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A Pilot Trial of Clofarabine Added to Standard Busulfan and Fludarabine for Conditioning Prior to Allogeneic Hematopoietic Cell Transplantation

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1.0 Hypothesis and Specific Aims:

1.1 Hypothesis: The addition of Clofarabine to standard Busulfan and Fludarabine prior to allogeneic hematopoietic cell transplant (HCT) will be safe and will improve engraftment rates of children with non-malignant diseases or decrease relapse rates of patients with high-risk myeloid malignancies.

1.2 Specific Aims:

1.2.1 Primary Objective: To determine the toxicity of the addition of Clofarabine to a conditioning backbone of Busulfan plus Fludarabine in patients with myeloid malignancies and non-malignant diseases undergoing allogeneic HCT.

1.2.2 Secondary Objectives:

a. To determine if the addition of Clofarabine to a conditioning backbone of Busulfan plus Fludarabine improves the engraftment rate of patients with non-malignant diseases (Stratum A) undergoing allogeneic HCT as compared to historic controls.

b. To determine if the addition of Clofarabine to a conditioning backbone of Busulfan plus Fludarabine improves the full donor chimerism of patients with high-risk myeloid malignancies (Stratum B) undergoing allogeneic HCT as compared to historic controls.

c. To evaluate the pharmacokinetics (PK) of Clofarabine and Fludarabine in a combination nucleoside analogue preparative regimen and define exposure-response relationships with clinical outcomes.

2.0 Background:

2.1 Clinical Need for an Improved Conditioning Regimen:

Although outcomes following allogeneic hematopoietic cell transplantation (HCT) have improved during recent years due to advances in supportive care techniques, certain categories of patients continue to do poorly. One major group of patients with poor outcomes are those with non-malignant diseases for whom an increased immunologic barriers to engraftment exists. This includes both intrinsic barriers to engraftment, such as patients with Hurler's Syndrome, or patients who are allo-immunized pre-HCT due to administration of large numbers of blood products, as well as those patients for whom we have been unable to identify a 10/10 HLA-matched donor. Because most patients with non-malignant diseases (excluding patients with SCID) have at least partially-intact immune systems and, unlike most patients with hematologic cancers, have not been treated with immunosuppressive chemotherapy prior to HCT, the risk of graft rejection in these patients can be very high.

The classic conditioning regimen for a patient with a non-malignant disease is the combination of targeted Busulfan with Cyclophosphamide. However, while immunoablative, the addition of Cyclophosphamide to Busulfan is associated with high rates of organ toxicity, especially to the liver.[1] In order to eliminate the toxicities of Cyclophosphamide, the UCSF Pediatric BMT team has conducted two trials of Busulfan plus Fludarabine for pre-HCT conditioning. The first trial ran from September 2000 to June 2004 and evaluated Busulfan plus Fludarabine plus Thymoglobulin (rATG) in 19 patients.[2] While certainly less toxic than Busulfan plus Cyclophosphamide, a finding that has been confirmed by other trials[3], this regimen was not sufficiently immunoablative to reliably establish engraftment in patients with mismatched donors. Therefore, from 2005

to 2010 a follow-up trial was performed in 35 patients that substituted rATG with Alemtuzumab, a monoclonal antibody directed against CD52, a protein found on the surface of most lymphocytes, with the hypothesis that it would be more effective at ablating the host immune system prior to HCT and would thus be associated with improved engraftment rates.[4] Although Alemtuzumab was found to be superior to rATG at preventing chronic GVHD, and thus its use has been adopted by the UCSF BMT team as standard of care for the conditioning of patients with non-malignant diseases, it too failed to reliably establish engraftment in patients with mismatched donors.

Although some patients that experience primary graft rejection are able to be salvaged with an emergency rescue second transplant,[5] this results in a significantly increased use of patient resources, prolonged hospitalizations, and is not always successful. Due to other advances in supportive care, graft rejection is becoming the major cause of death in certain subgroups of patients with non-malignant diseases undergoing allogeneic HCT. In order to make further advances in the care of these patients, a novel conditioning regimen will need to be employed.

Another group of patients with less than optimal outcomes following allogeneic HCT are those with certain types of myeloid malignancies. One group that appears to be at high-risk for relapse is patients with AML and detectable minimal residual disease at the time of initiation of HCT conditioning.[6,7] Presumably these patients begin HCT with a large enough disease burden that the graft-versus-leukemia (GVL) effect does not have sufficient time to develop and thereby control the underlying AML. Similarly, some myeloid malignancy variants, including MDS, JMML, and CML often come to HCT having received little to no pre-HCT chemotherapy. Therefore, not only are these patients potentially at increased risk for graft rejection when a mismatched donor is utilized,[8] but with their large disease burden they also may need an improved conditioning regimen in order to allow sufficient time for the GVL effect to develop. The UCSF Pediatric BMT team has published that relapses following HCT can be predicted by the degree of donor cell engraftment.[9] Patients with full-donor chimerism have only a 11% risk of relapse, as compared to a 71% in patients with mixed-donor chimerism.[9] UCSF Protocol CC# 09082: “A phase II study of preemptive fast withdrawal of immunosuppression and donor lymphocyte infusions for achieving complete donor chimerism following allogeneic transplant for pediatric hematologic malignancies” is currently testing whether fast withdrawal of immunosuppression plus possible donor lymphocyte infusions, can eliminate residual host cells and thereby convert patients to full-donor chimerism. However, by amplifying the early GVL effect, this approach also has a risk of increased rates of GVHD. Thus, a pharmacologic modification of the conditioning regimen that produces a greater degree of full-donor chimerism may be associated with lower rates of GVHD. Furthermore, since relapse following HCT can occur as late as 2-years out, the degree of full-donor chimerism at Day 100 post-HCT is a faster endpoint to utilize for a clinical trial when trying to determine whether the novel therapy is making a significant impact on outcomes.

Finally, although dramatically different disease types, patients with both myeloid malignancies as well as non-malignant diseases receive Busulfan plus Fludarabine as the standard-of-care conditioning regimen at UCSF. Therefore, the toxicity of a common addition to this regimen that potentially reduces both graft rejection and relapse rates could be studied in a single trial with two different strata.

2.2 Clofarabine as an Effective Agent Against Lymphocytes and Myeloid Cells:

Clofarabine (2-chloro-2-fluoro-deoxy-9-D-arabinofuranosyladenine) is a rationally designed second-generation purine nucleoside analog FDA-approved for the treatment of pediatric patients 1 to 21 years old with relapsed or refractory acute lymphoblastic leukemia at a dose of 52 mg/m2/day for 5 consecutive days. It was designed to retain the antitumor properties of Fludarabine and Cladribine but to have an improved safety profile. It exerts its anticancer activity through the intracellular generation of triphosphate species, which inhibits DNA synthesis and ribonucloeotide reductase, thereby inducing apoptosis. The double halogen strategy confers resistance to adenosine deaminase, while increasing acid stability and bioavailability (in part through more rapid membrane transport). Clofarabine is more efficient than fludarabine at inhibiting DNA polymerase A, inhibiting ribonucleotide reductase (RR), and better at disrupting mitochondrial function leading to apoptosis via recruitment of APAF-1 (biology reviewed in Kirschbaum *et al.*[10]). This latter activity may explain the increased killing of non-dividing lymphocytes,[11,12] an advantage that would theoretically reduce the likelihood of rejection in the allogeneic setting, and perhaps augment the graft-versus-tumor effect of the donor graft.

Clofarabine appears to have reduced CNS penetration compared to older purine nucleoside analogs (reviewed in Long-Boyle *et al.*[13]). While this property may decrease its effectiveness against CNS leukemia, when being combined with two agents with significant CNS penetration and potential toxicity, this “downside” is likely to be minimal. However, from a toxicity standpoint for patients without CNS leukemia or with non-malignant diseases, an agent like Clofarabine, with minimal CNS penetration is clearly optimal.

Furthermore, Andersson *et al.* investigated the in vitro anti-AML cytotoxic properties of Clofarabine alone and in combination with Fludarabine and Busulfan in a human cell-line model of Busulfan-resistant AML.[14] They demonstrated that in this model, as well as in additional human AML lines and primary explanted AML blasts from leukemic patients, on a molar basis, Clofarabine is about 50-fold more potent than Fludarabine, and Clofarabine synergizes to a higher degree than Fludarabine with Busulfan. A combination of Clofarabine and Fludarabine exerted a much higher level of synergy than either nucleoside analog alone when combined with the alkylating agent. When Fludarabine and Clofarabine were followed by Busulfan, a major enhancement of the anti-leukemic efficacy was seen.[15] Furthermore, via three *in vitro* models of apoptosis in AML cell lines, the combination of Busulfan plus Fludarabine plus Clofarabine was synergistic compared to any agent alone or two agents in combination.[16]

Therefore, the use of Clofarabine in combination with standard fludarabine in the conditioning regimen prior to allogeneic HCT may provide enhanced immunosuppression as well as probable increased anti-leukemia effect.

