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A PHASE I/II DOSE ESCALATION TRIAL OF CLOFARABINE, IN ADDITION TO MELPHALAN AND THIOTEPA AS MYELOABLATIVE REGIMEN FOLLOWED BY AN ALLOGENEIC UNMODIFIED HEMATOPOIETIC STEM CELL TRANSPLANT FROM HLA-COMPATIBLE RELATED OR UNRELATED DONORS FOR THE TREATMENT OF HIGH RISK AND/OR ADVANCED HEMATOLOGIC MALIGNANCIES.

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a single arm phase I/II clinical trial to assess efficacy (the antileukemic potential and relapse rate), and safety (peri-transplant morbidity and mortality) of a novel cytoreduction regimen in preparation for allogeneic hematopoietic stem cell transplantation (HSCT). Patients with high risk or advanced hematologic malignancies will receive cytoreduction with a novel agent, Clofarabine, in addition to Thiotepa and Melphalan as preparation for an allogeneic HSCT. Tacrolimus and Methotrexate (MTX) will be given to bone marrow transplant (BMT) and peripheral blood stem cell transplant (PBSCT) recipients for graft versus host disease (GvHD) prophylaxis. For umbilical cord blood transplant (UCBT) recipients, Tacrolimus and Mycophenolate mofetil (MMF) will be given for GvHD prophylaxis.

Candidates for this trial will include patients with high risk or advanced forms of acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute biphenotypic or undifferentiated leukemia, infant leukemia or myelodysplastic syndrome (MDS) for whom an allogeneic marrow transplant is indicated.

Stem cell grafts will be unmodified marrow, peripheral blood stem cells derived from HLA-matched related donors or HLA-compatible unrelated donors, or umbilical cord blood from unrelated donors. Cord blood grafts will consist of two cord blood units. Candidates for transplant will be stratified according to their donor types. Patients will be monitored for engraftment, chimerism, incidence and severity of acute and chronic GvHD, regimen-related toxicity, characteristics of hematopoietic and immune reconstitution, as well as overall and disease-free survival.

The first component of this trial is a phase I dose escalation study to determine the maximum tolerated dose (MTD) of Clofarabine, a novel agent included in the cytoreductive regimen. A total of 3 dose levels will be explored in this study. A standard 3 + 3 dose escalation design will be employed with 6 patients at each dose level. All patients will be conditioned for transplantation with Clofarabine at a dose of 20, 30 or 40 mg/m^2/dose x 5, followed by standard transplant doses of thiotepa (10 mg/kg x 1 dose) and melphalan (70 mg/m^2/dose x 2 doses). All patients will also receive Tacrolimus and MTX (for BMT and PBSCT recipients) or Tacrolimus and MMF (for cord blood transplant recipients) as GvHD prophylaxis.

Following the dose escalation portion of the trial, the second phase of this trial will be the treatment of the subsequent patients with clofarabine at the MTD level if achieved early, or at the third and highest dose level if not. Six patients treated at the maximum tolerated dose and 24 additional patients will be evaluated for the phase 2 component of the trial.

A total of 30 patients will be accrued on the phase 2 portion of the trial. This will include six patients treated at the MTD during the phase I portion of the trial and an additional 24 patients treated at the MTD dose level. It is anticipated that the accrual will last 3 years. At the conclusion of the study, the 1-year disease-free survival probability can be estimated to within +/- 0.20 for the 30 patients on this study.

In order to reduce patient risk, the study design includes early termination of any trial group in the event of excessive regimen related toxicity and grade 4 acute graft-versus-host disease.

PROTOCOL SCHEMA

Phase 1

The dose of Clofarabine will be escalated with 3 Dose Levels:

- Dose Level 1: 20 mg/m^2/dose x 5
- Dose Level 2: 30 mg/m^2/dose x 5
- Dose Level 3: 40 mg/m^2/dose x 5

Dose level 1: Clofarabine at 20 mg/m^2/dose x 5 + THIO-MEL 6 patients

- If 0 or 1 of 6 patients experience dose limiting toxicity, proceed to dose level 2.
- If ≥ 2 patients experience a dose limiting toxicity, re-evaluate the protocol.

Dose level 2: Clofarabine at 30 mg/m^2/dose x 5 + THIO-MEL 6 patients

- If 0 or 1 of 6 patients experience dose limiting toxicity, proceed to dose level 3.
- If ≥ 2 patients experience a dose limiting toxicity, the MTD will be Dose Level 1.

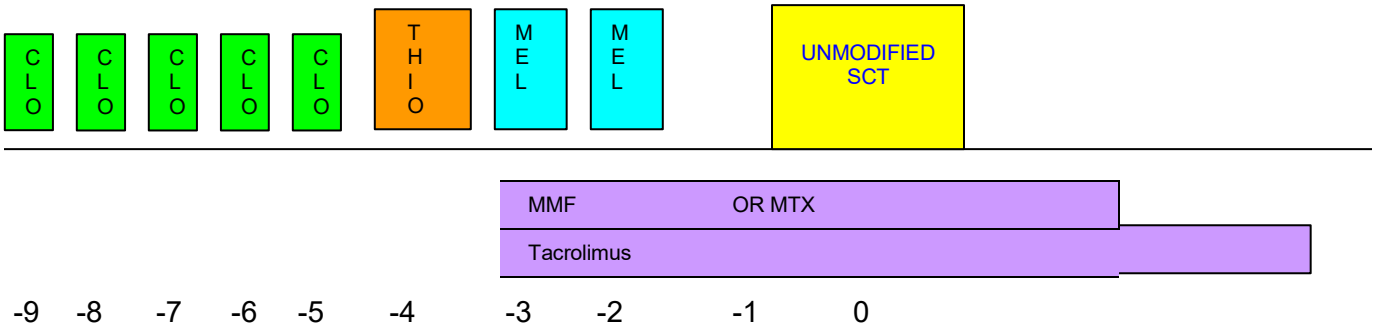
Dose level 3: Clofarabine at 40 mg/m^2/dose x 5 + THIO-MEL 6 patients

- If 0 or 1 of 6 patients experience dose limiting toxicity, this will be the dose level to be used for the rest of the trial.
- If ≥ 2 patients experience dose limiting toxicity, the MTD will be Dose Level 2.

Phase 2

6 patients treated at Dose Level of MTD (or dose Level 3 if MTD not reached) + 24 additional patients

ROAD MAP



DAYS

AGENT	DOSE	DAYS
Clofarabine (CLO)*	20, 30 or 40 mg/m^2/dose x 5	-9 to -5
Thiotepa (THIO)	10 mg/kg/dose x 1	-4
Melphalan (MEL)	70 mg/m^2/dose x 2	-3 and -2
+		
Tacrolimus	Dose per levels	Start -3

AND

UCBT:

For Adults and Children >12 yrs and > 50 kgs:

Mycophenolate mofetil (MMF)	1 gram IV bid	Start -3

For Adults and Teens >12 yrs but <50 kgs:

Mycophenolate mofetil (MMF)	15 mg/kg/dose po/IV bid	Start -3

For less than 12 yrs:

Mycophenolate mofetil (MMF)	20 mg/kg/dose po/IV bid to maximum of 1 gram IV bid	Start -3

OR

BMT/PBSCT:

Methotrexate (MTX)	15 mg/m2/dose	Day +1
	10 mg/m2/dose	Days +3, +6, +11

All patients will receive hydrocortisone prior to Clofarabine in an attempt to prevent inflammation-induced liver enzyme elevation

EXAMPLE OF ROAD MAP OF PREPARATION FOR BONE MARROW OR PERIPHERAL BLOOD STEM CELL TRANSPLANT

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
		-9 *hydrocortisone* Clofarabine	-8 *hydrocortisone* Clofarabine	-7 *hydrocortisone* Clofarabine	-6 *hydrocortisone* Clofarabine	-5 *hydrocortisone* Clofarabine
-4 Thiotepa	-3 Melphalan Tacrolimus	-2 Melphalan	-1	0 Unmodified Stem cell transplant	1 Methotrexate	2
3 Methotrexate	4	5	6 Methotrexate	7	8	9
10	11 Methotrexate					

EXAMPLE OF ROAD MAP OF PREPARATION FOR UMBILICAL CORD BLOOD TRANSPLANT

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
		-9 *hydrocortisone* Clofarabine	-8 *hydrocortisone* Clofarabine	-7 *hydrocortisone* Clofarabine	-6 *hydrocortisone* Clofarabine	-5 *hydrocortisone* Clofarabine
-4 Thiotepa	-3 Melphalan Tacrolimus Mycophenolate	-2 Melphalan	-1	0 Double unit cord blood transplant	1	2

2.1 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objectives:

1. Phase I: To determine the MTD of clofarabine used in combination with melphalan and thiotepa as a conditioning regimen

2. Phase II: To determine the 1 year disease-free survival (DFS) rate using this regimen.

Secondary Objectives

3. To evaluate the incidence and severity of non-hematologic toxicity and GvHD using this regimen.

3.1 BACKGROUND AND RATIONALE

Myeloablative therapy followed by allogeneic HSCT is potentially curative therapy in high risk hematologic malignancies including: very high risk acute lymphoblastic leukemia (ALL) in first remission (slow response to induction therapy and/or Philadelphia Chromosome positivity),[1-7] ALL in second or greater remission,[4,8-10] acute myeloblastic leukemia in first and second remission,[11-15] chronic myelogenous leukemia,[16-20] and myelodysplasia.[21-28] Hematologic malignancies associated with a lower risk of relapse following allogeneic HSCT include AML in first complete remission (CR1), ALL-CR1, ALL-CR2, and CML in first chronic phase. Allogeneic HSCT in these diseases are associated with a disease-free survival (DFS) rate as high as 70%[1,2,8-10,16-19]

Standard cytoreduction prior to allogeneic HSCT for hematologic malignancies includes cyclophosphamide in combination with either total body irradiation (TBI) or busulfan. DFS rates of 40 to 70% are reported in patients with good risk malignancies (AML CR1, ALL CR1 or CR2, or CML in first chronic phase) using these combinations.[1,2,8,10-12,16-19,29,30] The addition of thiotepa to standard cytoreduction with TBI and cyclophosphamide has resulted in disease-free survival rates of >70% in both children and adults with AML in CR1.[11] Patients with advanced disease have a significantly higher rate of recurrence and transplant-related toxicity(10-

40%).[8,31-35] At this center from 2000 to the present, 33 patients with advanced disease received unmodified stem cells following busulfan and melphalan for myeloablation. The disease-free survival among these very high-risk patients is 39% (median follow up is 12 months). With advances in supportive care, post transplant relapse remains the major barrier to cure in the advanced leukemias. Accordingly, transplant conditioning regimens for the advanced leukemias should include agents with anti-leukemic as well as myeloablative and/or immunosuppressive properties.

