NCT# 02458235

A phase II study of risk-adapted donor lymphocyte infusion and azacitidine for the prevention of hematologic malignancy relapse following allogeneic stem cell transplantation

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Principal Investigator

Co-Investigator

Statistician

Clinical Research Coordinator

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Protocol Signature Page

Protocol No.: 140813 Version Date: 11/17/2016

1. I agree to follow this protocol version as approved by the UCSF Protocol Review Committee (PRC), Committee on Human Research (CHR), and Data Safety Monitoring Committee (DSMC).
2. I will conduct the study in accordance with applicable CHR requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, and with local regulatory requirements. In accordance with the FDA Modernization Act, I will ensure the registration of the trial on the www.clinicaltrials.gov website.
5. I agree to maintain adequate and accurate records in accordance with CHR policies, Federal, state and local laws and regulations.

UCSF Principal Investigator / Study Chair

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Printed Name

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Signature Date

Investigator Site

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Printed Name

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Investigator Site

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Printed Name

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Signature Date

Participating Site(s)
All Children's Hospital, St. Petersberg, FL
Lucille Packard Children's Hospital, Palo Alto, CA

Abstract

The goal of this Phase II study is to determine whether post-transplant consolidation with azacitidine combined with donor lymphocyte infusion (DLI) is a safe and effective approach for the prevention of relapse in pediatric and young adult patients with hematologic malignancies who have undergone hematopoietic stem cell transplantation (HSCT).

We plan to enroll 67 children with hematologic malignancies (including acute myeloid leukemia, acute lymphoblastic leukemia, juvenile myelomonocytic leukemia, and myelodysplastic syndrome) at the time of HSCT at three institutions (including UCSF, All Children's Hospital, St. Petersburg, FL, and Lucile Packard Children's Hospital, Palo Alto, CA). Patients will be enrolled on the study by day +28, prior to withdrawal of immunosuppression or administration of donor lymphocyte infusion (DLI). They will have donor chimerism and minimal residual disease (MRD) testing from the peripheral blood (PB) and bone marrow (BM) on day +28 ± 7 (as per standard of care). Based on risk assessment, patients will receive one cycle of low-dose azacitidine (40mg/m^2 IV/SC daily x 4 days) alone as immunosuppression is tapered. After tapering immunosuppression, chimerism will be repeated and patients will receive up to 6 additional cycles of low-dose azacitidine. For patients who meet at high risk for relapse, azacitidine will be combined with escalating doses of DLI for a maximum of 7 cycles in total. Risk and safety assessments, including routine laboratory parameters, donor chimerism, minimal residual disease, and GHVD activity will be assessed following each cycle.

The primary outcome measures will be indicators of relapse risk and safety / toxicity. Patients will be followed for relapse as well as incidence of severe drug toxicities and acute and chronic GVHD until 2 years post-transplant. The study will be considered successful if the rate of relapse at 2 years is <25%, and the incidence of Grade IV GVHD and severe drug toxicity are <20%. Correlative studies will include the effect of azacitidine on T cell immune reconstitution, as well as prospective minimal residual disease estimation by gene expression / mutation panels in patients with AML.

Study Schema

Standard Risk:

- Active Grade I GVHD, OR
- Any history of >Grade II GVHD, OR
- Full Donor Chimerism AND Pre-HSCT MRD-neg AND Post-HSCT MRD-neg

High Risk:

- No GVHD or resolved Grade I GVHD, AND
- Mixed chimerism OR Pre-Tx MRD(+) OR Post-Tx MRD(+)

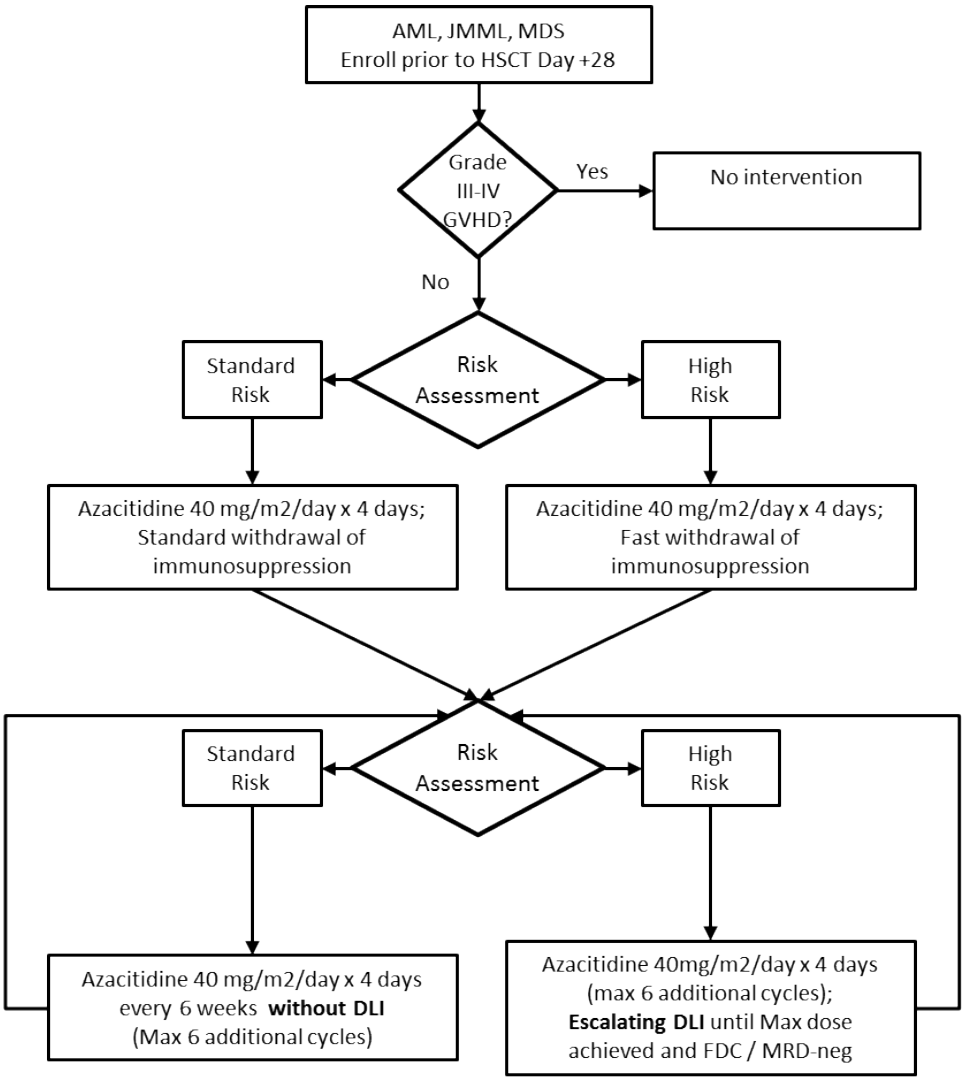

Figure 1a: Schema for myeloid malignancies. Patients will be enrolled on study prior to Day +28. Chimerism from PB and BM as well as MRD will be assessed. Patients with Grade III-IV GVHD will not receive study immunomodulatory therapy. Patients meeting criteria for High-Risk designation will receive azacitidine followed by fast withdrawal of immunosuppression. Standard-Risk patients will receive azacitidine with standard withdrawal of immunosuppression. Risk assessment will be completed following withdrawal of immunosuppression, and patients will be assigned to receive azacitidine alone or azacitidine in combination with DLI based on current risk. Patients may receive up to 6 additional courses of azacitidine with or without DLI after withdrawal of immunosuppression; the addition of DLI will be guided by risk assessments done prior to each subsequent course.