2.2.3 Alternate Agents to Combine with Busulfan and Fludarabine

Clofarabine is not the only possible agent to combine with Busulfan and Fludarabine in order to improve engraftment rates and relapse incidences following allogeneic HCT.

Melphalan is an alkylating agent with known in vitro activity against AML. It does not cross the blood-brain barrier and thus does not have additive CNS toxicities to

Busulfan and Fludarabine. However, its addition to HCT-conditioning regimens has been associated with increased toxicities. Helenglass *et al.* reported that a combination of TBI plus Melphalan was superior to TBI plus Cyclophosphamide in preventing relapse of AML post-HCT, however, the higher rates of TRM resulted in an identical OS rate.[17] Furthermore, although Melphalan does appear to possess immunosuppressive properties in addition to its myelosuppressive effects, in patients with JMML receiving HCT from mismatched donors, the rates of graft rejection following a Busulfan plus Fludarabine plus Melphalan conditioning regimen were still very high.[8]

Thiotepa is an alkylating agent with known in vitro efficacy against both solid tumors as well as hematologic malignancies, and it also caused severe myelosuppression in phase I studies (reviewed in Rosales *et al*.[18]). It does cross the blood-brain barrier, and it can be combined with standard HCT conditioning agents, including Busulfan or Total Body Irradiation. However, one pilot trial has concluded that the addition of Thiotepa to Busulfan and Cyclophosphamide did not improve outcomes compared to Busulfan and Cyclophosphamide alone.[18] Furthermore, Thiotepa has recently had significant supply issues that have limited its availability and use.

Both Melphalan and Thiotepa are alkylating agents, which in combination with Busulfan are known to increase the risk of sinusoidal obstruction syndrome post-HCT. Alkylators may contribute to increased risk of late effects of HCT, including endocrinologic damage and infertility. Therefore, based on the relatively limited benefit in terms of efficacy and the potential toxicity risks, neither Melphalan nor Thiotepa appear to be an optimal agent for combining with Busulfan and Fludarabine. Instead, preliminary clinical studies suggest that the purine nucleoside analogue Clofarabine may be the best available agent.

2.3 Preliminary Studies:

2.3.1 Efficacy Studies:

There have been several published trials of adding Clofarabine to pre-HCT conditioning regimens. Andersson *et al.* performed a 4-arm clinical trial in which escalating doses of Clofarabine were combined with de-escalating doses of Fludarabine, plus standard Busulfan in 51 primarily adult (though one patient was as young as 6 years old) patients with high-risk myeloid malignancies.[16] The dose of Clofarabine utilized was 10 to 40 mg/m^2/day for 4 consecutive days. All 51 patients engrafted and there was no significant difference between the four arms in terms of overall or progression-free survival. A direct comparison to their historical cohort that received only Busulfan and Fludarabine was not performed, however, some conclusions can be tentatively reached. For patients receiving BU-FLU-CLO, the overall survival (OS) was 48% in a population where 80% of patients had active disease at the time of transplant, compared to an OS of 42% for patients receiving BU-FLU only, where the population was slightly lower risk (71% with active disease at the time of transplant). Therefore, BU-FLU-CLO appears at least equal to a historical BU-FLU only cohort, with possibly a hint towards superiority.

Farag *et al.* performed a Phase I dose-escalation trial of Clofarabine in combination with Busulfan prior to allogeneic HCT in 15 adult patients with high-risk acute leukemias.[19] Clofarabine dosing started at 30 mg/m^2/dose, escalating to 60 mg/m^2/day, for 5 consecutive days. All patients engrafted and the maximally tolerated

dose (MTD) was not reached. The 1-year OS and event-free survival (EFS) were 60% and 53% in a high-risk population with an expected EFS of 25% or lower.

Kirschbaum *et al.* performed a Phase I dose-escalation trial of Clofarabine in combination with Melphalan prior to allogeneic HCT in 16 adult patients with AML.[10] Clofarabine dosing started as 30 mg/m^2/dose, escalating to 40 mg/m^2/dose, for 5 consecutive days. All patients engrafted. The 1-year OS and EFS were 73% and 61%.

Srivastava *et al.* performed a Phase I 3+3 dose-escalation trial of Clofarabine in combination with high-dose Cyclophosphamide and Etoposide prior to autologous HCT in 16 adult patients with high-risk Non-Hodgkin Lymphoma.[20] Clofarabine dosing started at 30 mg/m^2/dose, escalating to 70 mg/m^2/day, for 5 consecutive days. The MTD was not reached. The 1-year OS and PFS were 68% and 63%, in a high-risk patient population with an expected PFS of <20%.

Therefore, all four trials demonstrated, in a non-randomized fashion, at least equivalence and potentially superiority compared to non-Clofarabine based conditioning regimens for patients with malignancies.

There is considerably less in the literature regarding the use of Clofarabine for the conditioning of patients with non-malignant diseases undergoing allogeneic HCT. However, Long-Boyle *et al.* did report the pharmacokinetic data of 16 primarily pediatric patients (as young as 0.5 years of age) who received Clofarabine 40 mg/m^2/day for 5 consecutive days in combination with Melphalan and low-dose TBI as part of a brain-sparing conditioning regimen for patients with metabolic diseases.[13] The outcome data was not presented.

2.3.2 Safety Studies:

In general, the addition of Clofarabine to standard pre-HCT conditioning agents has been very well-tolerated. Andersson *et al.* demonstrated in 51 patients that the addition of Clofarabine to BU + FLU did not significantly alter the safety profile compared to their historic controls.[16] TRM rates were 15% at Day +365, compared to 12% in the historic BU-FLU cohort, and toxic deaths were solely limited to being caused by infections and GVHD, with no deaths due to direct organ toxicity. The most common toxicity was grade 2-3 mucositis in 80% of patients. There was only one case of reversible sinusoidal obstruction syndrome. Rates of grades II-IV acute GVHD were similar to historic controls: 31% for BU-FLU-CLO vs. 26% for BU-FLU only.

Farag *et al.* found that the major toxicity was a reversible Grade 3-4 elevation of liver transaminases occurring shortly after Clofarabine administration and resolving by Day +3 post-HCT in 67% of patients.[19] Grade 3-4 mucositis was also common (60%), and there was one case of hand-foot syndrome. Although two patients died of sepsis or cGVHD, there were no deaths due to direct organ toxicity.

Kirschbaum *et al.* found that 1 of 15 patients at the lower-dose Melphalan level expired from TRM (multi-organ failure).[10] Grade 1-2 liver toxicity was seen in all patients, though it fully resolved in all cases except the one that died of TRM.

Srivastava *et al.* found that most elevations of liver transaminases were Grade 1-2 in nature, and all resolved by Day +8 post-HCT.[20] There was a 31% incidence of hand-foot syndrome. All toxicities were reported to have resolved by Day +30 and there were no treatment-related deaths.

Therefore, all four trials demonstrated, in a non-randomized fashion, similar safety outcomes compared to non-Clofarabine based conditioning regimens for patients

with malignancies. TRM rates were not high, despite doses of Clofarabine that occasionally exceeded the FDA-approved dose, and most organ toxicities resolved fairly promptly. From the literature, the major toxicities that will need monitoring will be hepatic in nature.

