three (3) consecutive days that the ANC exceeds 0.5 x10^9 /L.

Delayed engraftment is defined as the evidence of engraftment beyond day 28 post hematopoietic stem cell (HSC) infusion achieved after the administration of therapeutic (high dose) hematopoietic growth factors.

Primary Graft failure is defined as failure to achieve an ANC > 0.5 x 10^9/L for 3 consecutive days by day 28 post HSC infusion, with no evidence of donor-derived cells by bone marrow chimerism studies in the absence of persistent or recurring disease.

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

Autologous reconstitution is defined by the presence of ANC > 0.5 x 10^9/L without evidence of donor-derived cells by bone marrow chimerism studies. This can occur at initial engraftment or later after initial engraftment has been documented.

Treatment Response

SITE	Complete Remission	Partial Remission
Nodes	None	≥ 50% decrease
Liver/Spleen	Not palpable	≥ 50% decrease
Symptoms	None	N/A
PMN	>1,500/μL	> 1,500/μL or >50% improvement from baseline
Platelets	>100,000/μL	> 100,0000/μL or >50% improvement from baseline
Hemoglobin (untransfused)	11.0 g/dL	> 11.0 g/dL or >50% improvement from baseline
Lymphocytes	<4,000/μL	>50% decrease
Bone Marrow aspirate Biopsy	<30% lymphocytes No lymphocyte infiltrate	N/A for PR <30% lymphocytes with residual disease on biopsy for nodular PR

Survival will be recorded by the day of death and the cause of death.

9.0 Off Study Criteria

1. Patient may be removed from study if in the judgment of the Principal Investigator further treatment is not in the best interest of the patient.
2. Unacceptable pattern of toxicity.
3. Patient withdraws informed consent.
4. Inability or unwillingness to have follow-up visits and/or laboratory tests required by this protocol.
5. Two years after treatment completion. Patients who experience graft failure or disease progression will continue on study for survival only.

10.0 Adverse Events Assessment

Assessment of the Adverse Events Severity.

The severity of the adverse events (AEs) will be graded according to the Common Terminology Criteria v3.0 (CTCAE).

Events not included in the CTCAE chart will be scored as follows:

General grading:

Grade 1: Mild: discomfort present with no disruption of daily activity, no treatment required beyond prophylaxis.
Grade 2: Moderate: discomfort present with some disruption of daily activity, require treatment.
Grade 3: Severe: discomfort that interrupts normal daily activity, not responding to first line treatment.

Grade 4: Life Threatening: discomfort that represents immediate risk of death

Grading for specific syndromes:
Veno-occlusive disease (VOD):
- Grade 3: Bili >2mg/dl with at least two of the following: increased weight >4% from baseline, ascites or hepatomegaly
- Grade 4: pulmonary and or renal failure

Pulmonary events not caused by CHF (interstitial pneumonitis (IP), pulmonary hemorrhage (DAH):
- Grade 1: CXR showing mild infiltrates or interstitial changes
- Grade 2: mild SOB
- Grade 3: requires supplemental oxygen, or is a documented infection
- Grade 4: requires intubation

Transplant related microangiopathy:
- Grade 1: No treatment required
- Grade 2:Requires steroids and/or plasma transfusions
- Grade 3: Requires plasma exchange

Cytokine storm or engraftment syndrome:
- Grade 1: No treatment required
- Grade 2: Treatment required
- Grade 3: Organ dysfunction
- Grade 4: Total Bilirubin >5

Hemorrhagic Cystitis:
- Grade 1: minimal or microscopic bleeding/pain
- Grade 2: gross bleeding/pain and spasms
- Grade 3: transfusion/irrigation required
- Grade 4: dialysis required

Casualty Assessment.
For the purpose of this study the treatment plan (preparative regimen followed by allogeneic stem cell transplantation) is defined as the "transplant package"; therefore adverse events known to be caused by components of the transplant package and its direct consequences will be scored as definitive related. Adverse events known to be related to drugs used for the treatment of GVHD and Infection episodes will be scored as probable related. When the relationship of the adverse event cannot be ruled out with certainty the AE may be considered possible related. Adverse events known to be related to drugs used for supportive treatment will be scored as unrelated.

The principal investigator will be the final arbiter in determining the casualty assessment.

List of most common expected adverse events.
1. Infections in the presence or absence of neutropenia: fungal, bacterial and or viral infections.
2. Fever: Non-neutropenic or neutropenic without infection
3. Acute graft versus host disease (aGVHD): most commonly manifested by skin rash, diarrhea and abnormal liver function tests could also present with some degree of fever,

upper gastrointestinal symptoms (nausea and vomiting) mucositis and eye dryness.