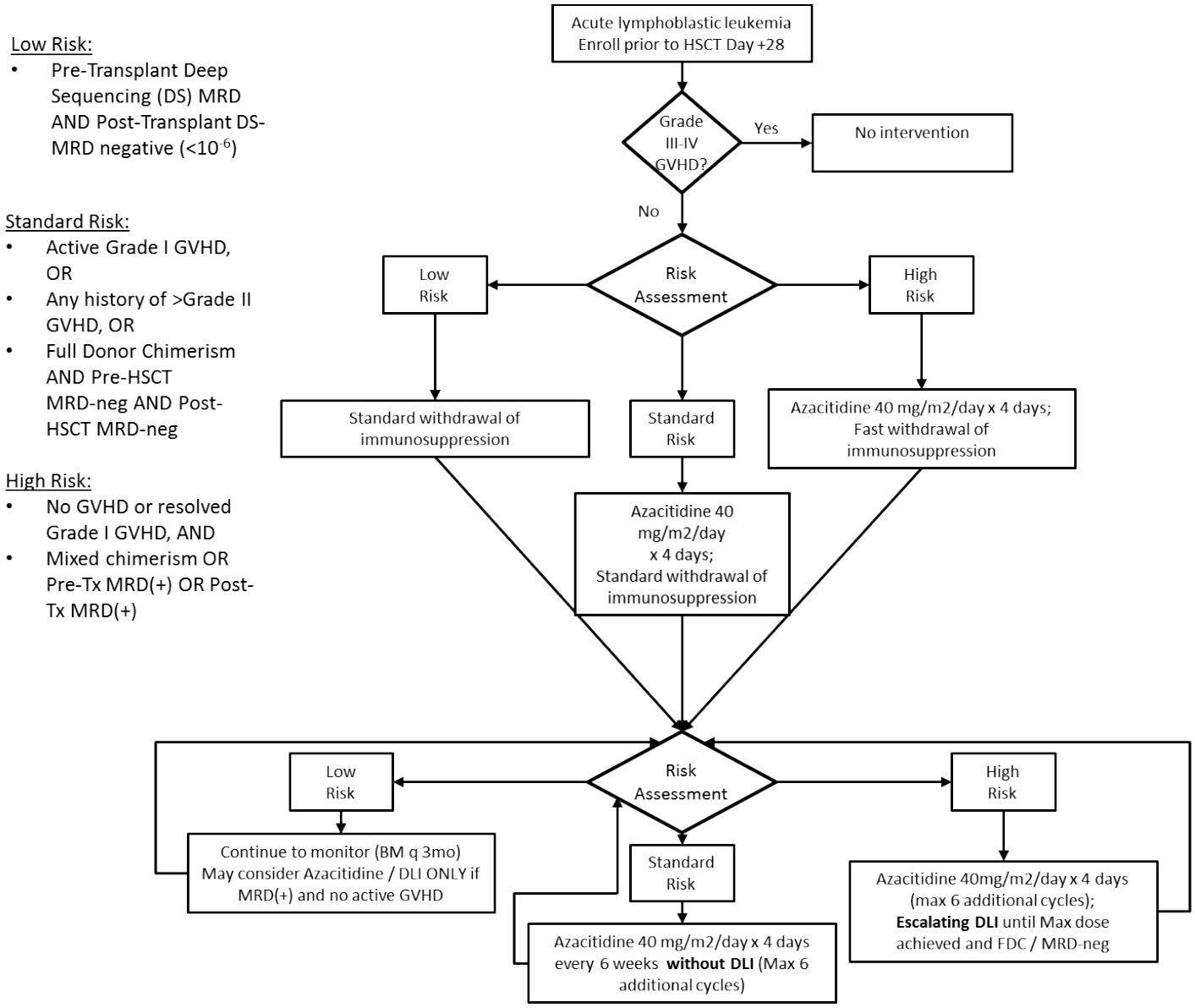

Figure 1b: Schema for acute lymphoblastic leukemia. Patients will be enrolled on study prior to Day +28. Chimerism from PB and BM as well as MRD (by conventional flow cytometry as well as deep sequencing when available) will be assessed. Patients with Grade III-IV GVHD will not receive study immunomodulatory therapy. Patients meeting criteria for High-Risk designation will receive azacitidine followed by fast withdrawal of immunosuppression. Standard-Risk patients will receive azacitidine with standard withdrawal of immunosuppression. Low-Risk patients will not receive study immunomodulatory intervention and will be treated per local SOP. Risk assessment will be completed following withdrawal of immunosuppression, and patients will be assigned to receive standard therapy, azacitidine alone, or azacitidine in combination with DLI based on current risk. Patients may receive up to 6 additional courses of azacitidine with or without DLI after withdrawal of immunosuppression; the addition of DLI will be guided by risk assessments done prior to each subsequent course.

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LIST OF ABBREVIATIONS

AE	adverse event
aGVHD	acute GVHD
ALL	acute lymphoblastic leukemia
ALT	alanine transaminase
AML	acute myelogenous leukemia
ANC	absolute neutrophil count
BM	bone marrow
cGVHD	chronic GVHD
CHR	Committee on Human Research (UCSF IRB)
CMV	cytomegalovirus
CRC	Clinical Research Coordinator
CRF	case report form
CSA	Cyclosporine A
CTC	Common Toxicity Criteria
CTMS	clinical trials management system
DLI	donor lymphocyte infusion
DSMC	Data and Safety Monitoring Committee
EBV	Epstein-Barr Virus
FDA	Food and Drug Administration
FWI	Fast Withdrawal of Immunosuppression
GCP	Good Clinical Practice
GVHD	graft-versus-host-disease
GVL	graft versus leukemia
HDFCCC	Helen Diller Family Comprehensive Cancer Center
HGB	hemoglobin
ICH	International Conference on Harmonization
IRB	Institutional Review Board
JMML	juvenile myelomonocytic leukemia
KPS	Karnofsky Performance Status
MC	mixed chimerism
MDS	myelodysplastic syndrome
MRD	minimal residual disease
NCI	National Cancer Institute
PB	peripheral blood
PBMTC	Pediatric Blood and Marrow Transplant Consortium
PI	Principal Investigator
PRC	UCSF Protocol Review Committee
RBC	red blood cell
SAE	serious adverse event
STR	short tandem repeats
WBC	white blood cell

1.0 Background and Rationale

1.1 Prevention of relapse in pediatric hematologic malignancies

Multiple hematologic malignancies, such as acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), juvenile myelomonocytic leukemia (JMML) and myelodysplastic syndrome (MDS) are treated with myeloablative hematopoietic stem cell transplantation (HSCT) in order to safely deliver high doses of anti-neoplastic therapy, and to take advantage of donor-derived immune cells to produce an allogeneic "graft-versus-leukemia" effect. As conditioning regimens and supportive care measures improve, transplant-related morbidity and mortality have decreased, such that relapse is the primary cause of treatment failure in these patients. Recent data for pediatric ALL, AML, and JMML demonstrate a 5-year relapse rate of 30-40% (*1-3*). Survival rates in patients who relapse following transplant are <20% at 4 years (*4*).

Additional strategies for the prevention of post-transplant relapse are needed. This study combines minimal residual disease by high throughput sequencing, multiparametric flow MRD, and donor chimerism to direct the application of post-transplant immunomodulatory therapy with azacitidine and donor lymphocyte infusion (DLI). By administering this therapy to patients at highest risk of relapse, this study aims to decrease relapse rates while maintaining a low risk of GVHD.