2.2.3 Clofarabine Pharmacokinetics

Recently, Long-Boyle et al. investigated the PK of Clofarabine in 16 pediatric patients undergoing HCT for the treatment of high risk inherited metabolic disorders.[13] All patients received a single nucleoside analogue preparative regimen consisted of Alemtuzumab, Clofarabine, Melphalan, and TBI. Parameter estimates for $AUC_{0-\infty}$ and C_{max} were consistent with the dose-proportional PK of Clofarabine characterized in phase I and phase II trials of pediatric patients receiving single agent monotherapy doses of 11.25-52 mg/m^2 daily over 5 days for treatment of refractory hematologic malignancies.[21] There was no evidence of accumulation of Clofarabine between the 1st and 5th dose in the patients with stable renal function.[13] This is similar to what has been reported when fludarabine is given daily at doses ranging from 30 to 40 mg/m^2 in adult HCT patients with adequate renal function.[22] Variability of 2-3 fold was observed in Clofarabine PK parameters including CL, $AUC_{0-\infty}$, and V_d and was not sufficiently explained by clinical covariates of body size and renal function.[13] Approximately 40-60% of Clofarabine is renally-eliminated unchanged in the kidneys through a combination of glomerular filtration and active tubular secretion via transporters.[23] Several transporters (ENT1, ENT2, CNT2 and CNT3) have been shown to influence *in vitro* distribution and accumulation of Clofarabine.[24,25] These transporters are expressed in both target tissues (e.g. B and T lymphocytes) and renal epithelial cells of the kidney and concomitant use with other nucleoside analogues such as fludarabine which share a similar metabolic pathway may impact PK and drug disposition through several mechanisms. To date, the impact the relationship between Clofarabine exposure and clinical outcomes in HCT remain undefined.

2.3.4 Choice of Clofarabine Dosing:

Although the FDA-approved dosing in children with ALL is 52 mg/m^2/dose for 5 consecutive days, this is as monotherapy. As noted above, several trials in adults utilized doses as high as 40 to 70 mg/m^2/day for 4 to 5 consecutive days with tolerable toxicity rates.[10,16,19] However, in order to not alter our backbone standard of care conditioning regimen of Busulfan plus Fludarabine, we will simply add 10 mg/m^2/dose of Clofarabine for 4 consecutive days. Therefore, we will only be altering one component of the conditioning regimen, which will improve our ability to compare the results of this trial with our historic controls. Furthermore, there was no apparent benefit to Clofarabine dose escalation (with simultaneous de-escalation of Fludarabine dosing) in the trial by Andersson *et al*.[16] so we chose to utilize the smallest published dose in order to maximize the safety of patients enrolling on this trial.

3.0 Eligibility:

3.1 Inclusion Criteria:

a. Patients must be ≥ 3 months and ≤30 years of age.

b. Stratum A: Non-Malignant Diseases, including:

i. Bone Marrow Failure Syndromes
ii. Hemoglobinopathies or transfusion-dependent RBC defects
iii. Congenital Immunodeficiencies
iv. Metabolic Diseases known to be treatable with HCT (e.g. Hurler's)
v. Other Bone Marrow Stem Cell Defects (e.g. Osteopetrosis)
vi. Severe Immune Dysregulation / Autoimmune Syndromes with at least transient prior response to immunosuppressive therapy

c. Stratum B: Myeloid Malignancies, including:

i. AML, in greater than first clinical remission, or in CR1 but with either (see Table 2):
   a. Detectable disease (≥0.1% Blasts by MRD or Flow, or Positive Cytogenetics), OR
   b. A matched sibling UCB donor , OR
   c. A mismatched unrelated donor.
ii. MDS
iii. JMML
iv. CML, with detectable disease by PCR

d. Patients must have a suitable donor based on the UCSF Pediatric BMT SOP. 10/10 (HLA-A, -B, -C, -DR, -DQ) matching will be done for related and adult unrelated donors; 8/8 (HLA-A, -B, -C, -DR) for umbilical cord blood donors. Patients with non-malignant diseases will generally be eligible only if they have a mismatched donor, or an accepted clinical reason to be considered high-risk for rejection (see Table 1 for details).

e. Liver transaminases (AST/ALT) and Direct Bilirubin less than twice the upper limit of normal within 2 weeks of admission.

f. Cardiac Shortening Fraction ≥27% within 4 weeks of admission.

g. Creatinine clearance by Schwartz formula, GFR or 24 hr urine collection ≥50 cc/min/1.73 m^2, within 4 weeks of admission.

h. Pulmonary diffusion capacity ≥50% of predicted corrected for anemia/lung volume within 4 weeks of admission. If unable to do PFT's, then no active lung disease by CXR and/or O2 Sat ≥90% on room air.

Table 1: Non-Malignant Diseases (Stratum A)

Stem Cell Source	Matched RD	10/10 (8/8) URD	< 10/10 (8/8) URD*
BM	BU600 + Fludarabine Alemtuzumab	BU600 + Fludarabine Alemtuzumab	**BU600 + Fludarabine Alemtuzumab Clofarabine**
PBSC	BU600 + Fludarabine Alemtuzumab	BU600 + Fludarabine Alemtuzumab	**BU600 + Fludarabine Alemtuzumab Clofarabine**
UCB	BU600 + Fludarabine rATG	BU600 + Fludarabine rATG	**BU600 + Fludarabine rATG Clofarabine**

* = Or Otherwise Determined to be at High Risk for Rejection Based on Clinical Criteria (i.e. Hurler's Syndrome, Osteopetrosis, etc.)
Shaded area = Low-risk patients not eligible for this trial, will receive a standard-of-care HCT.

Table 2: Myeloid Malignancies (Stratum B)

Stem Cell Source	AML CR1, MRD Negative		
	Matched RD	**10/10 (8/8) URD**	**< 10/10 (8/8) URD**
BM	BU900 + Fludarabine	BU900 + Fludarabine rATG	BU900 + Fludarabine rATG **Clofarabine**
PBSC	BU900 + Fludarabine rATG	BU900 + Fludarabine rATG	BU900 + Fludarabine rATG **Clofarabine**
UCB	**BU900 + Fludarabine Clofarabine**	BU900 + Fludarabine rATG	BU900 + Fludarabine rATG **Clofarabine**

Stem Cell Source	AML CR2 or MRD Positive; MDS; JMML; CML		
	Matched RD	**10/10 (8/8) URD**	**< 10/10 (8/8) URD**
BM	**BU900 + Fludarabine Clofarabine**	**BU900 + Fludarabine rATG Clofarabine**	**BU900 + Fludarabine rATG Clofarabine**
PBSC	**BU900 + Fludarabine rATG Clofarabine**	**BU900 + Fludarabine rATG Clofarabine**	**BU900 + Fludarabine rATG Clofarabine**
UCB	**BU900 + Fludarabine Clofarabine**	**BU900 + Fludarabine rATG Clofarabine**	**BU900 + Fludarabine rATG Clofarabine**

Shaded area = Low-risk patients not eligible for this trial, who will receive a standard-of-care HCT.

3.2 Exclusion Criteria:

1. Fanconi Anemia
2. Dyskeratosis Congenita
3. A known syndrome with increased sensitivity to radiation or alkylating agents
4. Severe Combined Immunodeficiency Disease eligible for a non-myeloablative HCT trial
5. A mismatched donor for whom *ex vivo* T-cell depletion of the donor stem cells is planned

4.0 Procedures:

4.1 Study Procedures/Treatments:

4.1.1 Stratum A (Non-Malignant / Stem Cell Defects)

Day	**Medication**	**Dose for ≥12 kg**	**Dose for <12 kg**
Day -13	Alemtuzumab	Test Dose (1 mg)*, then 0.5 mg/kg (max 15 mg)	Test Dose (1 mg)*, then 0.5 mg/kg (max 6 mg)
Day -12	Alemtuzumab	0.5 mg/kg (max 15 mg)	0.5 mg/kg (max 6 mg)
Day -11	Alemtuzumab	0.5 mg/kg (max 15 mg)	0.5 mg/kg (max 6 mg)
Day -10	Busulfan	TBD by Wt-based formula 1st dose at 9 pm	TBD by Wt-based formula 1st dose at 9 pm
Day -9	Busulfan	TBD by PK (goal CSS = 600)	TBD by PK (goal CSS = 600)
Day -8	Busulfan	TBD by PK	TBD by PK
Day -7	Busulfan	TBD by PK	TBD by PK
Day -6	Busulfan	TBD by PK: Last dose at 3 pm (16 doses total)	TBD by PK: Last dose at 3 pm (16 doses total)
Day -5	Fludarabine Clofarabine	40 mg/m^2 10 mg/m^2	1.33 mg/kg 0.33 mg/kg
Day -4	Fludarabine Clofarabine	40 mg/m^2 10 mg/m^2	1.33 mg/kg 0.33 mg/kg
Day -3	Fludarabine Clofarabine	40 mg/m^2 10 mg/m^2	1.33 mg/kg 0.33 mg/kg
Day -2	Fludarabine Clofarabine	40 mg/m^2 10 mg/m^2	1.33 mg/kg 0.33 mg/kg
Day -1	Rest		
Day 0	Transplant	To begin ≥48 hours from last dose of Flu and Clo	To begin ≥48 hours from last dose of Flu and Clo

* The test dose of Alemtuzumab (1 mg) will be given over 2 hours on the 1st day of conditioning. Four hours after the test dose, the first full dose of Alemtuzumab is given.