In 2003, Lacerda et. al[36]. reported a 50% overall survival rate in 14 patients with advanced hematologic malignancies transplanted with T-cell depleted bone marrow from haploidentical donors following cytoreduction with thiotepa, melphalan and fludarabine. Fludarabine is employed for its immunosuppressive effects to allow for the engraftment of a haplo-disparate graft. It is unlikely to add additional anti-leukemic activity. In the setting of a better-matched donor, this regimen may be improved upon by utilizing the potential anti-leukemic effect of a conventional graft and by replacing the fludarabine with a compound with more anti-leukemia activity.

Studies of clofarabine as a single agent demonstrate significant anti-leukemic activity when administered in 5 daily doses. The primary toxicity of both clofarabine and thiotepa is hepatotoxicity. The thiotepa will be given as a single 10mg/kg/dose, rather than the more common 5 mg/kg/dose x 2 doses in order to keep cytoreduction to a reasonable length without administering the drugs on the same day. The Italian group has used the single 10 mg/kg dose without any significant problems [37, 38]. The thiotepa will be followed by two daily doses of melphalan.

3.2 Overview of Clofarabine

Clofarabine ([2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine]; Cl-F-ara-A; CAFdA) is a rationally designed, second-generation purine nucleoside analogue. Clofarabine was designed as a hybrid molecule to overcome the limitations and incorporate the best qualities of both fludarabine (F-ara-A) and cladribine (2-CdA, CdA) both of which are currently approved by the FDA for treatment of hematologic malignancies. Because clofarabine has a chloro group at the 2-position of adenine, its chemical structure is more closely related to CdA than to F-ara-A. Halogenation at the 2-position of adenine renders this class of compounds resistant to cellular degradation by the enzyme adenosine deaminase. Substitution of a fluorine at the C-2'-position of the arabinofuranosyl moiety of clofarabine increases its stability in gastric acid[39,40] and decreases its susceptibility to phosphorolytic cleavage by the bacterial enzyme Escherichia coli purine nucleoside phosphorylase in the gastrointestinal tract.[39] Both of these factors may lead to enhanced oral bioavailability.

3.3 Clofarabine Mechanism of Action and Antitumor Activity

Like most other deaminase-resistant nucleoside analogues (see Figure 1), clofarabine requires intracellular phosphorylation by deoxycytidine kinase (dCK) to its active triphosphate form for cytotoxic and therapeutic activity.[40,41] The cellular distribution and substrate specificity of the nucleoside-activating enzyme, dCK, are highly species-specific. The activity of dCK has been reported to be 10-fold greater in human bone marrow than in

mice.[42] Thus, the toxicity of these nucleoside analogues is qualitatively the same among the various species, but the maximum tolerated dose (MTD) and dose level required to produce toxicity in a particular target organ may vary greatly.

Figure 1: Structure of 2 Nucleoside Analogues and Clofarabine

Clofarabine is a more efficient substrate for deoxycytidine kinase than CdA and the natural substrate deoxycytidine.[43] Similar to the other purine nucleosides, clofarabine potently inhibits DNA synthesis by inhibiting both DNA polymerase α and ribonucleotide reductase.[44] Clofarabine and CdA have demonstrated the unique ability to disrupt mitochondrial integrity, resulting in the release of pro-apoptotic proteins—cytochrome C and apoptosis-inducing factor.[45] This may be a factor in the cytotoxic effects of clofarabine towards non-dividing lymphocytes.

The precise mechanism of clofarabine, CdA, and F-ara-A on dividing and non-dividing cells is unknown. In dividing cells, the incorporation of the phosphorylated form of the halogenated nucleosides into DNA appears to be an important part of their activity in arresting cell division. Inhibition of DNA polymerase α and ribonucleotide reductase has been associated with their mechanism of cytotoxicity as well. Clofarabine has shown potent cytotoxic activity in a range of cell lines including CEM, K562, Hep2, and murine leukemia L1210.[46] Therapeutic activity has also been shown in murine tumor models (P388 leukemia, colon 36, and mammary 16/c).[47] Curative activity was noted in both advanced and early colon cancer in the tumor model colon 36.[47] The compound has also shown potential in the treatment of solid tumors[41,47] and nanomolar concentrations are cytotoxic to normal lymphocytes and macrophages, making it potentially useful in the treatment of autoimmune diseases.[40]

3.4 Clinical Studies of Clofarabine

In 1999, investigators at The University of Texas MD Anderson Cancer Center (MDACC) initiated the first Phase I, dose-escalation study (DM93-036) to evaluate safety, tolerability and maximum tolerated dose of clofarabine when administered to highly refractory and/ or relapsed adult patients with solid or hematologic malignancies. Doses ranged from 1.5 mg/m^2 to 55 mg/m^2 daily and were administered as a short intravenous infusion daily x 5 every 3 to 4 weeks up to a maximum of 12 cycles. According to the results published by MDACC, the dose limiting toxicity (DLT) in solid tumor patients was myelosuppression and the MTD was 2 mg/m^2. For patients with acute leukemia, the DLT was hepatotoxicity and the MTD 40 mg/m^2. Among 32 patients treated for acute leukemia, 2 achieved a complete response and 3 had a marrow complete response without platelet recovery, for an overall response rate of 16%.[48]

A phase I study (ID99-383) of clofarabine in pediatric patients with hematologic malignancies was performed by MDACC. MDACC observed the DLT to be reversible hepatotoxicity (hyperbilirubinemia, and elevated transaminases) and skin rash observed at the 70 mg/m^2 dose level. The MTD was determined to be 52 mg/m^2/day x 5 days for pediatric patients with relapsed or refractory acute leukemia. This was the recommended dose for Phase II studies. The higher MTD observed in pediatric patients compared to adult patients was attributed to better renal and hepatic function in pediatric

patients. A total of 25 patients were treated. Five patients achieved a complete remission and 3 achieved a partial remission, for an overall response rate of 32%.[49]

As of October 1, 2004, a total of 377 patients (245 adult and 132 pediatric patients) have been treated with clofarabine. Three hundred twenty one patients were treated for hematologic malignancies and 56 for solid tumors (including 17 patients that received the oral formulation). A summary of clinical studies conducted with clofarabine is listed in the table below.[50]

Protocol/ Status	Sponsor	Date Initiated	Patient Population/ # Patients Treated	Phase of Study	Disease Description
DM93-036 Completed	MDACC	Feb 1999	Adult 51 patients	Phase I	Solid and hematologic Malignancies
DM99-25 Completed	MDACC	Sep 1999	Adult 11patients	Phase II	CLL
ID99-383 Completed	MDACC	Aug 2000	Pediatric 25 patients	Phase I	Hematologic Malignancies
ID00-038 Completed	MDACC	May 2001	Adult 64 patients enrolled (62 treated)	Phase II	Acute Leukemia and Myelodysplastic Syndrome
CLO-221 Completed	Genzyme	Nov 2001	Adult 40 patients	Phase II	AML
CLO-212 Closed to accrual	Genzyme	Apr 2002	Pediatric 49 patients	Phase II	ALL
CLO-222 Closed to accrual	Genzyme	Jan 2002	Pediatric 35 patients	Phase II	AML
CLO-151 Ongoing	Genzyme	May 2002	Adult 26 patients	Phase I	Solid Tumors
CLO-152 Ongoing	Genzyme	July 2003	Adult 17 patients	Phase I	Oral formulation in Solid Tumors
CLO-141 Completed	Genzyme	June 2002	Adult 32 patients	Phase I/II	Clofarabine in combination with ara-C in AML, ALL, high risk MDS, CML blastic phase
Expanded Access	Genzyme	Jan 2002	Adult/Pediatric 29 patients	--	AML and ALL

Efficacy and safety in pediatric patients with ALL were demonstrated in a single multi-center Phase II trial that enrolled 49 patients, CLO-212. Most patients had received 2 to 4 prior regimens and 15/49 (31%) had undergone at least one transplant. The median age was 12 years. Clofarabine was given at a dose of 52 mg/m^2, intravenously, over 2 hours daily x 5 repeated every 2 to 6 weeks following recovery or return to baseline organ function. The study endpoints were the rate of complete response (CR) and the rate of complete response without platelet recovery (CRp). Six patients (12%) achieved a CR and 4 patients (8%) achieved a CRp, and 5 patients (10%) achieved a PR. Of the 15 responding patients, 6 had post-clofarabine bone marrow transplantation. Hence, response durations could not be determined. In the patients who were not transplanted, the response durations for CR were 43, 50, 82, 93+, and 160+ days; for CRp the response duration was 32 days.[50] Twelve (24%) of the patients were treated here at MSKCC.

CLO-222 was an identical Phase II trial in for pediatric patients with relapsed or refractory AML. Clofarabine was given at a dose of 52 mg/m^2, intravenously, over 2 hours daily x 5 repeated every 2 to 6 weeks following recovery or return to baseline organ function. The study endpoints were the rate of complete response (CR) and the rate of complete response without platelet recovery (CRp). A total of 35 patients were treated. No patient achieved a CR, 1 patient (2.9%) achieved a CRp, and 8 patients (22.9%) achieved a PR. Twelve of the 35 patients (34%) had post-clofarabine bone marrow transplantation. The responses of these 12 patients included 1 CRp, 6PR, 3 as not evaluable and 2 treatment failures. Time to transplant after clofarabine was 21 to 75 days, indicating these patients proceeded to transplant very rapidly. Seven of the 12 patients that underwent bone marrow transplant were still alive at last followup. Survival from treatment with clofarabine ranges from 16.4+ weeks to 93.6+ weeks and survival post transplant ranged from 0+ weeks to 64.4+ weeks.[50] Ten of the 35 patients (28%) were treated here at MSKCC.

The principal clofarabine toxicities were nausea, vomiting, hematologic toxicity, febrile neutropenia, hepatobiliary toxicity, infections and renal toxicity. Clofarabine can rarely produce systemic inflammatory response syndrome/capillary leak syndrome (SIRS), manifested by the rapid development of tachypnea, tachycardia, hypotension, shock, and multi-organ failure. Cardiac toxicity was characterized as transient left ventricular systolic dysfunction.[50]

Clofarabine was approved by the Food and Drug Administration on December 28, 2004 for use in children with relapsed and refractory acute lymphoblastic leukemia.