1.2 Predictors of post-transplant relapse

Mixed chimerism (MC) is the presence of both host's and donor's hematopoietic cells after allogeneic transplant. With the introduction of quantitative and frequent measurements of chimerism, the relationship between the presence of host cells and the relapse of acute leukemias after allogeneic transplant was recognized. In 1998, Bader et al. showed that pediatric patients with increasing amount of host (autologous) cells on their chimerism testing following allogeneic transplant have significantly increased risk of relapse (*5*). In their study, 36/54 children had complete donor chimerism and 19% of them relapsed, 10/54 children had increasing amounts of host cells post-transplant (increasing host chimerism) and 90% of them relapsed; in contrast, 8/54 children had decreasing amount of host cells following transplant (decreasing host chimerism) and none of them relapsed (*5*). Similar results were obtained by Barrios et al. in adults; 93% of patients with increasing host chimerism and 27% of patients with full donor chimerism relapsed, respectively (*6*).

Patients with increasing host chimerism sustained relapses between days 36 and 450 of their follow-up. Increasing host chimerism preceded relapse by a median of 74 days (*6*). The investigators concluded that frequent and sensitive measurement of chimerism after transplant is a useful tool for identifying a group of patients with a very high risk of relapse in whom further immunotherapy would be justified. Additionally, Bader and colleagues studied the origin of cells found in patients with mixed chimerism. In the early post-transplant period, mixed chimerism was caused predominantly by normal recipient hematopoietic cells. This finding supports the hypothesis that a state of mixed hematopoietic chimerism may reduce the clinical GVL effect of alloreactive donor-derived effector cells in patients with acute leukemias, and thus facilitate the proliferation of residual malignant cells that may have survived the preparative regimen (*7*).

The presence of minimal residual disease (MRD), either immediately prior to HSCT, or following HSCT, has been correlated with decreased survival and increased risk of relapse in both ALL (*8*) AML (*9*) patients (*10*). An MRD-guided strategy of reduction of immunosuppression without DLI or azacitidine resulted in overall survival of 72% in patients with MRD <0.1%, and 40.4% in those with MRD>0.1% (*11*). This protocol plans to build on

this approach through risk stratification based on MRD and chimerism, and post-transplant immunomodulation with not only fast withdrawal of immunosuppression, but also azacitidine and escalating doses of DLI.

1.3 Calcineurin inhibitor dose manipulation as a form of immunotherapy

In a randomized study that looked at the effect of dose of cyclosporine A (CSA) on leukemia relapse after transplant, disease-free survival was superior in patients receiving low dose CSA (1mg/kg/day) than in those receiving high dose CSA (5 mg/kg/day) (*12*). This benefit of low dose CSA persisted 10-years after transplant, but only in patients younger than 30 years of age, as the older patients receiving low CSA dose had an increased risk of transplant-related complications (*13*). A similar study in children comparing 1 mg/kg/day of CSA vs. 3 mg/kg/day showed relapse rates of 15% vs. 41% in low and high CSA dose groups, respectively (*14*). A study looking into the length of treatment with CSA (60 vs. 180 days) indicated that CSA can be safely stopped at 60 days in patients who did not have evidence of acute GVHD (*15*).

1.4 DLI as a form of immunotherapy

Donor lymphocyte infusions have been successfully used in patients with chronic myelogenous leukemia who relapse after transplant (*16, 17*). However, DLI is far less successful when given to acute leukemia patients who relapse after transplant. While the remission induction rate with DLI for CML is 80% and the effect appears to be prolonged, the remission induction rate for patients with acute leukemia is 15 – 25%, and often is of short duration (*18*). The failure of donor lymphocytes in the acute leukemia setting may be due to inadequate graft vs. leukemia effect in the presence of clinical relapse with a large leukemic cell burden. In order to avoid a large leukemic cell burden, investigators have moved to pre-emptive use of DLI in patients at a high risk for relapse. DLI have been extensively used in the setting of non-myeloablative or reduced toxicity transplantation (*19, 20*).

1.5 Azacitidine as a form of immunotherapy

Demethylating agents such as azacitidine and decitabine have various effects on multiple facets of the immune system. In vitro studies of AML and melanoma cells have demonstrated that co-culture with decitabine results in increased class I and II MHC expression (*21, 22*). Azacitidine administered to patients following allogeneic transplantation has been associated with a concomitant increase in cytotoxic CD8+ T cells and regulatory T cells, potentially resulting in increased GvL without commensurate increase in GVHD (*23*). Effects on NK cells are mixed: while decitabine has been shown to increase NKG2D-dependent sensitivity of AML cells to NK-mediated killing in vitro (*24*), azacitidine increases KIR expression (*25, 26*), and decreases expression of TRAIL, NKG2D, and NKp46 (*27*).

Azacitidine has been used in patients safely as monotherapy. In a dose-finding study, azacitidine was used as prophylactic / maintenance therapy in heavily pretreated refractory/relapsed AML/MDS patients following allogeneic transplant, and a dose of 32 mg/m^2 given for 5 days was shown to be safe for at least 4 cycles (*28*). 1 year EFS in this cohort was 58%. Azacitidine monotherapy has also been studied in AML patients following allogeneic transplantation as pre-emptive therapy for declining CD34 chimerism (RELAZA trial) (*29*). Azacitidine 75mg/m^2/day x 7 days was administered every 28 days in AML patients with declining chimerism post-transplant; of 20 patients, 10 achieved an increase in CD34+ donor chimerism, and 3 of these remained relapse-free for >6 months. Although there is less data for the use of demethylating agents in ALL, there is preclinical evidence that they may be effective (*30*), and there is early clinical indication that they may be effective in a subset of these patients (*31*).

1.6 Use of azacitidine with DLI as immunotherapy in patients with leukemic relapse

Azacitidine has been administered safely in combination with DLI for patients with AML relapse 30 days to 4 years post-transplant (*32*). The combination was shown to be active, inducing CR in 7 of 30 patients, with no increased risk of GVHD or azacitidine toxicity. Decitabine has also been used for relapse in 14 ALL, AML, and CML patients following transplant. High doses of 100-150 mg/m^2 Q12h x 5 days followed by DLI resulted in a response in 57% of patients, including one of two ALL patients (*33*). In addition, a lower (hypomethylating) dose of 20mg/m^2 IV x 5 days is also effective in patients with MDS (*34*).

1.7 Safety of Azacitidine

In the 2010 Phase I study of azacitidine used post-transplant in 45 very-high risk patients, the drug was well-tolerated at a maximum dose of 40mg/m^2/day x 5 days (*28*). In terms of hematologic toxicity, there was no correlation between white blood cell or platelet count at the start of maintenance and development of hematologic toxicities. They observed reversible grade 1-2 or 3 thrombocytopenia (n = 7 and n = 2), and in 1 of 2 patients receiving 40 mg/m^2. Grade 1-2 neutropenia was documented in 7 cases.

Other toxicities included: Grade 1 nausea (n = 9), Grade 2 fatigue (n = 6), Grade 1-2 transaminases elevation (n=3), Pruritus (n=1), cholecystitis (n=1), grade 1 confusion (n =2), grade 2 creatinine elevation (n = 1), oral ulcers (n = 2). There were also 3 cases of possible ocular toxicity: conjunctival erythema; retina hemorrhage with platelet count drop to 50,000/mm3 (possibly pre-existing); and papilledema.