4.1.2 Stratum B (Myeloid Malignancies)#

Day	Medication	Dose for ≥12 kg	Dose for <12 kg
Day -10	Busulfan	TBD by Wt-based formula 1st dose at 9 pm	TBD by Wt-based formula 1st dose at 9 pm
Day -9	Busulfan	TBD by PK (goal CSS = 900)	TBD by PK (goal CSS = 900)
Day -8	Busulfan	TBD by PK	TBD by PK
Day -7	Busulfan	TBD by PK	TBD by PK
Day -6	Busulfan	TBD by PK: Last dose at 3 pm (16 doses total)	TBD by PK: Last dose at 3 pm (16 doses total)
Day -5	Fludarabine Clofarabine	40 mg/m^2 10 mg/m^2	1.33 mg/kg 0.33 mg/kg
Day -4	Fludarabine Clofarabine rATG^	40 mg/m^2 10 mg/m^2 0.5 mg/kg	1.33 mg/kg 0.33 mg/kg 0.5 mg/kg
Day -3	Fludarabine Clofarabine rATG^	40 mg/m^2 10 mg/m^2 2.5 mg/kg (1 mg/kg if UCB)	1.33 mg/kg 0.33 mg/kg 2.5 mg/kg (1 mg/kg if UCB)
Day -2	Fludarabine Clofarabine rATG^	40 mg/m^2 10 mg/m^2 2.5 mg/kg (1 mg/kg if UCB)	1.33 mg/kg 0.33 mg/kg 2.5 mg/kg (1 mg/kg if UCB)
Day -1	rATG^	2.5 mg/kg (1 mg/kg if UCB)	2.5 mg/kg (1 mg/kg if UCB)
Day 0	Transplant	To begin ≥48 hours from last dose of Flu and Clo	To begin ≥48 hours from last dose of Flu and Clo

Because of limited data utilizing Alemtuzumab with Umbilical Cord Blood (UCB) Donors, this regimen will also be utilized for Stratum A Patients with UCB Donors (See Table 1), EXCEPT that their Busulfan goal CSS will still be 600.
^ Because of decreased GVL effect from matched related donors when using Bone Marrow or Umbilical Cord Blood as the stem cell source, only patients receiving Peripheral Blood Stem Cells from a matched related donor will receive rATG (See Table 1).
UCB recipients will receive “low dose” rATG (total dose of 3.5 mg/kg) based on recent data suggesting that “standard dose” rATG is associated with poor immunologic recovery.

4.1.3 Supportive Care Practices:

a. All USCF Pediatric BMT Supportive Care Guidelines will be followed

b. Premedications for Serotherapy (rATG and Alemtuzumab) will include: Acetaminophen (10 mg/kg), Diphenhydramine (1 mg/kg), and Dexamethasone (0.2 mg/kg).

c. Appropriate Seizure prophylaxis will be administered prior to 1st dose of Busulfan and for at least 12 hours after the final dose.

d. Fludarabine should be infused over 1 hour (9 am to 10 am) and Clofarabine should be infused over 2 hours immediately afterwards (10 am to 12 noon).

e. To prevent hand-foot syndrome with Clofarabine, hydrocortisone (100 mg/m^2; 100 mg max dose) will be administered 1-2 hours prior to each dose unless Dexamethasone has been given within the last 24 hours.
f. Ursodiol (6-7.5 mg/kg/dose twice daily) will be administered to all patients preferably 2 weeks prior to admission and no later than the day of admission. Ursodiol will continue until at least Day +30 or Day of Discharge, whichever is sooner, except for intolerance or side effects.
g. Standard GVHD prophylaxis per the UCSF BMT Guidelines will be administered starting on Day -1. If a patient is unable to receive methotrexate on Day +1 due to liver enzyme elevations, mycophenolate mofitil should be substituted until the liver enzyme elevation has resolved and methotrexate can be re-initiated.
h. Standard G-CSF administration will begin on Day +14, if needed.

4.2 Study Tests:

4.2. 1 Standard of Care Testing:

Most tests performed on this trial will be done as standard of care for any patient undergoing allogeneic HCT. This will include:

a. A minimum of twice weekly monitoring of CBC, chemistries, renal function, and liver enzymes during the first month post-HCT, and at least twice monthly for the first 100 days post-HCT.
b. Engraftment studies by STR analysis of cell subsets beginning on ~Day +30 and then monthly until stable.

4.2.2 Study Tests:

a. To monitor for liver toxicity while receiving Clofarabine, daily liver enzymes (AST, ALT, Total and Direct Bilirubin, Alk Phos, and GGT) will be obtained starting on Day -5 and continuing until at least Day +6, at which point, the UCSF Pediatric BMT standard operating practices resume, which includes monitoring of liver enzymes twice weekly. Because daily tests of electrolytes are standard of care, this does not requires any additional blood to be drawn.
b. Patients will undergo pharmacokinetics (PK) to evaluate both Clofarabine and Fludarabine ("nucleoside analogue") exposure.

i. **Blood volume and weight:** In total, 11 blood samples will be collected over the entire course of nucleoside analogue treatment to evaluate drug exposure and pharmacogenomics. The total amount of blood volume needed for PK sampling is based on the weight of the child. For children weighing < 8 kg a total of 16 ml of blood will be drawn over a 4 day treatment course. For children weighing ≥ than 8 kg, the amount of blood drawn over a 4 days treatment course will be a total of 24 ml.

ii. **DNA collection:** To assess the impact of genetic variants on nucleoside drug exposure a single blood collection for DNA and genotyping will occur on the day of admission prior to the start of chemotherapy. One (1 ml) of whole blood will be drawn into a 3 ml K_2EDTA tube, placed on wet ice, and sent to the UCSF CTSI Core DNA Bank for immediate DNA isolation, quantification, and storage until analysis is performed.

iii. **PK sampling:** To evaluate plasma and intracellular drug concentrations blood samples will be obtained at 6 times over the course of

nucleoside treatment for a total 10 blood samples. Per protocol, Fludarabine will be administered intravenously over 60 minutes, followed by Clofarabine infusion over 2 hours. For convenience, blood collection times are based on the administration time of fludarabine. Plasma and intracellular drug concentrations are processed independently from one another and therefore require two different blood collection tubes at several of the PK sampling times.

iv. **Day 1 of nucleoside analogue therapy:** Following the administration of Fludarabine, 1ml of whole blood will be obtained at **2, 4, 8,** and **24** hours after the start of infusion to evaluate **plasma** concentrations of both Clofarabine and Fludarabine. Blood samples for each plasma PK time point will be collected in a 2 mL spray dried K_2EDTA tube and immediately place on wet ice. Within 60 minutes of collection all samples must centrifuged at 3400 rpm for 10 minutes at 4ºC, the plasma removed, and stored at -70ºC until analysis. PK sampling for the analysis of intracellular fludarabine drug levels will occur concurrently at **2** and **24** hours for correlation of plasma and intracellular drug concentrations. Blood samples for **intracellular** drug quantification will be collected in a 4 mL cell preparation tube with sodium citrate (BD Vacutainer ® CPT Tube) and processed for recovery of peripheral blood mononuclear cells. For children <8 kg only 2ml of blood will be collected for intracellular analysis.

v. **Day 2, 3, or 4 of nucleoside analogue therapy:** To evaluate drug accumulation over the course of treatment and assess inter-occasion variability, PK sampling will be repeated on Day 2, 3 or 4 of nucleoside analogue treatment. Blood samples will be collected at 2 and 24 hours following administration of fludarabine for a total of 4 blood samples. Blood samples for each **plasma** PK time point will be collected in a 2 mL spray dried K_2EDTA tube and processed as previously described. As with Day 1 PK, blood collections for the analysis of intracellular nucleoside analogue drug concentrations will occur concurrently at **2** and **24** hours and processed for peripheral blood mononuclear cells.

vi. This PK sampling is identical to Study 10-00894 (Population Pharmacokinetics of Fludarabine in Pediatric Patients Undergoing Hematopoietic Cell Transplantation; Janel Long-Boyle, PI), thus patients will not need to co-enroll on that trial.