The highest dose to be used in this trial is to be 40 mg/m^2/dose, which is the MTD in adults with acute leukemia as defined by the Phase I study of clofarabine. In this study, 5 patients had a response. The doses received by these responders ranged from 11.25 to 55 mg/m^2/dose, with only one patient receiving more than 40 mg/m^2/dose. [48]

A Phase II trial of clofarabine at 40 mg/m^2/dose was conducted in adults with refractory or relapsed leukemia. Thirty two percent of patients achieved a CR, 2% achieved a PR and 15% achieved complete response with incomplete platelet recovery, for an overall response rate of 49%.[49]

The phase I Study in pediatric patients used doses ranging from 11.25 to 70 mg/m^2/dose. The MTD was defined as 52 mg/m^2/dose. Five patients achieved a complete response at doses of 30 (n=1), 40 (n=2), and 52 mg/m^2/dose (n=2). Seven other patients had a partial response or hematologic improvement; two patients received 40 mg/m^2/dose, four patients received 52 mg/m^2/dose and one patient received 72 mg/m^2/dose.[50]

GVHD Prophylaxis

In the current trial, Tacrolimus and MTX (for BMT and PBSCT recipients) or Tacrolimus and MMF (for cord blood transplant recipients**)** will be utilized as GVHD prophylaxis. The gold standard of GVHD prophylaxis is cyclosporine (CSA) and methotrexate (MTX), which has been shown to reduce the incidence and severity of acute GVHD.[52] The combination of tacrolimus and methotrexate has also demonstrated a reduction in the incidence and severity of acute GvHD in matched related and unrelated BMT recipients [53-54]. In 1998, a multi-institutional study demonstrated that the incidence of grade II-IV acute GvHD was significantly lower in patients who received methotrexate in combination with tacrolimus compared to those given methotrexate in combination with CSA (31.9% and 44.4%, respectively; p = .01). Grade III-IV acute GvHD was similar regardless of the prophylactic regimen (CSA: 17.1%; Tacrolimus: 13.3%) The incidence of chronic GvHD between the two groups was comparable (Tacrolimus: 55.9% and CSA: 49.4%; p = .8); however, a significantly higher proportion of patients in the CSA group had extensive chronic GVHD (p = . 03). Przepiorka et al[55], reported grade II-IV acute GvHD in 34% (95% CI, 17% to 52%) of patients and grade III-IV acute GVHD in 17% (95% CI, 3% to 31%) following tacrolimus and methotrexate for unrelated unmodified HSCT. These results are consistent with other studies utilizing CSA and methotrexate in similar unrelated patient populations.

A pilot study by Bornhauser et al[56] treated 14 patients with MMF (1g po bid from day 1 to 14) and CSA (started on day -1 to obtain levels of 200-300 ng/ml). They were followed for 14 days after allogeneic stem cell transplantation from an HLA-compatible sibling. These 14 patients were compared to a control group of 15 patients receiving MTX and CSA. Conditioning regimens consisted of busulfan and cyclophosphamide with or without etoposide. One patient's conditioning regimen included TBI. There were no significant differences in engraftment, toxicity, or acute GVHD.

A study by Niederwieser et al [57] used MMF in combination with cyclosporine to prevent rejection and GVHD in recipients of unrelated donor hematopoietic cell transplant after nonmyeloablative conditioning with low dose TBI and fludarabine. MMF was dosed at 15mg/kg twice daily; MPA levels were not monitored. CSA was dosed to obtain levels of 500ng/ml. It was tapered and then stopped on day 180 . There was a 12% rejection rate, which occurred primarily in MDS patients and those with low T cell counts in the graft. The 21% rate of Grade II-IV acute GVHD and 30% rate of chronic GVHD were acceptable.

A prospective randomized trial by Bolwell et al [58] compared CSA and short course methotrexate with CSA and MMF for GVHD prophylaxis. The preparative regimen consisted of oral busulfan and cyclophosphamide. The group receiving MMF had significantly less mucositis and a shorter median time to neutrophil engraftment. The incidence of acute GVHD was similar in both groups, as was overall and relapse-free survival. This study is limited by its small sample size.

A second study has compared CSA + MTX and CSA+ MMF after myeloablative allogeneic stem cell transplant from an HLA-identical sibling [59]. The preparative regimens consisted of high dose chemotherapy alone or in combination with TBI. Again, shorter time to neutrophil engraftment was demonstrated. There was no difference in overall survival, relapse rate, treatment-related mortality and acute or chronic GVHD. This study was retrospective and was limited by the lack of balance of parameters between the 2 groups.

The combination of tacrolimus and MMF to be used in this study has been shown to be an effective salvage therapy for steroid-resistant GVHD. [60-61]

Also, preliminary data from Pavletic et al [62] suggest the combination of tacrolimus and MMF possesses a superior safety profile, is associated with a lower incidence of acute GVHD and earlier myeloid and platelet engraftment compared with a CSA and low dose MTX regimen. Twenty-three adults with hematologic malignancies underwent myeloablative matched related donor allogeneic stem cell transplant. Tacrolimus was given via continuous infusion of 0.03 mg/kg/day until day +60. MMF was started on day +1 at a dose of 15mg/kg/dose po or IV twice daily until day +28. Outcomes were compared to 23 matched historical controls who received CSA/low dose methotrexate. The cumulative incidence of grades II to IV and grades III to IV acute GVHD in tacro/MMF vs CSA/MTX at 100 days post transplantation was 15% versus 62% (p=0.0003) and 6% versus 25% (p=0.025), respectively. The median time to neutrophil and platelet engraftment in study patients versus controls was 10 versus 15 days (p=0.001) and 13 versus 19 days (p=0.11) respectively. There was no difference in overall survival or 6 month cumulative incidence of relapse between the 2 groups.

A pilot study of tacrolimus and MMF for GVHD prophylaxis by Osunkwo et al [63] was well tolerated and may be an effective alternative to methotrexate and corticosteroids. Tacrolimus was administered via continuous infusion of 0.03 mg/kg/day. MMF was started on day +1 at a dose of 15mg/kg/dose po or IV twice daily. A total of 34 patients were enrolled, with age ranging from 0.5 to 21 years. Twenty-two patients had a malignant diagnosis. The donor of hematopoietic stem cells were umbilical cord blood (n=22), related bone marrow (n=6) and related peripheral blood stem cells (n=9). Conditioning regimens were diverse; 57% received myeloablative regimens, 43% received fludarabine-based reduced intensity regimens. Eleven patients (31%) received TBI.

Twelve of 27 patients developed grade $\geq$ II acute GVHD; 8 patients were grade III or IV. The probability of developing grade $\geq$ II acute GVHD and grade III to IV acute GVHD was higher in patients receiving related PBSC compared to cord blood or related bone marrow. Failure to achieve target MPA levels before day 30 was associated with a higher probability of developing moderate to severe acute GVHD (100% vs 16.7% $\pm$ 15.2%, p=0.02). The probability of developing chronic GVHD was 38.1 $\pm$ 19.7%. Four of 21

patients developed limited chronic GVHD and no patients developed extensive chronic GVHD.

Tacrolimus and MMF were well tolerated overall. Three patients (9%) had grade III to IV neurotoxicity (disorientation and leukoencephalopathy). Four patients (12%) developed grade III to IV nephrotoxicity; however, all were receiving concomitant nephrotoxins.

STEM CELL GRAFT

In the present study the stem cell graft can be obtained from one of three tissues: bone marrow, peripheral blood or cord blood.

Bone marrow or peripheral blood stem cells can be collected directly from related or unrelated donor and infused directly to the patient.

Cord blood is collected from healthy newborn babies and is being frozen. One cord blood collection is called a "cord blood unit." Multiple reports [64-70] have documented that cord blood stem cells are capable of reconstituting hematopoiesis after intensive myeloablative therapy. However, the cell content of a single cord blood collection may be low for adult or larger patients. To overcome this limitation, double unit cord blood transplants were pioneered at the University of Minnesota.[71] The results demonstrated that double unit UCBT can be performed safely in adults with improved engraftment and reduced TRM as compared to historical controls of single unit cord blood transplants. Interestingly, preliminary data also suggest that double unit UCBT may be associated with protection against relapse as compared to single unit UCBT.[72-73] Currently, all MSKCC protocols use double unit cord blood grafts, with the exception of 07-056, which is the BMT-CTN (0501) randomized study of single versus double cord blood unit grafts in pediatric patients with acute malignancies.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a single arm phase I/II clinical trial to assess the antileukemic potential and relapse rate (efficacy), and peri-transplant morbidity and mortality (safety) in patients with high risk or advanced hematologic malignancies following cytoreduction with a novel agent, clofarabine, in addition to thiotepa and melphalan as preparation for an allogeneic hematopoietic stem cell transplant. Tacrolimus and MTX (for BMT and PBSCT recipients) or Tacrolimus and MMF (for cord blood transplant recipients) will be given for GvHD prophylaxis.

Candidates for this trial will include patients with high risk or advanced forms of ALL, AML, CML, acute biphenotypic or undifferentiated leukemia, infant leukemia or myelodysplastic syndrome for whom an allogeneic marrow transplant is clearly indicated.

Stem cell grafts will be unmodified marrow, peripheral blood, or cord blood stem cells derived from HLA-matched related donors or HLA-compatible unrelated donors. Candidates for transplant will be stratified according to their donor types.

4.3 Intervention

The first component of this trial is a phase I dose escalation study to determine the maximum tolerated dose (MTD) of Clofarabine. A total of 3 dose levels will be explored in this study. A standard 3+3 dose escalation design will be employed with 6 patients at each dose level. All patients will be conditioned for transplantation with Clofarabine at a dose of 20, 30 or 40 mg/m^2/dose x 5, followed by thiotepa (10 mg/kg x 1 dose) and melphalan (70 mg/m^2/dose x 2 doses). All patients will also receive Tacrolimus and MTX (for BMT and PBSCT recipients) or Tacrolimus and MMF for cord blood transplant recipients) as GvHD prophylaxis.

The second component of this trial will be the treatment of the subsequent patients with clofarabine at the MTD level if achieved early, or at the third and highest dose level if the MTD is not reached. Six patients treated at the maximum tolerated dose and 24 additional patients will be evaluated for the phase 2 component of the trial, for a total of 30 patients.

5.1 THERAPEUTIC/DIAGNOSTIC AGENTS

5.2 Clofarabine (Clolar™)

FORMULATION
Clofarabine is formulated at a concentration of 1 mg/mL in sodium chloride (9 mg/mL), United States Pharmacopeia (USP) and water for injection, USP, qs to 1 mL. Clofarabine is supplied in a 1 mg/ml, 20 mL vial. 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.
DOSE, ADMINISTRATION, AND STORAGE
Vials containing undiluted Clofarabine for injection should be stored at controlled room temperature (15 to 30°C). Shelf-life studies of intact vials are currently ongoing. Clofarabine for injection should be filtered through a sterile 0.2 µm syringe filter and then further diluted with 5% dextrose injection USP or European Pharmacopoeia (EP) (D5W) or 0.9% sodium chloride injection USP or EP (normal saline [NS]) prior to IV infusion. The resulting admixture may be stored at room temperature, but must be used within 24 hours of preparation. Clofarabine will be administered by IV infusion over 2 hours daily for 5 consecutive days. Dosage of clofarabine will be based on a dose escalation format with cohorts of 3 to 6 patients. The starting dose will be 20 mg/m^2/day of Clofarabine. Dosage will escalate to 30 mg/m^2/day and finally to 40 mg/m^2/day. To prevent drug incompatibilities, no other medications should be administered through the same IV line.