The most serious possibly drug-related adverse event was 1 case of pulmonary hemorrhage because of fungal pneumonia, which occurred in a patient receiving a second HSCT, who evolved with thrombocytopenia and multiorgan failure. Infections that occurred during the treatment period were considered to be within the expected profile seen in this population.

1.8 Use of Azacitidine in Children

Azacitidine has been used safely in children. The maximum tolerated dose was examined in children as early as 1973. Children ages 2 to 17 years were treated with azacitidine monotherapy for 5 days every 14 days. The maximum tolerated dose was 150-200 mg/m^2 (*35*). Kalwinsky et al used azacitidine in 68 previously untreated pediatric AML patients at a dose of 300mg/m^2 on day 4 and 5 in combination with cytotoxic chemotherapy (*36*). A phase II study in 1996 randomized 41 pediatric patients with refractory disease to receive cytotoxic chemotherapy with or without azacitidine at a dose of 250mg/m^2/day x 2 days), with a significantly higher CR rate in the group of children who received azacitidine (*37*).

1.9 Lineage specific chimerism analysis

Monitoring chimerism in different hematopoietic cell lineages can increase the sensitivity of detecting the minority population when there are lineage differences in the extent of mixed chimerism. Zetterquist et al. showed that monitoring mixed chimerism in B-cell lineage correlated well with molecular confirmation of minimal residual disease and was detectable 2.5 months before morphologic relapse (*38*). Similarly, Mattson et al. showed that MC in CD13+ and CD33+ cell lineage in patients with AML could detect relapse a median of 66 days before hematologic relapse (*39*). Our feasibility study confirmed that leukemia-specific lineage mixed chimerism heralds relapse (*40*).

1.10 Minimal residual disease monitoring of AML in the post-transplant setting

In addition to chimerism analysis in the post allogeneic hematopoietic stem cell transplantation (allo-HSCT) setting, a variety of other high sensitivity methods for quantification of measurable disease burden in AML have been described and have recently been reviewed (*10, 41-43*), that allow for risk stratification when used prior to allo-HSCT and early prediction of impending

clinically evident relapse when used in surveillance monitoring post allo-HSCT. These high sensitivity measures of AML disease burden may also help quantify the efficacy of post allo-HSCT maintenance interventions (*29, 43*). The optimal MRD monitoring strategy in AML is a subject of current investigation.

1.11 Preliminary data

Our group has been studying the use of subset chimerism testing and pre-emptive immunotherapy with fast withdrawal of immunosuppression (FWI) and donor lymphocyte infusion (DLI) since 2005. Our prospective multi-institutional study evaluated the feasibility of longitudinal chimerism testing in a central laboratory (including WB, CD3+, and leukemia-specific lineage chimerism) in patients with a variety of hematologic malignancies, and evaluated the feasibility of fast withdrawal of immunosuppression based on WB chimerism results. Centralized chimerism testing was feasible and showed low inter-assay variability. Increasing mixed chimerism (MC) in WB was not useful as a predictor of relapse in our study. The presence of full donor chimerism in WB, CD3+ and leukemia-specific lineages on all measurements was related to a significantly lower risk of relapse than the presence of MC in either subset (11%% vs 71%, respectively; P=0.03). Increasing host chimerism in leukemia-specific lineage heralds relapse, but it was not detected early enough to allow immunotherapy. The conclusion of the study was that the goal of preemptive immunotherapy should be to achieve full donor chimerism in WB in CD3+ and leukemia-specific lineages, in an effort to prevent relapse before donor chimerism declines (*40*).

In a recent prospective UCSF analysis, 43 children (25 AML, 18 ALL,) underwent myeloablative BMT or PBSCT followed by immunomodulatory therapy (IT) between 2009 and 2012 (*44*). Patients with FDC in BM and PB at day 30 or evidence of GVHD were assigned to observation only (N=12). Patients with mixed chimerism (regardless of MRD) were assigned to intervention. Intervention consisted of fast withdrawal of immunosuppression (FWI) in 26 patients; 14 of these also received DLI. Five patients could not be assigned due to early death or relapse. Overall survival at a median of 30 months was 54%. Toxicity was acceptable, with an acute GVHD rate of 19% in the intervention arm, and a toxic death rate of 4%. Relapse was observed in 10/38 patients; 8 of the 10 relapses occurred in the intervention arm (associated with mixed chimerism). Four of these occurred late at >24 months post-transplant.

The most powerful predictor of post-transplant relapse was BM chimerism in the leukemia-specific subset. Mixed chimerism was associated with a 41% relapse rate, compared to a 6% rate in patients with full donor chimerism (p=0.001). Mixed chimerism in the BM CD34 subset was also significantly associated with increased risk of relapse (38% vs 10%, p=0.04). Interestingly, no patients who demonstrated full donor chimerism in all subsets (CD3, CD34, and leukemia-specific CD3 or CD19) relapsed (0 out of 7 patients), compared to a relapse rate of 37% in patients demonstrating mixed chimerism in any subset (p=0.02).

1.12 Rationale

Our most recent approach to decrease the risk of relapse post-transplant has focused on immunomodulatory therapy including fast withdrawal of calcineurin inhibitor (FWI) and administration of donor lymphocyte infusion (DLI). Our data has demonstrated that FWI with or without DLI administered to patients with mixed chimerism clearly prolongs survival. However, patients who were full donor chimeras remained at risk for relapse if they did not develop GVHD, as they were not eligible for immunomodulatory therapy. Therefore, additional therapy is required for this group of patients with higher risk of relapse.

An attractive option for post-transplant consolidation that has the potential to synergize with immunomodulatory therapy is the administration of a hypomethylating agent such as azacitidine. The main advantages to using this class of agents compared to other agents are:

- They are relatively safe / tolerable, so can be used in patients with recovering organ function post-transplant.
- They are potentially synergistic with immunotherapy, augmenting the Graft versus Leukemia (GvL) effect.
- They may provide protection against GvHD (the primary risk of post-transplant immunomodulation) even while preserving GvL effect.
- They can be applied indiscriminately to most types of AML and other myeloid malignancies (they do not require a specific target, unlike newer "targeted" therapies).

2.0 Objectives

2.1 Primary Objectives

2.1.1 To evaluate the efficacy of the risk-adaptive approach to relapse reduction (based on the relapse rate) with a combination of azacitidine and donor lymphocyte infusion (DLI) in pediatric patients with AML, ALL, or MDS treated with stem cell transplantation.

2.1.2 To investigate the safety and toxicity of the treatment with a combination of azacitidine and donor lymphocyte infusion (DLI) in pediatric patients with AML, ALL, or MDS treated with stem cell transplantation.

2.2 Secondary / Exploratory Objectives

2.2.1 To evaluate the effect of risk-adapted azacitidine and DLI on other outcomes such as relapse-free survival and median time to relapse.

2.2.2 To evaluate the effect of post-transplant azacitidine on immune function, including T cell function.

2.2.3 To evaluate the feasibility and utility of a gene-expression platform for the detection of minimal residual disease with high sensitivity in AML patients following HSCT.

2.3 Hypotheses

2.3.1 In patients with acute leukemia, the use of azacitidine and DLI is associated with a lower rate of relapse at two years compared with historical controls.

2.3.2 In patients at high risk for post-transplant relapse, the use of azacitidine and DLI is associated with a low rate of Grade III-IV GvHD or drug-related severe adverse events.