4. Gastrointestinal (GI tract): the GI tract manifestations could be not only due to direct damage from the preparative regiment but also be a manifestation of GVHD or infections. Therefore, the time course and its presentation are crucial when assessing these as adverse events. Nausea/vomiting, mucositis, diarrhea when presented within first 7 to 10 days most likely will be related to the preparative regimen.
5. Skin rash: not related to GVHD could be caused by chemotherapy used for the preparative regimen or antibiotics used a supportive treatment.
6. Transaminitis: liver function test elevation.
7. Pulmonary events: not related to CHF most likely caused by drug injury or infection. These could present with a pneumonitis pattern manifested with shortness of breath, pulmonary infiltrates on chest radiograph, sometimes accompanied by fever and cough and progress to acute respiratory insufficiency and a diffuse bilateral alveolar pattern.
8. Cytokine Storm/ engraftment syndrome: most likely caused by released cytokines.
9. Hemorrhagic cystitis: not related to chemotherapy agents used in the proposed preparative regimen is most likely caused by viral infection.
10. Thrombotic thrombocytopenic purpura (TTP).
11. Veno-occlusive Disease of the Liver (VOD): could be caused by busulfan. Some antimicrobial agents have been also incriminated in its development.
12. Fluid overload due to hydration required for conditioning regimen, blood product transfusions and or IV alimentation
13. Graft failure.
14. Chronic GVHD.
15. For the purpose of this study the following events would not be considered adverse events and would not be recorded in the database:
    1. Flu-like symptoms not associated with infection
    3. Abnormal laboratory findings considered associated to the original disease
    4. Isolated changes in laboratory parameters such as electrolyte, magnesium and metabolic imbalances, uric acid changes, elevations of ALT, AST, LDH and alkaline phosphatase.

Adverse events considered serious.

1. Prolonged hospitalization due to infections and/or organ failure requiring extensive supportive care (i.e. dialysis, mechanical ventilation).

2. Readmissions from any cause resulting in a prolonged hospitalization (>10 days).

3. Graft Failure/ rejection.

4. Any expected or unexpected event resulting in an irreversible condition and/or leading to death.

Adverse events data collection.

From the start of preparative regimen up to D+100 the collection of adverse events will reflect the onset and resolution date and maximum grade; beyond this point some events considered related to chronic GVHD or late complications post transplant might be recorded only with the first date of their awareness with no grade or resolution date.

Intermittent events should be labeled as such and followed until resolution.

If a patient is taken off study while an event is still ongoing, this will be followed until resolution unless another therapy is initiated. Pre-existing medical conditions will be recorded only if an exacerbation occurs during the active treatment period. Co-morbid events will not be

scored separately.

As stated in the treatment plan, patients treated on this protocol will required supportive care treatment (concurrent medication). These medications are considered standard of care and have no scientific contributions to the protocol, therefore no data will be captured on the various medications needed or their sides effects.

AE and Protocol Deviations Reporting Requirements.

Adverse events will be reported accordingly to MDACC (HSRM chapter 15.001) policy and procedures.This study will be conducted in compliance however in the event of any protocol deviations or violations these will be reported accordingly to MDACC (HSRM chapter 25).

11.0 Statistical Considerations

1. Preliminaries and Background. This is a phase I-II trial of Clofarabine + Gemcitabine + Busulfan as a preparative regimen for chronic lymphocytic leukemia (CLL) patients whose disease was found to be resistant or refractory to previous chemotherapy and who are undergoing an allogeneic stem cell transplant (allosct). Busulfan will be given daily on each of four consecutive days, -6, -5, -4, -3 prior to allogeneic transplant at day 0, at a fixed per-day dose of AUC 4000 (or 100 mg/m2). Clofarabine also will be given daily on days -6, -5, -4, -3, at a fixed per-day dose of 30 mg/m2. Gemcitabine will be given twice, on days -6 and -4, at a per-day dose to be determined in the phase I portion of this clinical trial. Thymoglobulin will be given on days -2 and -1 for patients with matched unrelated donors. The dose of Gemcitabine will be varied adaptively in phase I, and the optimal dose of Gemcitabine selected in phase I will be used to treat all patients in phase II.

2. Outcomes. Primary outcomes include toxicity in phase I and 100-day treatment success in phase II. Secondary outcomes include progression-free survival (PFS) time, overall survival (OS) time, time-to-engraftment, and graft-versus-host disease (GVHD). For phase I dose-finding, toxicity is defined as *any* grade 3 or higher non-hematologic toxicity, non-infectious toxicity or death attributable to Gemcitabine/Clofarabine/Busulfan/ATG, as well as grade 3 mucositis and grade 3 skin toxicity lasting for more than 3 days at their peak severity (i.e., grade 3), occurring within 30 days from transplant. Consideration of DLT will exclude neutropenic fever and asymptomatic, self-limited elevation of the transaminases as well as laboratory serum metabolic values not reflecting end-organ function within 30 days from transplant. For phase II, the 100-day success event, S100, is defined as *all* of the three efficacy outcomes that the patient is (i) alive, (ii) engrafted, and (ii) without grade 3 or 4 (i.e.,

severe) GVHD at 100 days post allogeneic transplant. OS, PFS, and time-to-engraftment all are defined from the day of allogeneic transplant, to the day of the event.