TOXICITY

1. Reversible hepatotoxicity is the major dose-limiting toxicity. This is most commonly demonstrated by a transient elevation of transaminases. Hyperbilirubinemia was less common.
2. Myelosuppression
3. acral erythema, dermatitis, pruritus
4. capillary leak syndrome, systemic inflammatory response syndrome
5. nausea, vomiting , diarrhea, abdominal pain

6. headache, fatigue, dizziness, somnolence, tremor
7. fever, rigors
8. myalgias, arthralgias
9. anorexia, weight loss
10. infection
11. shortness of breath

5.2 Thiotepa (Thioplex®)

FORMULATION:
15 mg vial lyophilized powder; must be diluted prior to infusion.

RECONSTITUTION DIRECTIONS:
Add 1.5ml of Sterile Water for Injection to 15mg vial to yield 10mg/ml. Solutions, which are grossly opaque or contain a precipitate, should not be used. In order to eliminate haze, solutions should be filtered through a 0.22-micron filter prior to administration.

STORAGE AND STABILITY:
1. Store vials in refrigerator and PROTECT FROM LIGHT.
2. Refrigerated: Prepare Infusion in NSS; stable for 14 days.
3. Room temperature: Prepare Infusion in NSS; stable for 7 days

PREPARATION:
1. Standard IV fluid: NSS
2. Final concentration range up to: 5mg/ml.
3. IV piggyback volume: 500 cc
4. Spike infusion bag with IMED 2200 tubing, primed with non-chemo containing fluid (ie; NSS).

CLINICAL CONSIDERATIONS:
Hydration: NA

TOXICITY

1. Myelosuppression is the major dose-limiting toxicity
2. Nausea, vomiting, diarrhea, anorexia, abdominal pain
3. Cutaneous erythema and bronzing
4. CNS toxicity manifested by headache, mild cognitive dysfunction, disorientation, confusion, irritability, and bizarre behavior.
5. Fever
6. Mucositis
7. Transient hepatic transaminase elevations are occasionally seen, but rarely severe.
8. Amenorrhea and impaired spermatogenesis
9. Anaphylaxis
10. Interstitial pneumonitis
11. Alopecia

INCOMPATIBILITIES: cisplatin, filgrastim (G-CSF), vinorelbine

5.3 Melphalan (Alkeran™)

FORMULATION: Melphalan is supplied as a freeze-dried powder containing 50 mg melphalan and 20 mg povidone per vial. A sterile diluent is provided for reconstitution.

RECONSTITUTION DIRECTIONS: The sterile diluent contains sodium citrate 0.2 g, propylene glycol 6 ml, ethanol (96%) 0.52 ml, and water for Injection for a total of 10 ml.

STORAGE AND STABILITY:

- Store vials at room temperature.
- Protect from light.
- Do not refrigerate the reconstituted product; a precipitate forms if the reconstituted solution is stored below 41^0 F.
- Reconstituted solutions must be further diluted immediately, discard unused portion.
- Drug administration must be completed within 60 minutes of initial reconstitution.

PREPARATION: Reconstitute by rapidly injecting 10 ml of the supplied diluent into the vial to yield a final concentration of 5 mg/ml. Shake vigorously until the solution is clear. Immediately dilute the dose to be administered in 0.9% Sodium Chloride, USP, to a concentration no greater than 0.45 mg/ml. Refrigerated storage of the reconstituted product results in precipitation.

CLINICAL CONSIDERATIONS:

Hydration: Pre-Dose – 2x maintenance for 2 hours
Post-Dose – 1.5x maintenance for 2 hours

TOXICITY

1. myelosuppression
2. hemolytic anemia
3. nausea, vomiting, diarrhea
4. mucositis
5. hepatotoxicity including veno-occlusive disease (rare)
6. syndrome of inappropriate antidiuretic hormone (SIADH) induced hyponatremia
7. pulmonary fibrosis, interstitial pneumonitis
8. alopecia
9. fever
10. hypersensitivity reactions
11. renal dysfunction
12. sterility
13. secondary leukemia
14. seizures

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

- **Diagnosis and Status**

DISEASE	DISEASE STATUS	
AML	**CR1**	**Poor risk [not t(15,17), not inv 16, not t(8,21)] OR not candidates for TBI* OR Any Infant AML in CR1**
	CR2	**All patients**
	> CR2 or Relapse / Refractory	**All patients Blast percentage >5% and < 25% in BM at time of SCT**
ALL	**CR1**	**Poor risk - t(9;22), t(4;11) - no CR after 7-28 days of induction* OR not candidates for TBI* OR Any Infant ALL in CR1**
	CR2	**All patients**
	> CR2 or Relapse / Refractory	**All patients Blast percentage >5% and < 25% in BM at time of SCT**
AUL OR ABIPL	**SAME as above**	
CML	**>1stCP**	**All patients**
MDS	**High Risk Primary MDS**	**Stage: ≥ RAEB1**
	High Risk Secondary MDS Any stage	**All patients**
	JMML	**All patients**

*The patients' initial diagnostic material must be submitted and made available for the confirmation of the patients' diagnosis and status at the time of transplant.

- Patients must be less than 55 years of age. Patients over the age of 55 years may be considered on a case-by-case basis. There is no lower age restriction.
- Patients may be male or female and of any ethnic background.

- Patients must have adequate cardiac, hepatic, renal, and pulmonary function as defined below:
  - Cardiac: a left ventricular ejection fraction at rest of >50% or a fractional shortening of ≥ 29%
  - Hepatic: SGOT of less than <2x upper limits of normal, and serum bilirubin < 1.5 m/dl unless the liver is involved with disease.
  - Renal: Serum creatinine within normal range for age or creatinine clearance of >60 ml/min/1.73 square meter
  - Pulmonary: asymptomatic with no prior risk factors or if symptomatic, diffusion capacity of >50% of predicted, corrected for hemoglobin.
- Patients may have had a prior allogeneic or autologous stem cell transplant. Interval between primary and secondary transplant must be >6 months.
- Patients or their guardians must be able to understand the nature and risk of the proposed study, and be able to sign informed consent.

6.3 Donor Inclusion Criteria

All donor and recipient pairs will be tested by DNA typing methods at high-resolution level for HLA – A, B, C, and DRB1.

HLA-compatible Related donors

- Patients who have an HLA-matched related donor are eligible for entry on this protocol. This will include a healthy related donor who is genotypically or phenotypically matched at least 7 or 8 of the A, B, C, and DRB1 alleles.

HLA-compatible Unrelated donors

- Patients who do not have an available healthy related HLA-matched donor as defined above but have an unrelated donor who is HLA-matched at
  - all 8 of 8 alleles matched
  - FOR PATIENTS < 18 years only: 7 or 8 alleles matched with the mismatch at only one A, B, C, or DRB1 allele.

HLA-compatible Unrelated Cord Blood Units

- 2 UCB units will be selected according to current MSKCC unit selection algorithm. HLA testing to be done using molecular techniques: A and B antigen to at least intermediate resolution and DRB1 allele at high-level resolution.
- Each unit will be at least 4/6 HLA-A,B antigen and DRB1 allele matched with the recipient
- In addition, each unit will have a cryopreserved dose of at least 1.5×10^7 total nucleated cells/recipient body weight (TNC/kg).
- Units with attached segments for confirmatory typing will be given preference.

Donors who are seropositive for HIV1/2 or HTLV I or II and female patients who are pregnant or breast-feeding will not be eligible for this study.

6.4 SubjectExclusion Criteria

- Karnofsky/Lansky score <70% (APPENDIX A)
- Female patients who are pregnant or breast-feeding
- Active CNS disease
- Active uncontrolled viral, bacterial, or fungal infection.
- Use of hydroxyurea within 2 week prior to initiation of cytoreduction
- Patients whose peripheral blast count doubles in two weeks
- Patients seropositive for HIV1/2 or HTLV I or II
- Patients who have undergone a prior allogeneic or autologous stem cell transplant within the previous 6 months

7.0 RECRUITMENT PLAN

One of the BMT attendings will see the patient in consultation; typically, this is the outpatient BMT attending. As part of the consultation, the BMT attending will present the patient with the risks and benefits of different types of cytoreduction and transplants. The BMT attending will then recruit patients who fulfill the eligibility criteria as listed in Section 6.0 for this study. After confirmation of patient eligibility by the medical or pediatric clinical trials office, one of the participating investigators authorized to obtain consent will obtain informed consent. A copy of the signed informed consent will be placed in the chart, as well as in the adult and pediatric clinical trials office.

8.1 PRETREATMENT EVALUATION

8.2 Pretreatment evaluation of the patient

In general, the following tests must be performed within 30 days of initiating cytoreduction:

1. Complete history, review of systems, and physical exam.
2. Bone marrow aspirate for morphology, surface markers (if warranted), cytogenetics, FISH and molecular studies (when warranted) for documentation of disease status.
3. Spinal or intra-Ommaya tap in patients with acute leukemia at risk for CNS disease.
4. Complete blood count and differential, comprehensive panel (as defined by HICFA), and for patients not in remission, LDH, serum uric acid and phosphorous
5. Coagulation profile
6. Type and Screen
7. EKG
8. Echocardiogram or MUGA (if clinically indicated)
9. Chest x-ray (Chest x-ray need not be performed if the patient has had a chest CT scan to evaluate pulmonary disease and the location of the central line tip is evident.)
10. Assessment of performance status
11. Blood will be tested for antibodies for CMV (IgG and IgM), HIV-1,2, HTLV1, 2, toxoplasmosis, Hep B, Hepatitis C, Hepatitis B surface antigen, Herpes Simplex, Herpes

Zoster, Epstein-Barr Virus, syphilis, as well as pcr testing for HIV 1-2, hepatitis C, and West Nile Virus PCR
12. Pregnancy test for females of childbearing age.

The following tests may be performed within 90 days of initiating cytoreduction as long as there has been no interval therapy given for the underlying disease and there has been no change in the patient's clinical status:

1. Dental evaluation
2. Pulmonary function testing when feasible

Research Studies for UCBT recipients:
1. Within approximately 60 days preceding cytoreduction, 3 green top tubes should be sent to Dr. Katharine Hsu's lab in Z745D.