5.0 Statistics & Sample Size:

5.1 Primary Objective:

a. Severe Toxicity will be defined as death or Grade IV (by CTC AE 4.0) pulmonary or hepatic failure (including moderate veno-occlusive disease,aka VOD) related to the transplant conditioning regimen (as defined in Section 8.2) within 100 days post-HCT.

i. VOD will be defined by standard criteria. Patients must have Bilirubin >2.0 plus Hepatomegaly and/or RUQ pain plus Weight gain >5%.

b. The historic control will be the 37 patients detailed below, who have been treated with BU-FLU-Serotherapy based protocols at UCSF from 2000 – 2010 (and who would have fit the eligibility criteria for this trial). Of these 37 patients, 1 experienced TRM, but there were no cases of Grade IV pulmonary or hepatic failure, for a cumulative incidence of severe toxicity of 3% (90% upper confidence bound: 10%).

c. Sample size justification is based on establishing that observed rates of toxicity with the addition of Clofarabine to a backbone of BU-FLU-Serotherapy are not worse than historical control levels. Since formal establishment of noninferiority is not possible given feasible sample sizes, expected toxicity rates are compared to historical rates in terms of the 90% exact binomial upper confidence bound for the observed proportion of events. For our planned sample size of 31, 3 or fewer events would lead to an upper bound within 10% of the corresponding upper bound for historical control levels. We consider a difference of 10% or smaller as evidence of similar levels of toxicity.
d. If there are more than 3 events in the first 15 patients enrolled, the trial will be closed early for failing to achieve its safety goal.

5.2 Secondary Objectives:

5.2.1 Engraftment in patients with non-malignant diseases (Stratum A):

a. Engraftment will be defined as the development of an ANC >500 for 3 consecutive days plus donor CD14/15 cells >70%.
b. The historic control for Stratum A will be as follows:

Patients	**# of High-Risk Patients**	**Engraftment**	**Overall Survival**
Enrolled on BU-FLU-rATG[2]	5	2	4
Enrolled on BU-FLU-C[4]	5	2	2
Treated with BU off study (2005-10)	5	3	5
Summary	**15**	**6**	**11**

c. Therefore, the engraftment rate in this population was only 40%. If the observed engraftment rate of this trial is as large as 83% (10/12), we would need a minimum of 12 patients in Stratum A to show a significant improvement (alpha < 0.05) in engraftment rate compared to historic controls at the 5% level.
d. If 3 patients in Stratum A experience graft rejection, we will close this stratum early for failing to achieve superior engraftment compared to standard-of-care.

5.2.2 Mixed-Chimerism in patients with high-risk myeloid malignancies (Stratum B):

a. Full-donor chimerism will be defined by as ≥99% donor cells by STR analysis in all cell lines (CD3, CD14/15, and CD19) in peripheral blood.
b. The historic control for Stratum B will be the 20 patients who were transplanted from 2005 – 2010 with BU-based regimens and who retrospectively would have been eligible for the current trial. Of these 20 patients, at 100 days post-HCT, only 8 (40%) patients had full-donor chimerism (B. Horn, unpublished data).
c. If the observed cumulative incidence of full-donor chimerism at Day 100 is at least 79%, we will need a minimum of 19 patients in Stratum B to show a

significant improvement (alpha < 0.05) in incidence of full donor chimerism compared to historic controls at the 5% level.

d. If 5 patients in Stratum B experienced mixed-donor chimerism at Day 100, we will close this stratum early for failing to achieve superior donor cell engraftment compared to standard-of-care.

5.2.3 Fludarabine and Clofarabine PK

Fludarabine and clofarabine drug levels and potential covariates influencing drug exposure such as renal function and genetic variants involved in drug metabolism, distribution, and activation will be analyzed using standard population pharmacokinetic methods using non-linear mixed effects modeling (NONMEM) software.

5.3 Sample Size:

a. We will plan to accrue 12 patients to Stratum A and 19 patients to Stratum B, for a total of 31 patients.

b. Feasibility: From 2005 to 2010, we transplanted 15 patients that would have met the criteria for enrollment in Stratum A, and 22 patients that would have met the criteria for Stratum B. Presuming at least 90% participation of future patients, this trial should be able to accrue in approximately 6 years if referrals for transplant stay constant, or faster if they increase.

6.0 Risks, Benefits, and Alternatives:

6.1 Risks: Risks and side effects seen with standard-dose Clofarabine are listed on the following table. Side-effects reported in four trials of Clofarabine use during the conditioning regimen prior to HCT are listed first and are **bolded**.

Likely	Less Likely	Rare But Serious
Nausea and/or vomiting	**Inflammation and/or sores in the mouth that may make swallowing difficult and are painful (painful mouth sores)**	**Inflammation or damage to the liver which can be severe and life-threatening and which may lead to an enlarged liver and spleen, bleeding from the veins in the esophagus (the passage that leads from the throat to the stomach), a yellow appearance to the skin and fluid collection in the abdomen which makes it look larger**
Diarrhea	**Increased levels of a chemical (creatinine) in the blood which could mean kidney damage**	Severe loss of water from the body (dehydration) which if untreated may cause low blood pressure and severe loss of salts such as sodium and potassium from the body and could lead to the kidneys failing which could be life-threatening

Elevation in the blood of certain enzymes found in the liver and bilirubin (a substance that comes from the liver breaking down waste products) which may mean the liver is not working as well as normal	**The skin and the whites of the eyes appears yellow as a result of too much bilirubin (a substance that comes from the liver breaking down waste products) in the blood**	Capillary leak syndrome: a condition in which fluid and proteins leak out of tiny blood vessels and flow into surrounding tissues, resulting in dangerously low blood pressure. Capillary leak syndrome may lead to multiple organ failure such as kidney, heart or liver failure and shock
Fever	**Severe rash with redness and pain on the palms of the hand and soles of the feet (Hand-Foot Syndrome)**	Inflammation of the pancreas (an organ in the abdomen which produces insulin and certain digestive chemicals) which may affect the function of the pancreas and which may cause pain in the abdomen (belly) which can be severe and may increase the blood sugar
Skin rash	**Numbness and tingling in the fingers and toes**	Severe inflammation and damage to the large intestine wall which can be life threatening
A fast heartbeat which may cause pain in the chest	**Rash with redness or red bumpy rash**	Abnormal clotting of the blood that can lead to formation of blood clots and/or bleeding with abnormal findings on neurologic exam
A feeling of extreme tiredness not relieved by sleep	The rapid death of large numbers of tumor cells which can cause the potassium and phosphate salts and the uric acid in the blood to rise quickly and this could lead to a life-threatening irregular heart beat or damage to the kidneys	Severe rashes which can result in loss of skin and damage to mucous membranes and which may be life-threatening (occurred in combination of other drugs known to cause this effect)
A decrease in blood pressure	Fluid build-up in the tissues	Severe kidney damage (which may be permanent)
Pain in the abdomen (belly)	Sleepiness and weakness	Failure of the bone marrow to produce blood cells and platelets which can be life threatening
Fever with a low white blood cell count which could mean that you have an infection and might require hospitalization and treatment with antibiotics	Changes to your emotions such that you feel depressed, anxious, agitated, irritable or confused	A change to the heart such that it does not pump the blood as well which may make you tired, weak, feel short of breath, and retain fluid
Chills	Shaking chills	

Anxiety	High blood pressure	
Loss of appetite	Cough or shortness of breath	
Headache	Reddening of the face with feelings of warmth when the drug is infusing	
Skin rash with inflammation	A fast rate of respiration that may cause pain in the chest	
Itching	Allergic reaction	
Red spots on the skin from low platelets	A change in alertness, concentration, and memory	
Bloody nose	Constipation	
Fewer white blood cells, red blood cells and platelets in the blood (may be prolonged) A low number of white blood cells can make it easier to get infections A low number of red blood cells can make you feel tired and weak A low number of platelets causes you to bruise and bleed more easily	A life-threatening form of severe blood infection that usually results from the presence of bacteria and their toxins in the bloodstream and is characterized especially by persistent hypotension with reduced blood flow to organs and tissues and often organ dysfunction	
Abnormal levels of potassium or magnesium in the body which may require that you take extra potassium by mouth or vein	Tremor (shakiness usually of the hands)	
An increase in an enzyme (called lipase) that helps break down fats in the body	Difficulty sleeping or falling Asleep	
	Fainting	
	Low levels of oxygen in the blood which may make you feel short of breath	
	Pain including back, bone, arm, or leg pain	
	Weight loss	
	Aches and pains in the muscles and joints	
	Bleeding from the bladder, gut, mouth, or gums	
	Vomiting or coughing blood	
	Fluid build-up in the lungs that can make you feel short of breath	
	A life threatening condition in which the level of oxygen in the blood becomes too low or the level of carbon dioxide in the blood becomes too high	
	Damage to the sac around the heart which can lead a build-up of fluid around the heart which may be painful	

	and affect the ability of the heart to work normally but in most cases is only mild and temporary	
	Bruising of the skin from low Platelets	
	Occasional sudden sharp pain in the rectal area	
	Infections including those caused by bacteria, virus, and fungus and can be found in the lung, the blood, the skin and other places in the body	
	Dizziness	
	Abnormal high level of potassium in the blood which may cause irregular heart beat and require drug treatment to lower the level	
	An increase in an enzyme (called amylase) that helps break down starch and sugars in the body	
	A problem in nerve function that may cause pain, numbness, tingling, and muscle weakness in various parts of the body and may affect the ability to perform tasks that require fine movements	