3. Phase I. An optimal dose of Gemcitabine will be determined from the four doses {100, 150, 200, 250} mg/m2 administered on days -6 and -4, hereafter referred to as dose levels {1, 2, 3, 4}. All gemcitabine doses will be preceded by a bolus gemcitabine dose of 75 mg/m2. The Bayesian-model-averaging continual reassessment method (BMA-CRM) of Yin and Yuan [28, 29] will be used to determine an optimal dose, with target toxicity probability 0.20, cohort size 3, starting with the first cohort treated at dose level 3 (total dose 275 mg/m2/administration), and maximum phase I sample size 30. For implementing the BMA, since there are only four dose levels, two probability skeletons will be considered, (0.05, 0.1, 0.2, 0.35) and (0.08, 0.2, 0.3, 0.45), under the model with pk,j = Prob(toxicity at dose level j | skeleton k=1,2) = {pk,j}^{exp(a)}, for k=1,2 (skeleton, i.e. model) and j=1,2,3,4 (dose level), where the prior is a ~ N(0,2). Under the BMA model, pj = probability of toxicity at dose level j = 1,2,3,4 is a Bayesian data-weighted mixture of p1,j and p2,j. The BMA elaboration is used to obtain a more robust CRM. The phase I portion of the trial will be terminated early if the lowest Gemcitabine dose level is found to be excessively toxic, formally if Pr(p1 > .20 | data) > .85. This dose-finding method will be implemented using the Department of Biostatistics Clinical Trials Conduct Website.

The operating characteristics of the BMA-CRM are summarized in Table 1.

Table 1. Operating characteristics of the BMA-CRM dose-finding design.

Dose Level	**True Prob(tox)**	**Selection Probability**	**# of Subjects Treated**	**# of Toxicities**
Scenario 1 PET = 0.01, mean Number of toxicities = 5.6				
1	0.050	0.02	3.3	0.2
2	0.100	0.23	6.5	0.6
3	0.200	0.58	13.8	2.7
4	0.350	0.15	6.1	2.1
Scenario 2 PET = 0.00, mean Number of toxicities = 4.4				
1	0.040	0.00	1.3	0.0
2	0.080	0.04	2.6	0.2
3	0.100	0.26	9.4	0.9
4	0.200	0.70	16.6	3.2
Scenario 3 PET = 0.05, mean Number of toxicities = 6.2				
1	0.080	0.15	7.3	0.6
2	0.200	0.56	10.8	2.1
3	0.320	0.20	8.3	2.6
4	0.400	0.03	2.3	0.9
Scenario 4 PET = 0.35, mean Number of toxicities = 6.6				
1	0.200	0.47	13.5	2.7
2	0.350	0.17	4.8	1.7
3	0.450	0.01	4.2	1.9
4	0.600	0.00	0.6	0.4

Scenario 5 PET = 0.93, mean Number of toxicities = 5.2				
1	0.400	0.07	7.0	2.8
2	0.500	0.01	0.9	0.4
3	0.600	0.00	3.1	1.8
4	0.700	0.00	0.2	0.2

4. Phase II. All patients in phase II will be treated at the MTD for Gemcitabine chosen by the BMA-CRM in phase I. The phase II sample will include all phase I patients treated at the MTD, plus an additional 30 patients. The method of Thall and Sung [30] will be used to monitor S100 in phase II. Accrual will be terminated in phase II if the 100-day success rate is unacceptably low compared to the historical rate 60%. Formally, denoting q100 = Pr(S100), if Pr(q100 > .60 | data) < .05 then the trial will be terminated. This rule will be applied using the a beta(600, 400) distribution to represent the cut-off .60, a beta(.60, .40) prior for q100, and it will be applied assuming a maximum sample size of 40 (10 from phase I treated at the MTD + 30 more) when S100 has been evaluated for 10, 20, and 30 patients. It implies that the trial will be stopped in phase II if [# patients with S100] / [# patients evaluated] is less than or equal to 3/10, 8/20, or 13/30. No computer program will be needed to implement this stopping rule, which will be the responsibility of the trial PI. The operating characteristics of this rule are summarized in Table 2.

Table 2. Operating characteristics of the 100-day success rate futility stopping rule.

True Pr(S100)	Pr(Stop Early)	Sample Size Quartiles
.40	.78	10, 20, 30
.50	.38	20, 40, 40
.60	.10	40, 40, 40
.70	.01	40, 40, 40

5. Data Analysis. The probability of toxicity as a function of dose will be estimated using the BMA-CRM model. All events, including GVHD and S100, will be cross-tabulated with dose. All unadjusted time-to-event distributions will be estimated by the method of Kaplan and Meier [31].

Time-to-event distributions as function of patient baseline covariates will be evaluated using Bayesian time-to-event regression modeling.[32]

12.0 References

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