8.3 Pretreatment evaluation of the donor

Family Donor

Any consenting healthy family donor who is HLA compatible with the recipient will be given highest priority as a potential donor for marrow or PBSC transplant. The donor (or the donor's parents for minors) must also provide signed informed consent for a marrow harvest under general anesthesia, or alternatively, to receive a 5-day course of G-CSF followed by 2-3 leukaphereses.

In preparation for the stem cell donation, the donor will provide informed consent and then undergo the following evaluation. In general, the following tests must be performed within 30 days of initiating cytoreduction:

1) Complete history, review of systems, and physical exam.
2) Complete blood count and differential
3) Comprehensive panel (as defined by HICFA)
4) Coagulation profile
5) Type and Screen
6) EKG and chest x-ray (if clinically indicated)
7) Serum will be tested for antibodies for CMV (IgG and IgM), HIV-1,2, HTLV1, 2, toxoplasmosis, Hep B, Hepatitis C, Hepatitis B surface antigen, Herpes Simplex, Herpes Zoster, Epstein-Barr Virus, VDRL
8) West Nile Virus PCR, Hepatitis C PCR and HIV PCR
9) Pregnancy test for females of childbearing age.

Before admission for bone marrow harvest (only in context of BMT and not PBSC), it is recommended that all donors at the discretion of the transplant physician, or at least all female donors be referred for autologous blood donation. The autologous blood product will be held in reserve for the donor, to be transfused in lieu of allogeneic blood products (unless emergency indications dictate otherwise) at the completion of the marrow harvest. Because of small donor size in many pediatric cases, autologous blood donation will be recommended at the discretion of the pediatric bone marrow transplant attending physician.

Unrelated donor

Unrelated donors will undergo preparation for marrow or peripheral blood stem cell collection as per the standards of the National Marrow Donor Program (NMDP). The donor will undergo pretransplant work-up and will sign consent for either G-CSF administration followed by leukapheresis or for bone marrow harvest at the donor center, also according to standard procedure as dictated by the NMDP and institutional guidelines.

Unrelated cord blood unit

Units will be selected according to the current MSKCC unit selection criteria, under the direction of Dr. Juliet Barker and Dr. Andromachi Scaradavou. Infectious disease testing and eligibility assignment will be reviewed by the above physicians.

9.0 TREATMENT/INTERVENTION PLAN

Patients will be admitted the Pediatric or Adult Bone Marrow Transplant Service. Patients will be maintained in reverse isolation as per the BMT clinical care guidelines. Prior to the administration of the pretransplant cytoreductive regimen, a double or triple lumen central venous catheter will be inserted under general or local anesthesia. A separate consent form will be obtained for this procedure.

9.1 Cytoreductive regimen and transplant

1. **Clofarabine**
   Clofarabine will be administered via a 2 hour intravenous infusion at a dose of 20, 30 or 40mg/m^2/day for 5 doses (days -9, -8, -7, -6. -5).

   Clofarabine will be administered after premedication with hydrocortisone. Hydrocortisone was added to the amended protocol in an attempt to prevent hepatic inflammation and elevation of transaminases.

   Hydrocortisone will be dosed at 50 mg/m2 dose IV once daily pre-clofarabine (maximum 100 mg).

2. **Thiotepa**
   Thiotepa will be administered via a 4 hour intravenous infusion at a dose of 10mg/kg/day for 1 dose (day –4). Dose should be adjusted if patient is > 125% ideal body weight and should be calculated on adjusted ideal body weight per MSKCC standard of care guidelines.
   .

3. **Melphalan**
   Melphalan will be administered via a 30 minutes infusion at a dose of 70mg/m^2/day for 2 doses (days -3, and -2). Dose should be adjusted if patient is > 125% ideal body weight and should be calculated on adjusted ideal body weight per MSKCC standard of care guidelines.

4. **Day 0:** patients will receive a T-cell replete allogeneic BMT or PBSCT or an allogeneic double unit CB graft.

Phase I

The dose of Clofarabine will be escalated with 3 Dose Levels:

- Dose Level 1: 20 mg/m^2/dose x 5
- Dose Level 2: 30 mg/m^2/dose x 5
- Dose Level 3: 40 mg/m^2/dose x 5

Dose level 1: Clofarabine at 20 mg/m^2/dose x 5 + THIO-MEL 6 patients

- If 0 or 1 of 6 patients experience dose limiting toxicity, proceed to dose level 2.
- If ≥ 2 patients experience a dose limiting toxicity, re-evaluate the protocol.

Dose level 2: Clofarabine at 30 mg/m^2/dose x 5 + THIO-MEL 6 patients

- If 0 or 1 of 6 patients experience dose limiting toxicity, proceed to dose level 3.
- If ≥ 2 patients experience a dose limiting toxicity, the MTD will be Dose Level 1.

Dose level 3: Clofarabine at 40 mg/m^2/dose x 5 + THIO-MEL 6 patients

- If 0 or 1 of 6 patients experience dose limiting toxicity, this will be the dose level to be used for the rest of the trial.
- If ≥ 2 patients experience dose limiting toxicity, the MTD will be Dose Level 2.

Phase II

6 patients treated at Dose Level of MTD (or dose Level 3 if MTD not reached) + 24 additional patients

9.2 Graft versus host disease prophylaxis

BONE MARROW AND PERIPHERAL BLOOD STEM CELL TRANSPLANT RECIPIENTS

1. **Tacrolimus**

   Tacrolimus will be started day –3 at an approximate dose of 0.03 mg/kg/24 hours as a continuous infusion. The dose of tacrolimus will be adjusted to achieve a level between 5 and 15 ng/ml. Once oral medications are tolerated, patients may be converted to oral tacrolimus at 3-4 times the current IV dose divided every 8 to 12 hours.

   For recipients of matched related transplants without GvHD, tacrolimus will be continued for approximately 60 days and will then be tapered by approximately 10% per week. It will be discontinued at approximately 6 months post-transplant.

For recipients of unrelated or mismatched related transplants without GvHD, tacrolimus will be continued for approximately 6 months and will then be tapered by approximately 5% per week. It will be discontinued at approximately 1 year post-transplant.

In case of major toxicity, such as CNS symptomatology, tacrolimus may be discontinued earlier and substituted with other immunosuppressive medications as per clinical care guidelines.

The immunosuppressive regimens on this protocol may conform to current practice where additional agents may be introduced in addition to Tacrolimus.

2. Methotrexate

A. Methotrexate (MTX) Methotrexate will be given at a dose of 15 mg/m2/dose on day +1 post transplant and 10 mg/m^2 intravenously on days +3, +6 and +11 to recipients of related or unrelated donor bone marrow or peripheral blood stem cell transplants BUT not to Cord Blood transplant recipients.

Methotrexate dose adjustment will be as follows:

a. Creatinine	< 1.5	100%
	1.6-1.8	50%
	1.9-2.0	25%
	> 2.0	hold
b. Bilirubin	< 2.0	100%
	2.1-3.0	50%
	3.1-5.0	25%
	> 5.0	hold

c. For patients with severe mucositis ≥ grade 3, decrease or hold Methotrexate as clinically indicated.

B. Methotrexate will be given at a dose of 5 mg/m2/dose intravenously on day +1, days +3, +6 and +11 post transplant to all adult recipients of related or unrelated donor bone marrow or peripheral blood stem cell transplants BUT not to Cord Blood transplant recipients.

If Methotrexate is to be modified or discontinued, GvHD prophylaxis may conform to current practice and additional agents can be introduced.

1. **Tacrolimus**

Tacrolimus will be started day –3 at an approximate dose of 0.03 mg/kg/24 hours as a continuous infusion. The dose of tacrolimus will be adjusted to achieve a level between 5 and 15 ng/ml. Once oral medications are tolerated, patients may be converted to oral tacrolimus at 3-4 times the current IV dose divided every 8 to 12 hours.

For recipients of umbilical cord blood transplants without GvHD, tacrolimus will be continued for approximately 6 months and will then be tapered 5% per week. It will be discontinued at approximately 1 year post-transplant.

In case of major toxicity, such as CNS symptomatology, tacrolimus may be discontinued earlier and substituted with other immunosuppressive medications as per clinical care guidelines.

The immunosuppressive regimens on this protocol may conform to current practice where additional agents may be introduced in addition to Tacrolimus.

2. **Mycophenolate mofetil (MMF)**

MMF will be started on day -3 to +45 then tapered.

- Dose is 1 gram IV q8 hours for adults and children who are both >12 years and >50 kg. Adults and teens >12 years but <50 kgs, give 15 mg/kg IV q8 hours. If less than 12 years, give 20mg/kg IV q8 hours to a maximum of 1 gram IV q8 hours. MMF from day -3 to hospital discharge (must be at least day +14) should be IV. In preparation for discharge, switch to oral route (CellCept or generic mycophenolate mofetil and same dosing as IV) and round to tablet size.
- No dose adjustments for renal or liver disease are needed routinely unless severe organ dysfunction. Measurements of serum blood levels of MMF should be done routinely as per current MSKCC guidelines to guide assessment of potential MMF toxicity in the event of unexpected myelosuppression. If patient ≥ +28 days and slow engraftment, consideration can be made to reduce dosing to q12 after discussion with PI or co-PI. See MSKCC guidelines for further information about MMF levels.
- Taper to 500 mg TID (or 7.5 mg/kg/dose if < 50 kg) on day +46 and continue to +70. Then taper to 250mg TID (3.75 mg/kg/dose) till day 100 and stop.
- If the patient cannot tolerate therapeutic doses of CSA due to renal dysfunction, do not taper MMF on day +46.
- If the patient has acute GVHD requiring systemic therapy, MMF should only be tapered on days 46 and 70, or stopped on days 100, if control of GVHD has been obtained (eg resolution of skin rash, vomiting, and diarrhea).
- If persistent disease or disease progression occurs prior to day 100, early taper or cessation of MMF can be considered with close observation for acute GVHD.

For patients with active GvHD, tacrolimus and MMF may be continued for longer periods of time according to clinical care guidelines.

9.3 Stem Cell Transplant

BMT

Bone marrow is harvested from the donors in the Operating Room according to standard BMT guidelines. The marrow is delivered to the Cytotherapy Laboratory. In the Cytotherapy Laboratory, cell counts will be performed. Red cells or Plasma will be depleted in case of ABO mismatch according to standard BMT guidelines. Volume depletion will be performed in Pediatric patients to obtain a final volume of 10-12 ml/Kg of recipient's weight.

PBSCT

Peripheral blood stem cells are collected from the donors in the Donor Room according to standard BMT guidelines. The peripheral blood is delivered to the Cytotherapy Laboratory. In the Cytotherapy Laboratory, cell counts will be performed. Red cells or Plasma will be depleted in case of ABO mismatch according to standard BMT guidelines. Volume depletion will be performed in Pediatric patients to obtain a final marrow volume of 10-12 ml/Kg of recipient's weight.