3.0 Study Design and Eligibility Criteria

3.1 Study Design (see also Study Schema, page 3)

3.1.1 We propose a Phase II single-arm trial of azacitidine (IV or SC) in combination with escalating donor lymphocyte infusions (DLI). This study will be open to patients with ALL, AML, JMML, or MDS.

3.1.2 The first cycle of azacitidine will be administered during withdrawal of immunosuppression for patients not developing severe organ toxicity or Grade III-IV GVHD.

3.1.3 After the initial cycle during withdrawal of immunosuppression (duration is based on day 28 PB chimerism), azacitidine is given for 4 days in 6-week cycles (+/- 2 weeks) at a dose of 40 mg/m^2/day.

3.1.4 For patients meeting High Risk criteria who have available cell product, DLI is given on day 5 of each cycle in escalating doses. Peripheral blood chimerism and MRD is assessed after week 4, prior to initiating the next cycle. BM chimerism and MRD is

obtained at regular intervals prior to specified cycles as per the study schedule. Patients will receive up to 7 cycles as tolerated.

3.1.5 Patients will be followed by laboratory monitoring and physician evaluation prior to each cycle. Weekly labs will be obtained and will include toxicity monitoring (CBC, LFTs, and electrolytes) and correlative studies, such as immunophenotype and T cell function by PHA. Patients will be followed for two years to study toxicity and GVHD outcomes as well as relapse incidence.

3.2 Inclusion Criteria

3.2.1 Patients age 0 – 29.9 years undergoing allogeneic stem cell transplant using a peripheral blood stem cell source.

3.2.1.1 Patients receiving bone marrow or umbilical cord blood as a stem cell source may also be considered for enrollment with acknowledgement that if there is insufficient product available for DLI, the patient will receive azacitidine without DLI per Standard-Risk treatment.

3.2.2 Patients with one of the following diagnoses:

3.2.2.1 Acute myeloid leukemia (AML)

3.2.2.2 Acute lymphoblastic leukemia (ALL)

3.2.2.3 Juvenile myelomonocytic leukemia (JMML)

3.2.2.4 Myelodysplastic syndrome (MDS)

3.3 Exclusion Criteria:

3.3.1 Patients who have had a prior transplant

3.3.2 Patients receiving a haploidentical/T cell depleted transplant

3.3.3 Patients with Fanconi anemia or other cancer-predisposition syndromes

3.3.4 Patients with expected survival <12 weeks

3.3.5 Lansky score <60%

4.0 Patient Registration

4.1 Recruitment:

Patients will be recruited to the study by a bone marrow transplant physician during pre-transplant clinic visits, or during hospital admission. Patients will be consented for study any time between pre-transplant consent conference and day +28; enrollment will occur before day +28 and prior to withdrawal of immunosuppression.

4.2 Registration:

All prospective patients will undergo an informed consent conference during which their transplant physician will explain risks, benefits and alternatives to the study participation. The assent of the recipient will be obtained when age appropriate, as well as parental permission. To register patients, the attending physician will contact the Study Chair, [redacted] The investigator will complete the eligibility forms (provided in the Case Report Form packet) and fax them per the instructions on the form to either the UCSF PI or UCSF Study Coordinator. Pertinent information will be maintained at UCSF on each enrolled patient.

5.0 Investigational Intervention Plan

5.1 Collection of Patient Demographic Information

5.1.1 Age

5.1.2 Disease type / cytogenetics / genetic risk classification

5.1.3 Disease status at HSCT (site of disease, MRD status at each site)

- Pre-transplant MRD must be collected within 28 days prior to transplant.

5.1.4 Conditioning regimen (Bu/Flu, TBI/Cy, others)
5.1.5 Donor (related versus unrelated)
5.1.6 HLA match
5.1.7 GVHD prophylaxis (use of steroids or other agents; use of calcineurin inhibitor and target; use of serotherapy)

5.2 Schedule of Testing

5.2.1 BM studies to be obtained with each BM exam (not including correlative samples):

- Chimerism:
  - Chimerism <100% in any lineage / cell subset will be designated "Mixed Donor Chimerism" (MDC)
  - Chimerism of 100% in all lineages / subsets will be designated "Full Donor Chimerism" (FDC)
- Morphology
- Minimal residual disease (MRD):
  - Multiparametric flow cytometry, FISH, PCR, or deep sequencing (DS-MRD, Sequenta ClonoSIGHT, Adaptive) should be sent as clinically indicated, but results are not required prior to initiation of next cycle. Preliminary risk stratification can be assigned and adjusted when results are available.
    MRD positivity is defined as detectable disease by the most sensitive method used for each patient. In some cases this will be limited to flow cytometry. PCR and deep-sequencing MRD may also define MRD positivity in patients for whom these tests are available.

5.2.2 First BM and PB chimerism tests are due at Day +28 but can be done between Day +21 and Day +42 when ANC >500 x 3 days.

- A minimum of 8 ml of each BM and PB in ACD tube will be sent to the UCSF or local Immunogenetics clinical laboratory for chimerism testing.

5.2.3 When available locally, whole blood chimerism and subset (CD3, CD14/15, and CD19) chimerism will be tested and prioritized according to underlying disease:

- For T-cell malignancy: CD3 > CD14/15
- For B-cell malignancy: CD3 > CD19 > CD14/15
- For myeloid malignancy: CD3 > CD14/15

5.2.4 Bone marrow subset chimerism will be similarly prioritized:

- For T-cell malignancy: CD34> CD3
- For B-cell malignancy: CD34 > CD19 > CD3
- For myeloid malignancy: CD34 > CD33 > CD3

5.2.5 If PB converts to FDC, BM must be obtained for chimerism prior to starting the next cycle in order to determine eligibility for further DLI.

Time point	**Required Study**	**Comments**
Day +21 through Day +42	PB Chimerism PB MRD* **BM Chimerism** **BM MRD**	Required prior to Cycle 1 (Aza alone)
2-8 weeks following completion of withdrawal of immunosuppression.	PB chimerism PB MRD*	Required prior to Cycle 2 (Aza +/- DLI)
4-8 weeks following initiation of Cycle 2	PB Chimerism PB MRD* **BM chimerism**	Required prior to Cycle 3 (Aza +/- DLI)

Time point	Required Study	Comments
	BM MRD	
4-8 weeks following initiation of Cycle 3	PB chimerism PB MRD*	Required prior to Cycle 4 (Aza +/- DLI)
4-8 weeks following initiation of Cycle 4	PB chimerism PB MRD* **BM chimerism** **BM MRD**	Required prior to Cycle 5 (Aza +/- DLI)
4-8 weeks following initiation of Cycle 5	PB chimerism PB MRD*	Required prior to Cycle 6 (Aza +/- DLI)
4-8 weeks following initiation of Cycle 6	PB chimerism PB MRD* **BM chimerism** **BM MRD**	Required prior to Cycle 7 (Aza +/- DLI)

Table 1: Schedule of chimerism and MRD analysis for each cycle. PB DS-MRD is recommended for ALL patients when available. May omit PB MRD if concurrent BM MRD sample is sent. Peripheral blood MRD by flow cytometry as clinically indicated.

5.3 Risk Stratification

5.3.1 Risk is evaluated and assigned prior to each azacitidine cycle based on clinical testing specific to that patient's disease.

5.3.2 For patients with MDS, any evidence of disease may be used to stratify the patient accordingly. In the absence of evidence of persistent disease, chimerism will be used to stratify the patient. MDS patients with no evidence of disease and 100% chimerism will be stratified as standard risk.