6.2 Benefits:

a. There is a possible benefit to the patient in that the addition of low-dose Clofarabine may decrease the risk of either graft rejection or relapse. If that occurs, then there would likely be a survival benefit to the patient as well.
b. The characterization of Clofarabine PK and knowledge of associations between drug exposure and outcomes will facilitate the development of individualized dosing strategies for use in pediatric HCT. We expect individualized dosing strategies will lead to improved rates of stem cell engraftment and reduced treatment-related mortality and thus will be a potential future benefit to the segment of society in need of receiving these agents.

6.3 Alternatives:

The UCSF standard of care for patients eligible for enrollment on this study would be to receive Busulfan and Fludarabine, without the addition of Clofarabine, for the pre-HCT conditioning regimen.

7.0 Subject Recruitment and Consent Process

7.1 Subject Recruitment

a. Source: Subjects are referred from outside physicians and immunology and hematology clinics and specialists.
b. Initial contact: Usually, patients and parents are initially seen in the pediatric BMT outpatient clinic. Occasionally, the initial contact is as an inpatient.

7.2 Consent Process:

There is an initial consultation evaluation in the BMT outpatient clinic for all prospective patients and their parents at which the basic concepts of transplantation are reviewed including the overall risks and benefits of the procedure, the donor search, and the main aspects of the transplant, i.e., the estimated length of hospital stay, administration of chemotherapy, and the major side effects and complications, both acute and long term. When it is apparent that a donor is available, a second outpatient meeting occurs with the family, and the protocol is discussed in more detail including who the likely donor will be (if known based upon HLA typing). This is generally within 2-3 weeks of the potential admission to the BMT unit. If the parents want to proceed, the evaluations are done on the patient and donor. Once this is complete and at least 3 days prior to admission to the BMT unit, a final informed consent conference is held to review the results of the work-up and to obtain final signed agreement. At that meeting, the attending discusses the donation procedure with the donor (if related).

All participants must be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Participants must sign the IRB-approved informed consent form prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

8.0 Reporting and Documentation of Adverse Events

8.1 Definitions of Adverse Events

8.1.1 Adverse Event

An adverse event (also known as an adverse experience) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. More specifically, an adverse event (can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

8.1.2 Adverse reaction

An adverse reaction is defined as any adverse event caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

8.1.3 Suspected adverse reaction

A suspected adverse reaction is defined as any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, “reasonable possibility” indicates that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

8.1.4 Unexpected

An adverse event or suspected adverse reaction is considered *unexpected* if it is not listed in the investigator brochure or package insert(s), or is not listed at the specificity or severity that has been observed, or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

“Unexpected,” as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse events that would be anticipated to occur as part of the disease process are considered *unexpected* for the purposes of reporting because they would not be listed in the investigator brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the investigator brochure.

Some adverse events are listed in the Investigator Brochure as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered *unexpected* until they have been observed with the drug under investigation. For example, although angioedema is anticipated to occur in some patients exposed to drugs in the ACE inhibitor class and angioedema would be described in the investigator brochure as a class effect, the first case of angioedema observed with the drug under investigation should be considered *unexpected* for reporting purposes.

8.1.5 Serious

An adverse event or suspected adverse reaction is considered *serious* if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function
- Congenital anomaly/birth defect

Important medical events that may not result in death, are life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.1.6 Life-threatening

An adverse event or suspected adverse reaction is considered *life-threatening* if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

8.2 Recording of an Adverse Event

All Grade 3 – 5 adverse events will be entered into OnCore®, whether or not the event is believed to be associated with use of the study drug. Data about these events and their severity will be recorded using the NCI CTCAE v4.0.

The Investigator will assign attribution of the possible association of the event with use of the investigational drug, and this information will be entered into OnCore® using the classification system listed below:

Relationship	Attribution	Description
Unrelated to investigational drug/intervention	Unrelated	The AE *is clearly NOT related* to the intervention
	Unlikely	The AE *is doubtfully related* to the intervention
Related to investigational drug/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

Signs or symptoms reported as adverse events will be graded and recorded by the Investigator according to the CTCAE. When specific adverse events are not listed in the CTCAE they will be graded by the Investigator as *none*, *mild*, *moderate* or *severe* according to the following grades and definitions:

Grade 0	No AE (or within normal limits)
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local, or noninvasive intervention (e.g., packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL)
Grade 3:	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4:	Life-threatening consequences; urgent intervention indicated
Grade 5:	Death related to AE

8.3 Follow-up of Adverse Events

All Grade 3 – 5 adverse events will be followed with appropriate medical management until resolved. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. For selected adverse events for which administration of the investigational drug was stopped, a re-challenge of the subject with the investigational drug may be conducted if considered both safe and ethical by the Investigator.

8.4 Adverse Events Monitoring

All Grade 3 – 5 adverse events, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be entered into OnCore®, as noted above.

The Investigator will assess all adverse events and determine reportability requirements to the UCSF Data and Safety Monitoring Committee (DSMC) and UCSF's Institutional Review Board, the Committee on Human Research (CHR); and, when the study is conducted under an Investigational New Drug Application (IND), to the Food and Drug Administration (FDA) if it meets the FDA reporting criteria.

All adverse events entered into OnCore® will be reviewed by the Helen Diller Family Comprehensive Cancer Center Site Committee on a weekly basis. The Site Committee will review and discuss at each weekly meeting the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s).

In addition, all adverse events and suspected adverse reactions considered "serious," entered into OnCore® will be reviewed and monitored by the Data and Safety Monitoring Committee on an ongoing basis, discussed at DSMC meetings which take place every six (6) weeks, and prior to dose escalation.

8.5 Expedited Reporting

Reporting to the Data and Safety Monitoring Committee

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and it is determined to be related either to the study drug(s) or to a study procedure, the Investigator or his/her designee

must notify the DSMC Chair (or qualified alternate) within 1 business day of knowledge of the event. The contact may be by phone or e-mail.

Reporting to UCSF Committee on Human Research (Institutional Review Board)

The Principal Investigator must report events meeting the UCSF CHR definition of "Unanticipated Problem" (UP) within 10 business days of his/her awareness of the event.

9.0 Data and Safety Monitoring Plan

9.1 The UCSF-HDFCCC Data Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and patient safety for all UCSF-HDFCCC institutional clinical studies. A summary of DSMC activities for this study includes:

- Review of subject data • Review of suspected adverse reactions considered "serious"
- Monitoring every six months (depending on study accrual)
- Minimum of a yearly audit

9.1.2 Monitoring and Reporting Guidelines

Investigators will conduct continuous review of data and patient safety at monthly study group or site committee meetings where the results of each patient's treatment are discussed and the discussion is documented in the minutes. The discussion will include the number of patients, significant toxicities as described in the protocol, doses adjustments, and observed responses.

All institutional Phase 2 studies are designated with a moderate risk assessment. The data is monitored twice per year with twenty percent of the subject's monitored (or at least three subjects if the calculated value is less than three).

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AEs, subject participation data, deviations and clinical data from electronic case report forms (CRFs) will be reviewed and signed off by the Investigator quarterly/monthly.

9.1.3 Adverse Event Review and Monitoring

All grade(s) 3-5 adverse events, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be entered into OnCore®, UCSF's Clinical Trial Management System.
All grade(s) 3-5 adverse events entered into OnCore® will be reviewed on a monthly basis at the Site Committee meetings. The Site Committee will review and discuss the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s).