The goal of stem cell collection is $\geq 2 \times 10^6$ CD34+ cells per kilogram patient weight. As this value cannot be obtained while the patient is in the OR, the harvesting physician will aim to collect at least 2×10^8 total nucleated cells per kilogram patient weight.

UCBT

- UCB grafts will be received at the MSKCC Cytotherapy Laboratory prior to the start of the preparative regimen.
- Units will be thawed by and released from the Cytotherapy Laboratory according to current standards of practice (SOPs) and release criteria. Units will undergo albumin dilution, except in case of small children with wt less than 20 kg, when the cord blood units will be washed to remove the cryoprotectant, and the stem cells will be resuspended in appropriate volume (for the wt of the patient). As per standard practice, ABO blood group, total nucleated cells (TNC), CD34+ and CD3+ cell number and viability, sterility and colony-forming units (CFU) will be measured post-thaw.
  A small amount of cells (<5% of the post-thaw TNC of each unit) will be used for laboratory research studies.
- Units should be administered immediately upon arrival to the patient care unit by IV infusion by the nursing staff under supervision of a BMT attending physician. UCB infusion nursing guidelines should be followed.
- Units should be given consecutively as per nursing guidelines.
- Pre-medication should include acetaminophen and diphenhydramine or hydroxyzine dosed as appropriate for patient age. Do not give Hydrocortisone. Anti-emetics may be necessary and can be given prn.
- IV hydration equal to twice standard maintenance should be given for 6 hours prior to and at least 12 hours post UCB graft infusion with close monitoring of fluid balance, per MSKCC guidelines.
- IV Hydralazine should be used to treat hypertension associated with UCB infusion, if necessary.

Following infusion the bag and tubing from each UCB unit must be submitted to the Microbiology Laboratory. Specimen for Diagnostic Molecular Pathology will be forwarded by the personnel in the Cytotherapy Laboratory.

GROWTH FACTOR (G-CSF) AFTER SCT
G-CSF will be given to all patients, regardless of transplant type, from day +7 if absolute neutrophil count (ANC) is < 500. Continue until ANC is >2000 for 3 consecutive days. GCSF may be given earlier post transplant if clinically indicated at the discretion of the Attending Physician. G-CSF doses will be rounded according to institutional guidelines.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

10.1 Prophylaxis against Infection

Patients will be treated according to the allogeneic BMT standard of care guidelines and will be given prophylaxis against 1) Pneumocystis carinii, 2) Herpes simplex and Herpes Zoster, and 3) fungal infections. Patients who are CMV sero-negative pre-transplant should receive CMV seronegative blood products, if possible. Patients with CMV viremia will be treated according to the allogeneic BMT standard of care guidelines with ganciclovir or foscarnet.

10.2 Prophylaxis against Graft versus Host Disease

All patients will receive Tacrolimus and MTX (for BMT and PBSCT recipients) or Tacrolimus and MMF (for cord blood transplant recipients) as GvHD prophylaxis as follows:

Tacrolimus will be started day –3 at a dose of 0.03 mg/kg/24 hours as a continuous infusion. Trough tacrolimus levels will be drawn 30 minutes prior to the dose in patients receiving divided doses. In patients receiving continuous infusion tacrolimus, steady state levels can be drawn at any time. Care must be given not to draw levels through IV tubing that has been exposed to tacrolimus. Plasma levels will be determined by whole blood assay. Tacrolimus dosing will be monitored as clinically appropriate. Trough levels or steady state levels will be adjusted to try to keep tacrolimus levels between 5-15 ng/ml. Adjustments of tacrolimus levels are to be approved by the supervising BMT Attending. Tacrolimus dose may be decreased in case of hyperbilirubinemia, unexplained elevation of BUN and creatinine, or other major toxicity felt to be related to this drug. Once the patient can tolerate oral medications, tacrolimus will be converted to an oral form at four times the current IV dose divided every 8 to 12 hours. In case of major toxicity (eg CNS neurotoxicity documented by MRI), tacrolimus should be discontinued. Patients may be re-challenged at a later date if thought clinically appropriate. Substitution with other immunosuppressive medications as prophylaxis must only be done in consultation with the Principle Investigator.

The immunosuppressive regimens on this protocol may conform to current practice where additional agents may be introduced in addition to Tacrolimus.

The duration of tacrolimus is dependent on the type of donor. Patients whose donors were HLA-matched related donors will continue tacrolimus until day approximately +60. As long as there are no signs or symptoms of GVHD, the dose will be tapered by approximately 10% per week and discontinued at approximately 6 months post-transplant

Patients whose donors were unrelated donors or mismatched related donors will continue tacrolimus until approximately day +180. As long as there are no signs or symptoms of GVHD, the dose will be tapered by approximately 5% per week and discontinued at approximately 1 year post-transplant.

Mycophenolate mofetil (MMF) will be started on day –3 and continued through day +45 then tapered. The dose is 1 gram IV q8 hours for adults and children who are both >12 years and >50 kg. Adults and teens >12 years but <50 kgs, give 15 mg/kg IV q8 hours. If less than 12 years, give 20mg/kg IV q8 hours to a maximum of 1 gram IV q8 hours. Subsequently, MMF may be administered orally once other oral medications are tolerated. The dosing is the same as the IV formulation but will be rounded to the nearest tablet size. No dose adjustments for renal or liver disease are required. MMF will be tapered after day +45 by 50%, as long as there are no signs or symptoms of acute GVHD present. **The aim is to be off MMF by day 100 in the absence of uncontrolled GVHD**. If the patient has acute GVHD requiring systemic therapy by day +45, MMF may be stopped 7 days after control of GVHD (e.g. resolution of skin rash, vomiting, and diarrhea).

Methotrexate (MTX) will be given at a dose of 15 mg/m2/dose on day +1 post transplant and 10 mg/m^2 intravenously on days +3, +6 and +11 to recipients of related or unrelated donor bone marrow or peripheral blood stem cell transplants BUT not to Cord Blood transplant recipients.

10.2 CNS leukemia

Patients with ALL, those with M4/M5 AML, and those transplanted with AML in bone marrow relapse may receive intrathecal infusions of cytarabine at monthly intervals, beginning approximately 2 months posttransplant, and thereafter on a monthly basis for a total of 5 doses. Patients with a prior history of CNS leukemia may receive 11 doses of IT cytarabine from approximately 2 until 12 months post-transplant. Intrathecal Cytarabine will be dosed according to age:

AGE	DOSE
<1 year	20 mg
1 to <2 years	30 mg
2 to <3 years	50 mg
≥ 3 years	70 mg

10.4 Transfusions

Following initiation of the pretransplant cytoreduction, all blood products for transfusion, with the exception of the stem cell graft, will be irradiated to 3,000 cGy to inactivate lymphocytes capable of initiating lethal GvHD. Blood products are irradiated in the blood bank, using a cesium gamma emitter. Patients who are Cytomegalovirus (CMV)-seronegative pretransplant will not be permitted to receive any CMV-seropositive blood products, except in an emergency. Platelets will be administered for clinical evidence of active hemorrhage. To minimize bleeding, platelets will be transfused prophylactically in order to maintain a platelet count greater than 20,000/mm3. Posttransplantation, the marrow donor or selected family donors will be utilized, whenever possible, as platelet donors. Packed irradiated red blood cells will be administered as clinically indicated.

10.5 Prophylaxis against metrorrhagias

All post-pubertal females will receive hormonal therapy to suppress menses. This therapy may include Ovral™ (Ethinyl estradiol + norgestrel) daily at a dose of one daily tablet orally, estrogen patch +/- oral progesterone, or another regimen which has been successful for the patient. Hormonal suppression will continue throughout the transplant until the platelet count is above 50,000. The administration of hormonal therapy will be determined based on the patient's clinical status.

10.6 Nutritional Support

The physician will monitor nutritional status, and high-calorie parenteral alimentation will be introduced as needed. Vitamin supplements will be administered as clinically indicated.

10.7 Post transplant evaluation*

During the first 100 days or 3 months post transplant:

1. Regular physical examination as per standard of care until discharge, CBCs at least 3x per week until discharge.
2. Acute GVHD will be assessed and graded according to MSKCC criteria. To determine the severity of acute GVHD data will be collected approximately every 2 weeks for the first 3 months (Appendix B).
3. Blood chemistries, including liver function tests, will be performed at least twice weekly, until discharge after discharge,
4. Physical examination, CBC, and blood chemistries, including liver function tests will be obtained every 2-4 weeks, or as clinically indicated.
5. Bone marrow aspiration with cytogenetic analysis for chimerism and disease status (if indicated) will be performed at approximately 1 month and 3 months posttransplant.
6. Recipients of cord blood grafts will undergo BM evaluation by aspirate, as well as evaluation of donor-host chimerism, on approximately day +21. Evaluation of PB chimerism will be performed on approximately days +28, +60, and +100.
7. Cord blood transplant recipients will have research blood (approximately 20 cc) be obtained to assess the recovery of NK cells on approximately days +28, +60 and +100.
8. Cord blood transplant recipients will be closely monitored for any infections that may be associated with delayed engraftment.

**Tests may be held in case of severe toxicity or if patient is without counts.*

10.8 Evaluation > 100 days post transplant*

1. Physical examinations, CBC, blood chemistries and liver function test at a minimum of approximately every 6 weeks until 6 months, then at a minimum of approximately every three months for one year, then at approximately 3-6 month intervals until 2 years post transplant. The patient's referring physician, in consultation with a bone marrow transplant physician, may perform follow-up.
2. Chronic GVHD will be diagnosed and graded according to the criteria of Sullivan et al [67], (Appendix C). Assessments will be obtained at approximately day 100, 180 and 365. Patients who develop chronic GVHD will be treated according to current standard of care.
3. Bone marrow aspiration with cytogenetic analysis for chimerism and disease status (if indicated) should be performed approximately every 3-6 months until one-year post transplant, and then as clinically indicated.

4. Recipients of cord blood grafts will undergo evaluation of PB chimerism at 6 months, 1 year, and 2 years post-transplant.
5. Lymphoid immunophenotyping and function will be evaluated as per BMT standard of care guidelines.
6. For cord blood transplant recipients, research blood (approximately 20 cc) will be obtained to assess the recovery of NK cells on days 180 and 1 year post-transplant.
7. Cord blood transplant recipients will be closely monitored for any infections that may be associated with delayed engraftment.