5.3.3 Peripheral blood disease assessment will be done as clinically indicated. Peripheral blood MRD by deep sequencing (if positive) may be used to risk-stratify ALL patients instead of bone marrow MRD. Any evidence of disease in peripheral blood for patients with AML/JMML or MDS will prompt BM evaluation as clinically indicated. In this instance, study treatment may continue after BM evaluation is complete.

5.3.4 Risk stratification criteria:

- Low Risk
  - May only be applied to acute lymphoblastic leukemia patients
  - All of the following
    - Pre-transplant deep sequencing MRD negative, AND
    - All post-transplant deep sequencing MRD samples negative, AND
    - Any chimerism or GVHD status
      - ALL patients with negative deep-sequencing MRD pre- and post-transplant will be treated as low-risk regardless of chimerism

- Standard Risk
  - Active Grade I GVHD, OR
  - Any history of Grade II (or higher) GVHD, OR
  - All of the following
    - Full Donor Chimerism AND
    - Pre-HSCT MRD $<10^{-5}$ (bone marrow) AND
    - Post-HSCT MRD $<10^{-5}$ (bone marrow), unless 3-fold increase is observed

- High Risk
  - No active acute or chronic GVHD, AND
  - No history of Grade II (or higher) acute GVHD or chronic GVHD, AND

- Any of the following:
  - Mixed chimerism OR
  - Pre-HSCT MRD > 1 x 10^{-5} OR
  - Post-HSCT MRD at day 28 or later >=10^{-5} once, OR
  - Post-HSCT MRD at day 28 or later >=10^{-6} with >=3-fold increase on any subsequent measurement (need not be consecutive).
    - Post-HSCT MRD <1 x 10^{-5} is treated as Standard Risk until a 3-fold increase is documented.

5.4 Chimerism assessment

5.4.1 Chimerism assessment will be done at the UCSF Immunogenetics and Transplantation Laboratory [redacted], or at the local institution for collaborating sites. Chimerism will be assessed using short tandem repeats (STR) on whole blood and bone marrow and on cell subsets when such testing is available. Peripheral blood subset analyses will include whole blood CD3+, and CD14/15 subsets for all patients. Patients with B-cell malignancies will also have CD19+ subset tested. For bone marrow specimens, CD34+ will also be analyzed in all patients, in addition to disease-specific subsets.

5.4.2 Patients with any degree of host chimerism detected in any subset from either peripheral blood or bone marrow will be considered high risk.

5.5 Criteria for standard or fast withdrawal of immunosuppression (SWI versus FWI)

5.5.1 GVHD, MRD, and chimerism status must be assessed prior to each cycle and applied to risk stratification as above.

5.5.2 If the patient meets High-Risk criteria, then fast withdrawal of immunosuppression (FWI) will be initiated as early as Day +30.

5.5.3 If patient meets Standard-Risk criteria (or Low-Risk criteria for ALL patients), standard withdrawal of immunosuppression (SWI) will be initiated.

5.6 Schedule of standard and fast withdrawal of immunosuppression (SWI versus FWI)

5.6.1 Standard withdrawal of immunosuppression will be performed per institutional standard procedure. Suggested course of taper is over 10 weeks starting at day 30-50.

5.6.2 Fast withdrawal of immunosuppression will be performed per institutional standard procedure. Suggested course of taper is over 2-4 weeks starting at day 30-50.

5.7 Schedule of azacitidine administration

Azacitidine may be held or dose-adjusted based on the parameters below. If azacitidine is held > 6 weeks, the patient will remain on study but will be included in a separate analysis with regard to safety and efficacy.

5.7.1 Cytopenias prior to Cycle 1 require dose reductions as outlined below. Cytopenias following subsequent cycles require dose adjustments based on nadir counts as outlined in appendix 5.

- For platelets <30,000/uL, 50% dose reduction is recommended.
- If platelet transfusion dependent, hold therapy until platelets >30,000/uL.
- For neutrophils <750 not responsive to GCSF, 50% dose reduction is recommended.
- For neutrophils <500 not responsive to GCSF, hold therapy until toxicity improves.

5.7.2 Renal insufficiency:

- Creatinine <2X baseline: No dose adjustment is required.
- Creatinine 2-3X above baseline: Hold therapy until AKI resolves. Restart therapy at 50% dose reduction. If well-tolerated, full dose may be administered.

5.7.3 Hepatotoxicity:

- AST/ALT <5x ULN: No dose adjustment is required.
- AST/ALT 5-20x ULN: Hold therapy until transaminitis resolves. May consider 50% dose reduction and close monitoring.
- AST/ALT >20x ULN: Hold therapy until transaminitis resolves
- Total bilirubin 3-10x ULN: Hold therapy until resolution; may consider 50% dose reduction and close monitoring
- Total bilirubin >10x ULN: Hold therapy until resolution.

5.7.4 For other toxicities, repeat test twice weekly:

- Other laboratory abnormalities to be evaluated on a per-patient basis. Options include holding azacitidine until resolution, versus starting azacitidine at 50% dose reduction at the PI and treating physician's discretion.

5.7.5 Except for ALL patients classified as Low Risk, administration of azacitidine will commence during SWI or FWI if criteria are met (see section 6.2 for dose adjustment guidelines).

- Azacitidine will be given at a dose of 40mg /m^2 IV or SC daily for 4 days

5.7.6 Administration of azacitidine <u>following</u> SWI or FWI:

- PB and BM chimerism and MRD will be obtained as above and risk will be re-assessed.
- <u>*No Intervention:*</u> For Low Risk ALL patients, azacitidine will be deferred. Patients will receive standard treatment and will be followed by BM every 3 months for 9-12 months.
- <u>*Azacitidine without DLI:*</u> Patients meeting Standard Risk criteria will receive azacitidine without DLI at a dose of 40mg/m^2 IV or SC x 4 days every 6 weeks. DLI should be considered for patients with resolved Grade 2 GHVD if pre- or post-transplant MRD is positive.
- <u>*Azacitidine with DLI:*</u> Patients meeting High Risk criteria, who also have available cells, will receive DLI in addition to azacitidine. Patients with history of Grade 2 GVHD (which has resolved) may also receive DLI if pre- or post-transplant MRD is positive at the discretion of the treating physician.
    - Administration of azacitidine following withdrawal of immunosuppression will commence (typically on a Monday) 2-8 weeks following *completion* of SWI/FWI (see section 6.2 for dose adjustments).
    - Azacitidine will be given at a dose of 40mg /m^2 IV or SC daily for 4 days every 6 weeks.
    - Escalating doses of DLI will be administered (typically on a Friday) within 5 days following the last dose of azacitidine every cycle (see section 5.8 for DLI schedule).
    - For patients with negative pre- and post-transplant MRD:
        - Once MDC resolves to FDC in both blood and bone marrow, DLI will be discontinued and patient will continue to receive azacitidine alone for a maximum of 7 cycles as tolerated.
        - If FDC is detected in blood, bone marrow chimerism must be obtained and confirmed to be mixed before proceeding with DLI.
- If DLI is administered without azacitidine during cycles 2 through 7, the subject should continue with all subsequent study assessments, but will be analyzed separately with regard to safety and efficacy.
- Cycles may be delayed for reasons such as toxicity, as per dose adjustment section 6.0.
- For all patients who receive azacitidine, a total of 7 cycles may be administered as tolerated.