In addition, all adverse events or suspected adverse reactions considered "serious," entered into OnCore® will be reviewed and monitored by the Data and Safety Monitoring Committee on an ongoing basis and discussed at DSMC meetings, which take place every six (6) weeks.
If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and it is determined to be related either to the study drug(s) or to a study procedure, the Investigator or his/her designee must notify the DSMC Chair within **1 business day** of knowledge of the event. The contact may be by phone or e-mail.

9.1.4 Increase in Adverse Event Rates

If an increase in the frequency of Grade 3 or 4 adverse events (above the rate reported in the Investigator Brochure or package insert) is noted in the study, a report should be submitted to the DSMC at the time the increased rate is identified. The report will indicate if the incidence of AEs observed in the study is within the range stated in the Investigator Brochure or package insert.
If at any time the Investigator stops enrollment or stops the study due to safety issues the DSMC Chair and administrator must be notified within **1 business day** via e-mail. The DSMC must receive a formal letter within **10 business days** and the CHR must be notified.

Data and Safety Monitoring Committee Contacts:

DSMC Chair: [redacted]
Phone [redacted]
Email [redacted]
Box [redacted]

DSMC Monitors
Box 1297
UCSF Helen Diller Family Comprehensive Cancer Center

9.2 Assessment of Risk: This study is deemed to be a pilot phase II clinical trial that present a moderate degree of risk to the study subjects. Because this study will enroll pediatric HCT patients using an agent with limited experience when combined with Busulfan and Fludarabine, safety monitoring will be conducted by the UCSF Data and Safety Monitoring Committee (DSMC). Utilizing the UCSF DSMC will enable the data to be reviewed and thus any serious adverse events can be linked to the pharmacological agent being investigated.

9.2.1 Anticipated Adverse Events: Clofarabine has previously been demonstrated to be safe for human therapy in pediatric cancer patients, further supporting the assignment of a moderate degree of risk to this study. However, like most chemotherapy agents, Clofarabine does have many known side-effects (see Section 6.1). Some "side-effects," such as hematologic toxicity and immunosuppression, are actually desired effects of

Clofarabine on this HCT trial. Some potential side-effects of Clofarabine, such as vomiting or mucositis, will be very difficult to distinguish from known side-effects of the simultaneously administered agents Busulfan and Fludarabine, and thus their incidence will not be tracked. Other potential side-effects of Clofarabine, such as Hand-Foot Syndrome, may be relatively unique to this agent but are not of sufficient severity to dissuade future use of this agent in the HCT setting if it shows potential benefit for prevention of graft rejection or relapse, potentially fatal outcomes. In the primarily adult trials of Clofarabine use as part of a HCT conditioning regimen, the most notable and commonly seen adverse event was the development of an early and reversible hepatic transaminitis. The trial has been designed to specifically monitor for this adverse event, but unless it leads to the development of Grade IV hepatic failure or moderate VOD, this will not be considered a significant event for the safety analysis of the trial. Myeloablative HCT in general, whether done as part of a clinical trial or as a standard-of-care treatment, is a inherently risky procedure, with a known risk of TRM and many non-fatal toxicities, independent of the potential addition of novel agent(s). Given that only patients at high risk of graft rejection or relapse (frequently fatal events) are eligible for this trial, only a significant increase in TRM or very severe (Grade IV hepatic or pulmonary) toxicities would be sufficient cause to suspend further development of Clofarabine to potentially improve overall outcomes following HCT.

9.3. Safety Monitoring Plan: Subjects will remain in the hospital for at least 3 weeks following administration of the study medication Clofarabine. This will allow sufficient time to monitor for the development of severe adverse events potentially attributable to Clofarabine. During this time, as part of routine post-HCT standard of care, patients will have daily physical exams, frequent vital sign monitoring, and laboratory testing at least twice weekly (and daily during the initial period). The safety laboratory studies (i.e. hepatic transaminases) will be reviewed on a continuous basis by the investigators. Following discharge from the hospital and until Day +100, patients will be followed closely in clinic at least twice monthly, with laboratory studies done at least once weekly, all per the UCSF Pediatric BMT SOP. Patients will also have a contact number to report any potential adverse events that occur in between scheduled clinic visits. Should a serious adverse event occur during the course of the study, it will be reported immediately to the Committee on Human Research (Institutional Review Board) at the University of California San Francisco (UCSF) in accordance with current University guidelines for reporting possible adverse events.

9.3.1 Frequency of Safety Reviews: Safety monitoring will be conducted monthly on each patient.

10.0 Study Management

10.1 Prestudy Documentation

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

Before initiating this trial, the Investigator will have written and dated approval from the Institutional Review Board for the protocol, written informed consent form, subject recruitment procedures (e.g., advertisements), and any other written information to be provided to subjects before any protocol related procedures are performed on any subjects.

The clinical investigation will not begin until either FDA has determined that the study under the Investigational Drug Application (IND) is allowed to proceed or the Investigator has received a letter from FDA stating that the study is exempt from IND requirements.

The Investigator must comply with the applicable regulations in Title 21 of the Code of Federal Regulations (21 CFR §50, §54, and §312), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

10.2 Institutional Review Board Approval

The protocol, the proposed informed consent form, and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the Committee on Human Research (CHR) (UCSF's IRB). Prior to obtaining CHR approval, the protocol must be approved by the Helen Diller Family Comprehensive Cancer Center Site Committee and by the Protocol Review Committee (PRC). The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

10.3 Changes in the Protocol

Once the protocol has been approved by the UCSF Committee on Human Research (CHR), any changes to the protocol must be documented in the form of an amendment. The amendment must be signed by the Investigator and approved by PRC and the CHR prior to implementation.

If it becomes necessary to alter the protocol to eliminate an immediate hazard to patients, an amendment may be implemented prior to CHR approval. In this circumstance, however, the Investigator must then notify the CHR in writing within five (5) working days after implementation.

10.4 Handling and Documentation of Clinical Supplies

The Investigator shall maintain complete records showing the receipt, dispensation, return, or other disposition of all investigational drugs. The date, quantity and batch or code number of the drug, and the identification of patients to whom study drug has been dispensed by patient number and initials will be included. The sponsor-investigator will maintain written records of any disposition of the study drug.

The Investigator shall not make the investigational drug available to any individuals other than to qualified study patients. Furthermore, the Investigator will not allow the investigational drug to be used in any manner other than that specified in this protocol.

10.5 Case Report Forms (CRFs)

The Principal Investigator and/or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data

pertinent to the study. Study specific Case Report Forms (CRFs) will document treatment outcomes for data analysis (see Appendix 1). All study data will be entered into OnCore®, UCSF's Clinical Trial Management System (CTMS) via standardized CRFs in accordance with the CTMS study calendar, using single data entry with a secure access account. The Clinical Research Coordinator (CRC) will complete the CRFs as soon as possible upon completion of the study visit; the Investigator will review and approve the completed CRFs.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the patient's medical records maintained by UCSF personnel. All source documentation should be kept in separate research folders for each patient.

In accordance with federal regulations, the Investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered onto CRFs. Each CRF must be reviewed for accuracy by the Investigator, corrected as necessary, and then approved. Alternatively, the Investigator may sign individual, printed CRFs. These signatures attest that the information contained on the CRFs is true and accurate.

All source documentation and CTMS data will be available for review/monitoring by the Data and Safety Monitoring Committee (DSMC) and regulatory agencies.

The Principal Investigator will be responsible for ensuring the accurate capture of study data. At study completion, when the database has been declared to be complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement among the Principal Investigator, the Trial Statistician, and the Protocol Project Manager.

10.6 Oversight and Monitoring Plan

The Helen Diller Family Comprehensive Cancer Center Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study. The DSMC will monitor the study in accordance with the NCI-approved Data and Safety Monitoring Plan (DSMP). The DSMC will regularly review all adverse events and suspected adverse reactions considered "serious" and protocol deviations associated with the research to ensure the protection of human subjects. The DSMC will audit study-related activities to ensure that the study is conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). Significant results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as applicable.

10.7 Record Keeping and Record Retention

The investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects, as well as written records of the disposition of the drug when the study ends.

The investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study. Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

In accordance with FDA regulations, the investigator shall retain records for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

Appendix I. Case Report Form:

Patient's Medical Record Number: _____

Patient Name (last, first): _____

Date of Birth: _____/_____/_____ **Gender** ☐Male ☐Female

Date of Transplant: _____/_____/_____

1. Toxicities Through Day +100
   a. Treatment-Related Mortality: ☐ No ☐ Yes Date: _____
   b. Grade IV Pulmonary Toxicity: ☐ No ☐ Yes Date of onset: _____
   (Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated)
   c. Grade IV Hepatic Toxicity: ☐ No ☐ Yes Date of onset: _____
   (Moderate to severe encephalopathy; coma; life-threatening consequences)
   d. Moderate or Severe VOD: ☐ No ☐ Yes Date of onset: _____
   (Bilirubin >2.0 plus Hepatomegaly and/or RUQ pain plus Weight gain >5%; typically requires treatment with Defibrotide)
   e. Required Liver Enzyme Studies:

	AST	ALT	TBili	DBili	AlkPhos	GGT
Day -5						
Day -4						
Day -3						
Day -2						
Day -1						
Day 0						
Day +1						
Day +2						
Day +3						
Day +4						
Day +5						

2. Outcomes
   a. First Engraftment Study (~Day +30): Report % Donor Cells Date: _____
      a. Whole Blood _____
      b. CD3 _____
      c. CD14/15 _____
      d. CD19 _____
   b. ~Day +100 Engraftment Study: Report % Donor Cells Date: _____
      a. Whole Blood _____
      b. CD3 _____
      c. CD14/15 _____
      d. CD19 _____

Appendix II. IND Exemption Determination:

This is a new use of an already approved drug. Clofarabine's FDA label is that it is indicated for the treatment of pediatric patients 1 to 21 years old with relapsed or refractory acute lymphoblastic leukemia after at least two prior regimens. The recommended pediatric dose and schedule is 52 mg/m^2 administered by intravenous (IV) infusion over 2 hours daily for 5 consecutive days. This trial meets the requirements for exemption from the Investigational New Drug regulations, 21 CFR 312, specifically because:

1. This drug is lawfully marketed in the United States.
2. This investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use of the drug product.
3. The investigation is not intended to support a significant change in advertising to an existing lawfully marketed prescription drug product.
4. The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product, as detailed in the preliminary data section.
5. The investigation will be conducted in compliance with the requirements for institutional review set forth in FDA regulations 21 CFR 56, and requirements for informed consent as set forth in FDA regulations 21 CFR 50.

REFERENCES:

1. Cantoni N, Gerull S, Heim D, et al. Order of application and liver toxicity in patients given BU and CY containing conditioning regimens for allogeneic hematopoietic SCT. Bone Marrow Transplant. 2011;46:344-349.
2. Horn B, Baxter-Lowe L, Englert L, et al. Reduced intensity conditioning using intravenous busulfan, fludarabine and rabbit ATG for children with nonmalignant disorders and CML. Bone Marrow Transplant. 2006;37:263-269.
3. Andersson BS, de Lima M, Thall PF, et al. Once Daily i.v. Busulfan and Fludarabine (i.v. Bu-Flu) Compares Favorably with i.v. Busulfan and Cyclophosphamide (i.v. BuCy2) as Pretransplant Conditioning Therapy in AML/MDS. Biol Blood Marrow Transplant. 2008;14:672-684.
4. Law J, Cowan M, Dvorak C, et al. Fludarabine, busulfan, and alemtuzumab as a reduced toxicity regimen for children with marrow stem cell defects and malignancy improves engraftment and graft versus host disease without delaying immune reconstitution. Biol Blood Marrow Transplant. 2011;17:S260.
5. Dvorak C, Gilman A, Horn B, Cowan M. Primary graft failure after umbilical cord blood transplant rescued by parental haplocompatible stem cell transplantation. J Pediatr Hematol Oncol. 2009;31:300-303.
6. Walter R, Gooley T, Wood B, et al. Impact of pretransplantation minimal residual disease, as detected by multiparametric flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. J Clin Oncol. 2011;29:1190-1197.
7. Ammon K, Cowan M, Horn B, Dvorak C. Pre-Allogeneic Hematopoietic Cell Transplant Minimal Residual Disease Predicts Post-Transplant Relapse in Pediatric Acute Myeloblastic Leukemia. ASBMT Annual Meeting Abstracts. 2011.
8. Yabe M, Sako M, Yabe H, et al. A conditioning regimen of busulfan, fludarabine, and melphalan for allogeneic stem cell transplantation in children with juvenile myelomonocytic leukemia. Pediatr Transplant. 2008;12:862-867.
9. Horn B, Soni S, Khan S, et al. Feasibility study of preemptive withdrawal of immunosuppression based on chimerism testing in children undergoing myeloablative allogeneic transplantation for hematologic malignancies. Bone Marrow Transplant. 2009;43:469-476.
10. Kirschbaum M, Stein A, Popplewell L, et al. A phase I study in adults of clofarabine combined with high-dose melphalan as reduced intensity conditioning for allogeneic transplantation. Biol Blood Marrow Transplant. 2011;[Epub ahead of print].
11. Carson D, Wasson D, Esparza L, Carrera C, Kipps T, Cottam H. Oral antilymphocyte activity and induction of apoptosis by 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine. . Proc Natl Acad Sci U S A. 1992;89:2970-2974.
12. Carson D, Wasson D, Taetle R, Yu A. Specific toxicity of 2-chlorodeoxyadenosine toward resting and proliferating human lymphocytes. . Blood. 1983;62:737-743.
13. Long-Boyle J, Huang J, Rydholm N, et al. Pharmacokinetics of clofarabine in patients with high-risk inherited metabolic disorders undergoing brain-sparing hematopoietic cell transplantation. J Clin Pharmacol. 2011;51:679-686.
14. Valdez B, Murray D, Ramdas L, et al. Altered gene expression in busulfan-resistant human myeloid leukemia. Leuk Res. 2008;32:1684-1697.

15. Valdez B, Andersson B. Interstrand crosslink inducing agents in pretransplant conditioning therapy for hematologic malignancies. Environ Mol Mutagen. 2010;51:659-668.
16. Andersson BS, Valdez BC, de Lima M, et al. Clofarabine ± Fludarabine with Once Daily i.v. Busulfan as Pretransplant Conditioning Therapy for Advanced Myeloid Leukemia and MDS. Biology of Blood and Marrow Transplantation. 2011;17:893-900.
17. Helenglass G, Powles R, McElwain T, Lakhani A, Milan S, Gore MA, Zuiable A, Perren T, Forgeson G, et al. Melphalan and total body irradiation (TBI) versus cyclophosphamide and TBI as conditioning for allogeneic matched sibling bone marrow transplants for acute myeloblastic leukaemia in first remission. Bone Marrow Transplant. 1988;3:21-29.
18. Rosales F, Naparstek E, Varadi G, Or R, Slavin S, Nagler A. The role of thiotepa in allogeneic stem cell transplantation in patients with leukemia. Leuk Res. 1999;23:947-952.
19. Farag S, Wood L, Schwartz J, et al. Phase I trial and pharmacokinetic study of high-dose clofarabine and busulfan and allogeneic stem cell transplantation in adults with high-risk and refractory acute leukemia. Leukemia. 2011;25:599-605.
20. Srivastava S, Jones D, Wood L, et al. A phase I trial of high-dose clofarabine, etoposide, and cyclophosphamide and autologous peripheral blood stem cell transplantation in patients with primary refractory and relapsed and refractory non-Hodgkin lymphoma. Biol Blood Marrow Transplant. 2011;17:987-994.
21. Bonate P, Craig A, Gaynon P, et al. Population pharmacokinetics of clofarabine, a second-generation nucleoside analog, in pediatric patients with acute leukemia. . J Clin Pharmacol. 2004;44:1309-1322.
22. Long-Boyle J, Green K, Brunstein C, et al. High fludarabine exposure and relationship with treatment-related mortality after nonmyeloablative hematopoietic cell transplantation. . Bone Marrow Transplant. 2011;46:20-26.
23. Ajavon A, Bonate P, Taft D. Renal excretion of clofarabine: assessment of dose-linearity and role of renal transport systems on drug excretion. Eur J Pharm Sci. 2010;40:209-216.
24. King K, Damaraju V, Vickers M, et al. A comparison of the transportability, and its role in cytotoxicity, of clofarabine, cladribine, and fludarabine by recombinant human nucleoside transporters produced in three model expression systems. . Mol Pharmacol. 2006;346-53.
25. Elwi A, Damaraju V, Kuzma M, et al. Transepithelial fluxes of adenosine and 2'-deoxyadenosine across human renal proximal tubule cells: roles of nucleoside transporters hENT1, hENT2, and hCNT3. Am J Physiol Renal Physiol. 2009;296:F1439-1451.