**Tests may be held in case of severe toxicity or if patient is without counts.*

11.1 TOXICITIES/SIDE EFFECTS

11.2 Clofarabine

Likely

- Reversible hepatotoxicity is the major dose-limiting toxicity. This is most commonly demonstrated by a transient elevation of transaminases. Hyperbilirubinemia was less common.
- Dermatitis and pruritus
- nausea, vomiting , diarrhea, abdominal pain
- myelosuppression and pancytopenia
- headache
- fever - chills or rigors

Less Likely

- acrodermatitis
- anorexia, weight loss myalgias, arthralgias
- headache, fatigue, dizziness, somnolence, tremor
- shortness of breath
- infections

Rare but serious

- Capillary leak syndrome
- Systemic inflammatory response syndrome

11.2 Thiotepa

Likely

- alopecia
- anorexia - nausea and vomiting – abdominal pain - diarrhea
- myelosuppression and pancytopenia
- sterility
- fever
- cutaneous erythema and bronzing

Less Likely

- dizziness
- transient hepatic transaminases elevation

Rare but serious

- CNS toxicity manifested by headache, mild cognitive dysfunction, disorientation, confusion, irritability, and bizarre behavior

- Anaphylaxis
- interstitial pneumonitis
- renal failure

11.3 Melphalan

Likely

- nausea and vomiting - Diarrhea
- mucositis
- myelosuppression and pancytopenia
- elevated liver function tests
- sterility
- alopecia
- fever

Less Likely

- Syndrome of Inappropriate anti-diuretic hormone (SIADH)
- Interstitial pneumonitis
- Pulmonary fibrosis

Rare but serious

- secondary leukemia
- anaphylaxis
- seizures
- kidney failure
- veno-occlusive disease of the liver (VOD):

11.4 Tacrolimus (FK506, Prograf™)

Likely

- renal dysfunction and electrolyte abnormalities of potassium and magnesium
- hypertension
- hepatotoxicity
- nausea and vomiting – constipation - anorexia
- tremors
- fatigue

Less Likely

- hemolytic anemia
- renal failure
- unsteadiness
- depression
- peripheral neuropathy

Rare but serious

- Seizures
- Ataxia – cortical blindness
- hemolytic uremic syndrome – thrombotic thrombocytopenic purpura
- post-transplant lymphoproliferative disorder
- anaphylaxis
- capillary leak syndrome

11.5 Mycophenolate mofetil (MMF)

Likely

- hypertension
- nausea and vomiting - diarrhea
- myelosuppression and pancytopenia

Less Likely

- headache
- insomnia
- peripheral edema
- confusion - tremor

Rare but serious

- skin cancer, not including melanoma
- gastrointestinal bleeding
- progressive multifocal leukoencephalopathy (PML), a progressive disease of the nervous system that can cause severe disability or death. A very small number of cases of PML have been reported in patients treated with MMF.

11.6 Methotrexate (MTX)

Likely

- Renal dysfunction: Decrease in creatinine clearance, renal wasting of magnesium and calcium, hypertension. Hypertension may be exacerbated by the concomitant use of corticosteroids.
- Hepatic dysfunction: Elevation in serum bilirubin and occasionally in serum transaminases.
- Mucositis: Mucositis secondary to the cytoreduction may be potentiated following the use of methotrexate post transplant.

11.7 G-CSF (Neupogen)

- Side effects of G-CSF are generally mild, include bone pain, headaches, body aches, fatigue, edema and nausea and are managed with supportive care. Pleuro- or pericarditis are seen rarely and are managed by cessation of the medication and corticosteroids if necessary.

11.8 CORD BLOOD UNIT INFUSION

Toxicities potentially associated with the infusion of the UCB graft include DMSO toxicity and side effects from red cells and may include changes in heart rate or rhythm, changes in blood pressure, fever, chills, sweats, nausea/vomiting, diarrhea, abdominal cramping, headache, dyspnea, presence of DMSO taste and odor, hemoglobinuria, and acute renal failure. However, due to the dilution step and pre-medications, these toxicities are unlikely.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Definition of events in the post-transplant course important for analysis and treatment

12.1. Leukemic Relapse

Relapse of leukemia is the primary endpoint of this study. It will be analyzed as to type and genetic origin of the leukemic cells. These will be defined by an increasing number of blasts in the marrow over 5%, by the presence of circulating peripheral blasts, or by the presence of blasts in any extramedullary site.

Cytogenetic analysis of the marrow and/or peripheral blood will also be obtained for the diagnosis of relapse. The presence of the original cytogenetic marker or molecular evidence of disease will be considered a relapse if the abnormality is present in two consecutive samples obtained 1 month apart or if treating physician initiates additional therapy for treatment of relapse.

For high-risk patients with advanced disease, an estimated relapse probability is approximately 50%.

12.2. Early post-transplant severe morbidity and mortality

Early severe post transplant regimen-related severe morbidity (grade IV non-hematologic toxicity) and/or mortality will be the second endpoint of this study. In the context of the agents or agent-combination used for cytoreduction, particular attention will be given to, and Dose Limiting Toxicity will be based on, grade IV toxicity involving (1) the liver, (2) the heart, (3) the oral mucosa and gastrointestinal tract, and (4) the skin. The grading for monitoring the morbidity and mortality will be based on the NCI/CTEP common toxicity criteria, CTCAE version 3.0.

12.3. Graft Failure or Rejection

Primary non-engraftment is diagnosed when the patient fails to achieve an ANC $\geq 500/\mu l$ at any time in the first 28 days post-transplant. If the patient's leukemia recurs during this interval, the patient is scored as having refractory leukemia. In such a situation, the absence of donor hematopoiesis is not evaluable for graft failure or rejection. If (1) after achievement of an ANC $\geq 500/mm^3$, the ANC declines to $<500/mm^3$ for more than 3 consecutive days in the absence of relapse, or, (2) there is absence of donor cells in the marrow and/or blood as demonstrated by chimerism assay in the absence of relapse, a diagnosis of secondary graft failure is made. If, however, recurrence of host leukemia is detected concurrently, the patient is not evaluable for graft failure or rejection. Patients with evidence of graft failure without evidence of recurrence of host leukemia will have additional studies drawn to ascertain cause and define relevant histoincompatibilities.

These analyses may include (1) evaluation of bone marrow aspirates and biopsies for residual or recurrent leukemia, when indicated, (2) culture and/or molecular analyses of marrow and blood for viral pathogens potentially causing graft failure including CMV, HHV6 and parvovirus B 19, and (3) immunophenotypic and genetic analysis of circulating T-cells and NK cells to ascertain their origin and potential function.

Patients who suffer graft failure will be considered for a secondary transplant. The need for additional immunosuppression or treatment for viral infection prior to the secondary transplant will be determined by the results obtained from chimeric and viral studies.

12.4. Graft Versus Host Disease

Standard clinical criteria as defined by MSKCC criteria and histological grading of skin, liver or gastrointestinal pathology as defined by Glucksberg et al[75], Slavin, et al [77] and Schulman et al. [78] will be used to establish and grade acute GvHD (Appendix B).

To determine the severity of acute GvHD, data will be collected approximately weekly to characterize the severity of symptoms and signs caused by GvHD and to evaluate possible confounding factors. Real time data collection will include

- descriptive characteristics of rash
- estimated body surface area involved
- extent of dermal/epidermal separation
- identification of concomitant causes of increased bilirubin other than GvHD
- presence or absence of nausea, vomiting or anorexia persistent after engraftment
- peak diarrhea volume with annotations concerning the presence after engraftment
- peak diarrhea volume with annotations concerning the presence or absence of urinary mixing and estimates of true diarrhea volume
- presence or absence of abdominal cramps
- presence or absence of frank blood in the stool or melena
- concomitant causes of GI symptoms other than GvHD
- biopsy results
- identification of any agents used for treatment
- autopsy results.

Patients will be observed for acute and/or chronic GvHD as long as they have not received donor derived leukocytes infusions (DLI) for the treatment of relapse or infections. If at any time, a patient receives DLI, that time will represent the end-time for evaluation of GvHD. Graft-versus-host disease occurring after DLI infusions will be analyzed separately.

Patients with moderate to severe acute GvHD (grade II-IV) will be treated in standard fashion with high-dose IV methylprednisolone (2-20mg/kg/day). Patients failing to respond to steroids will be considered for treatment with a human monoclonal antibody directed against the IL-2 receptor or with and/or experimental treatment available at the time of diagnosis of GvHD.

Chronic GvHD will be diagnosed and graded according to the criteria of Sullivan[76] (Appendix C) and treated with standard or experimental immunosuppressive therapy. Treatment will consist of corticosteroids, cyclosporin A, azathioprine, or combinations of these agents. Other novel treatments could be used if available, i.e. thalidomide, rituximab, psoralen/ultraviolet A phototherapy (PUVA).

12.5. Immunologic Reconstitution

Immunophenotyping of T-cells, B-cells, and NK cells, and T-cell proliferations in response to non-specific mitogens and specific antigens, will be performed at serial time points after transplant to measure immune recovery. Cord blood transplant recipients will have research

blood (approximately 20 cc) will be obtained to assess the recovery of NK cells on approximately days +28, +60, and +100. The studies will be performed at approximately 4 and 6 months post transplant and then every 6-12 months until normal for all patients. Patients may receive supplemental IV immunoglobulin (Ig) to prevent infections as clinically indicated and based on IgG levels. Subsequently, Ig levels will be tested at approximately 6, 12, 18 and 24 months post transplant to guide further IgG supplementation. Patients may be re-immunized as from 12 months post-transplant and the response to vaccination will be documented.

12.6 Response to Therapy

- A **complete response (CR)** will be defined as less than 5% bone marrow blasts in the setting of a neutrophil count of ≥1.0 K/ul and a platelet count of ≥75,000/ ul.
- A **complete response except platelets (CRp)** will be defined as less than 5% bone marrow blasts in the setting of a neutrophil count of ≥1.0 K/ul and a platelet count of ≤75,000/ul.
- A **partial response (PR)** will be defined as 5%-25% bone marrow blasts in the setting of a neutrophil count of ≥1.0 K/ul and a platelet count of ≥75,000/ul.
- A **partial response except platelets (PRp)** will be defined as 5%-25% bone marrow blasts in the setting of a neutrophil count of ≥1.0 K/ul and a platelet count of ≤75,000/ul.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Patients may be removed from the study at any point deemed appropriate by the principle investigator. However, once the melphalan is given on Day -4, patients will continue on study until after administration of the stem cells. Failure to rescue the patient with stem cells at this point in the cytoreduction would most likely be fatal.

14.0 BIOSTATISTICS

This is a phase1/2 clinical trial to determine the safety and efficacy of clofarabine, melphalan, and thiotepa followed by a hematopoietic stem cell transplant from HLA compatible related or unrelated donors for patients with advanced hematologic disorders.