5.8 Criteria for DLI

5.8.1 Deep Sequencing-MRD (ALL only)

- For Low-Risk ALL patients with Deep Sequencing-MRD negative on all measurements before and after transplant, azacitidine and DLI administration will not be given.
- Low-Risk ALL patients will undergo BM MRD and chimerism exams every 3 months for the first 9-12 months post-transplant. Intervention will be resumed if MRD becomes positive.

5.8.2 Mixed Chimerism

- Patients with persistent MDC following withdrawal of immunosuppression will receive DLI with the next cycle of azacitidine. Mixed chimerism of low purity should be repeated before proceeding with DLI and/or FWI in order to ensure mixed chimerism. If MDC persists after cycle 7, DLI may be administered without azacitidine after discussion with the study chair.
- Patients who revert to MDC on subsequent PB or BM testing will receive DLI with the next cycle of azacitidine.
- Patients with FDC are generally not eligible for further DLI except as described below.

5.8.3 Pre-Transplant MRD positive

- Patients with pre-transplant MRD $>10^{-5}$ are eligible to receive escalating DLI regardless of chimerism status if there is no active GVHD.
- If no active GVHD, DLI should continue until maximum DLI dose is achieved.

5.8.4 Post-Transplant MRD positive

- For patients with persistent MRD post-transplant ($>1x10^{-5}$ or $1x10^{-6}$ and increasing) and with no active GVHD, azacitidine and escalating doses of DLI should be administered.
- DLI should be escalated to maximum dose and administered while MRD remains positive. If MRD is positive after cycle 7, DLI may be administered with or without azacitidine after discussion with the study chair.

5.9 Intra-patient DLI dose escalation to be used on day 5 (following 4 days of azacitidine):

5.9.1 Mismatched related donor or any unrelated donor:

- 1st dose: 5×10^5 /kg -- 1×10^6 /kg of CD3+ cells
- 2nd dose: 1×10^6/kg -- 1×10^7 /kg of CD3+ cells
- 3rd and all subsequent doses: 1×10^7 /kg -- 5×10^7/kg of CD3+ cells

5.9.2 Matched related donor:

- 1st dose: 1×10^7 /kg of CD3+ cells
- 2nd and subsequent doses: 5×10^7 /kg of CD3+ cells

5.9.3 Peripheral blood chimerism will be repeated during or after week 4 of every cycle, and the next cycle will not be initiated until results are obtained.

5.10 Schedule of safety parameter studies

5.10.1 Pre-therapy laboratory evaluations should be completed within 7 days prior to each azacitidine cycle:

- Liver function (AST, ALT, bilirubin) and renal function (creatinine) tests
- Complete blood count (consider bone marrow aspiration if cytopenias develop if clinically indicated).
- T cell number / function: Lymphocyte phenotyping, quantitative regulatory T cells, T cell function (PHA)
- Chimerism testing as noted above.

- MRD (BM) by flow cytometry. Peripheral blood MRD (flow, DSMRD for ALL) may be sent at the discretion of the treating physician.

5.10.2 Pre-therapy clinic visit should be conducted within 14 days prior to the cycle start date.

5.10.3 On-therapy evaluations:

- Weekly liver function and renal function tests while receiving azacitidine. If patients have tolerated two cycles of azacitidine without requirement for further intervention, complete blood count, liver and renal function tests may be performed every two weeks during their remaining cycles.
- Bone marrow aspiration for MRD and chimerism as above every 2 cycles (or with each cycle if PB chimerism is 100% and BM chimerism remains mixed).

5.11 Schedule of correlative studies

5.11.1 T cell number and function with each cycle will be assessed while receiving azacitidine.

5.11.2 AML gene expression and mutation panel (AML-GEMP) will be assessed in those patients with a diagnosis of AML. Samples from BM and PB will be collected at day +30, +90 and +180 (+/- 14 days) (see Appendix 4).

Evaluation	Prior to enrollment	Prior to FWI/ SWI/ Day 28 (can be between Day 21 and Day 42	Prior to each azacitidine cycle	Weekly during azacitidine	3 and 6 months post HCT (+/- 28 days)[3]	From 9 months to 24 months post-HCT: Every 3 months (+/- 28 days)[3]
History and Physical (including GVHD assessment)	X	X	X		X	X
Routine labs[1]	X	X	X	X[1]	X	X
Peripheral Blood: Chimerism and Minimal Residual Disease		X	X			
Bone Marrow: Chimerism and minimal residual disease		X[2]	X[2]		X[2]	X[2]
T cell number / function		X	X[3]			
Peripheral Blood: AML gene expression and mutation panel (AML-GEMP) Optional		X			X	
Bone marrow: AML-GEMP Optional		X			X	

Table 2: Schedule of required investigations

1. Routine labs to include Complete Blood Count with differential, and chemistry including serum creatinine, AST, ALT, bicarbonate and total bilirubin within 1 week prior to azacitidine administration. If a patient has tolerated two cycles of azacitidine without requirement for

further intervention, complete blood count, liver and renal function tests may be performed every two weeks during their remaining cycles.

2. BM aspiration should be performed every other cycle (including day +30 and day +90). However, if patient is found to be FDC on peripheral blood sample, the next cycle that includes DLI cannot be initiated until BM chimerism is obtained and mixed BM chimerism is confirmed (unless DLI is indicated for (+) MRD). The following will be investigated with each BM exam (not including correlative samples): Chimerism, morphology, MRD (by flow cytometry, and/or FISH, PCR, and/or deep sequencing (DS-MRD). Whenever possible, the most sensitive MRD technique should be used. Correlative AML-GEMP will be collected and sent to the NIH on days +30, +90, and +180.
3. If a 3-monthly time point coincides with a pre-cycle assessment, a separate 3-monthly assessment does not need to be completed. However, bone marrow MRD/chimerism and AML-GEMP specimens, if applicable, must be included with the pre-cycle assessments. If azacitidine cycles have been deferred, patients should undergo the required assessments including chimerism and lymphocyte quantification and function at least every 3 months.

5.12 Duration of study participation and follow-up

All patients will be followed as part of routine clinical care at a frequency of no less than every 3 months for at least 2 years post-transplant. All patients will have a thorough history and physical exam as well as laboratory monitoring for signs of GVHD as part of routine clinical care for the duration of the study (2 years post-transplant).

6.0 Toxicity Management and Dose Modifications

6.1 DLI Toxicity: GVHD

- DLI will be GCSF-mobilized unless otherwise specified; DLI dose modifications may be made on a case-by-case basis. See Appendix 3 for additional details regarding toxicity management.
- As soon as GVHD is suspected, all further DLI or withdrawal of immunosuppression should be stopped and the patient monitored, regardless of chimerism or MRD status.
- Azacitidine may continue at the discretion of the treating physician for grade I-II acute GVHD (aGVHD) or mild chronic GVHD (cGVHD).
- If aGVHD or cGVHD is confirmed, the patient should initiate therapy as per institutional standard of care.
- All instances of aGVHD or cGVHD in patients in the intervention group should be reported to the study chair within 10 days of learning about them, and grade III-IV aGVHD and severe cGVHD should be reported within 24 hours of learning about them (see Adverse Events Section 11.0).
- At each instance of grade III-IV aGVHD or cGVHD reported in the study, subgroup analysis will be performed in the high-risk group in order to monitor safety of DLI, as outlined in section 8.7.