After the first 6 patients were enrolled on this study, it was decided by the transplant team to change (1) the stem cell sources and allow the use of cord blood transplants, (2) the indications for transplant and allow high risk acute leukemias in CR1, and (3) change the GvHD prophylaxis for recipients of marrow or peripheral blood stem cell grafts.
Because of these changes, the protocol accrual for the study will restart from zero.

This is an amendment for the phase 1 component of the study. Three dose levels will be explored in the study. To find the maximum tolerated dose (MTD), a dose escalation scheme will be employed with ***6*** patients per dose. Dose escalation is based on dose limiting toxicity (DLT), which is defined as grade IV toxicity involving (1) the liver, (2) the heart, (3) the oral mucosa and gastrointestinal tract, and (4) the skin (section 12.2)

If zero or one patient experiences a DLT at a given dose level, then dose assessment will proceed to the next dose level. If two or more patients experience a DLT at a dose level, the preceding dose will be declared the MTD. Escalation to the next dose level is probable if the risk of DLT is low, and the likelihood of escalation decreases as the risk of DLT increases.

True Risk of Toxicity	0.10	0.20	0.30	0.40	0.50
Probability of Escalation	0.89	0.66	0.42	0.23	0.11

Given 3 dose levels, a maximum of 18 patients will be entered onto this component of the trial.

The 6 patients treated at the MTD, plus an additional 24 patients (for a total of 30) will be evaluated for the phase 2 component of this trial. The primary objective is to estimate the probability of survival and disease free survival (DFS) in this patient population. We propose declaring the treatment active if the one year DFS in the population exceeds 0.50. A single stage design that differentiates between one-year DFS probabilities of 0.50 and 0.70 will be used to assess efficacy. The donor subgroups will be aggregated for the survival and disease free survival estimates. If nineteen or more patients survive free of disease for one year from the time of transplant, it is concluded that the transplant is sufficient effective to warrant further study. This design has power 0.89 for a one-year disease free survival probability of 0.70, using a one-sided test with size 0.10.

At the conclusion of the study, the overall and disease free survival endpoint will be estimated using the product limit estimate.

In order to reduce patient risk, the study design includes early termination of the trial in the event of excessive grade 4 non-hematologic toxicity and grade 2-4 acute graft versus host disease (aGvHD) during the accrual period. A constrained sequential probability ratio test, based on the binomial random variable, will be used to monitor the failure rates within each donor group. If the stopping boundary is met for one donor group, the study will continue accrual in the other donor group. At the conclusion of the study, these incidence rates will be summarized using the cumulative incidence function.

Tables 1-3 describe the stopping boundaries for this study. A single sequential stopping boundary is developed for the endpoint grade 4 non-hematologic toxicity (Table 1). For grade 2-4 acute graft versus host disease, separate stopping boundaries are produced for patients with related donors and patients with unrelated donors (Tables 2 and 3).

If the disease-free survival probability in this heterogeneous population is acceptable, and the trial is not terminated due to excessive levels of non-hematologic toxicity or graft versus host disease, then larger, potentially multi-institutional trials designed to ascertain the potential of this transplant approach for patients with advanced hematologic disorders would be indicated.

We anticipate a per year accrual of approximately 10 patients and expect the study to run three to four years.

Table 1 – stopping boundary for non-hematologic toxicity

Boundary based on an expected toxicity rate of 0.10 and an unacceptable toxicity rate of 0.30. The constrained probability ratio test using this boundary has size 0.10 and power 0.90.

Stop if observe	2	toxicities within the first	3	patients
	3		6	
	4		10	
	5		23	
	6		25	
	7		30	

Table 2 - stopping boundary for grade 2-4 acute GvHD – related donor

Boundary based on an expected acute GvHD rate of 0.15 and an unacceptable acute GvHD rate of 0.35. The constrained probability ratio test using this boundary has size 0.10 and power 0.90.

Stop if observe	2	GvHDs within the first	2	patients
	3		5	
	4		8	
	5		12	
	6		26	
	7		30	

Table 3 - stopping boundary for grade 2-4 acute GvHD – unrelated donor

Boundary based on an expected acute GvHD rate of 0.25 and an unacceptable acute GvHD rate of 0.50. The constrained probability ratio test using this boundary has size 0.10 and power 0.90.

Stop if observe	11	GvHDs at any time during the study

15.1 SUBJECT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. The PPR fax numbers are (646) 735-0008 and (646) 735-0003. Registrations can be phoned in or faxed. The completed signature page of the written consent/verbal script and a completed Eligibility Checklist must be faxed to PPR.

16.1 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA and principal investigator include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

16.2 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.3 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at:
http://mskweb2.mskcc.org/irb/index.htm

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for it's level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation

17.1 PROTECTION OF HUMAN SUBJECTS

17.2 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board.

17.3 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org containing the following information:

Fields populated from the CRDB:

- Subject's name
- Medical record number
- Disease/histology (if applicable)
- Protocol number

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following information:
  - A explanation of how the AE was handled
  - A description of the subject's condition
  - Indication if the subject remains on the study
  - If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.

17.2.1 Definition of SAE

An SAE is an undesirable experience that meets one of the following criteria:

- Is fatal or life-threatening
- Is disabling
- Results in hospitalization or prolongation of hospitalization
- Results in a congenital anomaly or occurrence of malignancy
- Important medical event that jeopardizes the participant AND requires medical or surgical intervention to prevent one of the outcomes above *Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.*

Attribution:

- Unrelated: The AE *is clearly NOT related* to the intervention
- Unlikely: The AE *is doubtfully related* to the intervention
- Possible: The AE *may be related* to the intervention
- Probable: The AE *is likely related* to the intervention
- Definite: The AE *is clearly related* to the intervention

Expected and Unexpected Event:

- Expected: Any experience *previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure or general investigational plan
- Unexpected: Any experience *not previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure or general investigational plan

UNEXPECTED EVENT:

- Grades 1-2: Adverse Event Reporting NOT required.
- Grades 3: Possible, Probable or Definite attribution to the drug and/or device.
- Grades 4 and 5: Regardless of Attribution. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution.

EXPECTED EVENT

- Grades 1 – 3: Adverse Event Reporting NOT required.
- Grades 4 and 5: Regardless of Attribution. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution.

*Reportable events are those which occur within 30 days from the last day of treatment on protocol. Events beyond 30 days will be reported at the discretion of the PI.

18.1 INFORMED CONSENT PROCEDURES

The individual listed as consenting professionals have completed the mandatory Human Subjects Education and Certification Program. The consulting BMT attending will obtain consent from the patient or the patient's guardian. The patient will sign three copies of the consent. One copy will be given to the patient to keep, one will be scanned into the patient's medical record and one copy will be stored in the patient's research file.

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form

meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.
Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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APPENDICES

A. Karnofsky and Lansky Performance Scales
B. Clinical staging and Grading of Acute Graft versus Host Disease
C. International Bone Marrow Transplant Registry Severity Scoring of Chronic GVHD

APPENDIX A

KARNOFSKY SCALE (≥16 y.o.)

The score is defined by the phrase which best describes the activity status of the recipient.

. Able to carry on normal activity; no special care is needed.

100 Normal; no complaints; no evidence of disease
90 Able to carry on normal activity
80 Normal activity with effort

. Unable to work; able to live at home, care for most personal needs; a varying amount of assistance is needed.

70 Cares for self; unable to carry on normal activity or to do active work
60 Requires occasional assistance but is able to care for most needs
50 Requires considerable assistance and frequent medical care

. Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.

40 Disabled; requires special care and assistance
30 Severely disabled; hospitalization indicated, although death not imminent
20 Very sick; hospitalization necessary
10 Moribund; fatal process progressing rapidly.

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LANSKY SCALE (<16 y.o.)

The score is defined by the phrase which best describes the activity status of the recipient.

. Able to carry on normal activity; no special care is needed.

100 Fully active
90 Minor restriction in physically strenuous play
80 Restricted in strenuous play, tires more easily, otherwise active

. Mild to moderate restriction

70 Both greater restrictions of, and less time spent in, active play
60 Ambulatory up to 50% of time, limited active play with assistance/supervision
50 Considerable assistance required for any active play; fully able to engage in quiet play

. Moderate to severe restriction

40 Able to initiate quiet activities.
30 Needs considerable assistance for quiet activity
20 Limited to very passive activity initiated by others (e.g. TV)
10 Completely disabled; not even passive play

APPENDIX B

CLINICAL STAGING AND GRADING OF ACUTE GRAFT VERSUS HOST DISEASE

ORGAN INVOLVEMENT

STAGE	Skin	Liver	Gut
1	**maculopapular rash <25% body surface**	**Bili 2.0 – 3.0 mg/dl**	***Diarrhea*** **500-1000 ml/d (children: 280-555 ml/m^2/d) OR *persistent nausea***
2	**maculopapular rash 25-50 % of body surface**	**Bili 3.1 – 6.0 mg/dl**	***Diarrhea*** **>1000 but ≤ 1500 ml/d (children: 556-833 ml/m^2/d)**
3	**maculopapular rash >50 % of body surface**	**Bili 6.1 - 15 mg/dl**	***Diarrhea*** **>1500 ml/d (children: >834 ml/m^2/d)**
4	**generalized erythroderma with bullous formation**	**Bili > 15 mg/dl**	**Severe abdominal pain ± Ileus**

GRADING

GRADE	Skin	Liver	Gut
0	**NONE**	None **AND**	**NONE**
I	Stage 1-2 **AND**	None **AND**	**NONE**
II	Stage 3 **AND/OR**	Stage 1 **AND/OR**	**Stage 1**
III	None **OR** Stage 3 **AND**	Stage 2-3 **OR**	**Stage 2-4**
IV	Stage 4 **OR**	**Stage 4**	**NA**

STAGING

- For skin GvHD: - Use "Rule of Nines or burn chart to determine extent of rash
- For liver GvHD: - Range of bilirubin given as total bilirubin.
- Downgrade one stage if an additional cause of hyperbilirubinemia is documented
- For gut GvHD: - Downgrade one stage if an additional cause of diarrhea is documented
- St 1: Persistent nausea, vomiting and anorexia in the absence of other known cause
Unless histology is negative

GRADING**- Criteria for grading given as minimum degree of organ involvement required to confer that grade**

Appendix C

International Bone Marrow Transplant Registry (IBMTR) Severity Scoring of Chronic GVHD

KPS at diagnosis of cGVHD	Mild (low risk)	Moderate (intermediate risk)	Severe (high risk)
≥80	No weight loss No diarrhea	Either weight loss or diarrhea	Both weight loss and diarrhea
< 80	No weight loss, no skin involvement (or) one or two of above with oral involvement	Diarrhea, weight loss and or skin involvement (one or two of above)	Weight loss, diarrhea and skin involvment (or) one or two of the above with oral involvement