6.2 Azacitidine Toxicity

Guidelines for adjusting azacitidine dose administration are based on the Common Terminology Criteria for Adverse Events (CTCAE version 4.0) applied to patient reports, history and physical, and laboratory evaluations performed each cycle. Laboratory evaluations for toxicity will be performed the week prior to each cycle. Dose adjustments are as follows (*28*):

6.2.1 Development of drug-related grade 4 organ toxicity or severe infection:

- Discontinuation of azacitidine

6.2.2 Hematologic toxicity:

- Grade I-II toxicity: No dose adjustment is required.

- For platelets <30,000/uL on a stable transfusion regimen, 50% dose reduction is recommended.
- For platelets <15,000/uL, hold therapy until toxicity improves.
- For neutrophils <750 not responsive to GCSF, 50% dose reduction is recommended.
- For neutrophils <500 not responsive to GCSF, hold therapy until toxicity improves.
- See Appendix 5 for guidelines on subsequent dose adjustments based on nadir counts.

6.2.3 Renal toxicity
- Grade I: No dose adjustment is required.
- Grade II: Hold therapy until Grade II toxicity resolves. Restart therapy at 50% dose reduction. If well-tolerated, full dose may be administered.

6.2.4 Hepatotoxicity
- Grade I-II: No dose adjustment is required.
- Grade III (AST/ALT >5x ULN): Hold therapy until Grade III toxicity resolves if clearly related to azacitidine administration. Otherwise, consider 50% dose reduction and close monitoring.

6.2.5 For other toxicities, repeat test twice weekly:
- If abnormality resolves within 2 weeks, restart azacitidine at 50% dose reduction (20mg/m^2) to complete 4 doses. Proceed with DLI after 4th dose.
- If abnormality does not resolve after 2 weeks, hold azacitidine but proceed with DLI if no signs / symptoms of GVHD. Azacitidine 50% dose reduction should be used at start of next cycle even if lab abnormality returns to baseline.

7.0 Criteria for Termination

7.1 Conditions for terminating the study:

The PI may terminate the study for any of the following reasons:

7.1.1 Significant toxicities are observed.
7.1.2 Stopping rules for interim toxicity and relapse futility are triggered as per Section 8.7.3.
7.1.3 It becomes clear that the study treatment is less effective than standard treatment.
7.1.4 All data have been collected.

7.2 Conditions for terminating individual patient participation in the study:

The PI may terminate the participation of an individual patient for any of the following reasons:

7.2.1 Withdrawal from the study by patient / parents or physician
7.2.2 Death
7.2.3 Leukemia relapse, defined as >5% leukemic blasts on bone marrow examination or more than 1% leukemic cells by immunoflow or FISH.
7.2.4 Loss to follow-up or non-compliance with study procedures.

8.0 Statistical Considerations

8.1 Primary Efficacy endpoint definitions

8.1.1 Relapse rate

8.2 Secondary / Exploratory endpoints

8.2.1 Relapse-free survival
8.2.2 Median time to relapse

8.3 Primary Safety Endpoint Definitions

8.3.1 National Cancer Institute CTCAE version 4.0 grade 3 or higher renal, hepatic, cardiac, pulmonary, or neurologic toxicity;
8.3.2 Grade III/IV acute GVHD or Severe/Extensive chronic GVHD
8.3.3 Serious infection
8.3.4 Severe hematologic toxicity/ graft failure
8.3.5 >2 dose reductions for any reason.

8.4 Sample size

8.4.1 The sample size is estimated using Bayesian sequential monitoring design with toxicity and response outcomes as multiple endpoints(45). It is assumed that the rate of relapse in pediatric acute leukemia post-transplant would be 40% (as shown in our pilot study), azacitidine +/- DLI would reduce the 2-year relapse rate by approximately 40% to a rate of 25%. A sample size of 67 evaluable patients is required to provide 80% power at a one-sided significance level of 0.05 to test the hypothesis based on the relapse rate of H0: relapse rate > 40% (historically-controlled) versus Ha: relapse rate < 25% (treatment-targeted). The study will enroll patients until accrual of 67 patients is achieved.

8.4.2 Enrollment of 67 patients will be attainable with enrollment at multiple centers. Investigators from All Children's Hospital, Lucille Packard Children's Hospital have written letters of support expecting to enroll 30-40 patients over 2 years.

8.5 Estimated Duration of the Study

8.5.1 67 patients are expected to enroll; enrollment over 2 years is anticipated. Study intervention and data collection (2 years) are expected to be completed by 4 years. Preliminary safety analysis will be available within 1 year.

8.6 Statistical Analysis

8.6.1 Patient demographics and baseline characteristics including age, sex, race, ethnicity and medical conditions will be summarized using descriptive statistics. For quantitative parameters, descriptive statistics will include the mean, standard deviation, minimum, median and maximum. For qualitative parameters, descriptive statistics will include the frequency and proportions.

8.6.2 The primary endpoint parameters: (1) relapse rate and (2) incidence of Grade III-IV aGVHD, severe cGVHD, and Grade IV toxicities, will be estimated using proportions with 95% CI.

8.6.3 The chimerism parameters (CD3+, CD14/15+, CD19+, CD33+, CD34+) and incidence of acute and chronic GVHD will also be analyzed using descriptive statistics including means, standard deviation and 95% CI.

8.7 Interim analysis and stopping rules for safety and efficacy

8.7.1 As a phase II study with 67 evaluable patients, the study is designed to have interim stopping rules based on the safety/toxicity evaluation (including death and incidence of severe GVHD). See Section 11.0 for reporting SAEs associated with study stopping rules.

8.7.2 The occurrence of any serious adverse events (SAEs) will be reported to the Coordinating Trial Center within 24 hours of learning about them, and within 10 days in writing. See Section 11.0 for SAE definitions and reporting requirements. A rate of SAEs of up to 20% is deemed acceptable in view of the potential benefit of the therapy. Accrual will stop if 3 patients with SAEs are observed in the first 3-6 patients undergoing the intervention, if 4 patients with SAEs are observed in the first 7-9 patients undergoing the intervention, or if 5 patients with SAEs are observed in the first 10-13 patients undergoing the intervention. The following table displays the minimum

number of SAEs for the study to be stopped at each accrual period and the probability of early stopping at the assumed SAE rate.

Patients on Study	4-6	7-9	10-13	14-17	18-21	22-27
Number of patients with SAE	≥3	≥4	≥5	≥6	≥7	≥8
15%SAE	1.2%-4.2%	1.1%-2.8%	0.9%-2.7%	0.9%-2.4%	0.9%-2.0%	0.8%-2.6%
20%SAE	2.6%-8.2%	2.9%-6.6%	2.6%-6.9%	3.2%-6.8%	3.5%-6.6%	3.6%-8.2%
25%SAE	4.7%-13.2%	5.8%-11.7%	5.8%-12.6%	7.3%-12.8%	8.2%-12.7%	8.9%-14.2%

8.7.3 Stopping rules for interim toxicity and relapse futility

- Study data will be analyzed for relapse rates within 3 months for efficacy assessment. Such early futility assessment will be done when the first 14 patients have completed the 3-month follow-up in the study. If there are 9 or more relapses out of 14 patients during the futility assessment, enrollment will be stopped due to futility. Otherwise an additional 54 patients will be accrued for a total of 67 patients in the study. By the end of the study, if there are 32 or more relapses observed, further investigation will not proceed. In this study the rate of relapse in pediatric acute leukemia post-transplant is assumed to be 40% (as shown in our pilot study), azacitidine +/- DLI would reduce the 2-year relapse rate by approximately 40% to a rate of 25% and that the toxicity rate in pediatric acute leukemia post-transplant under azacitidine +/- DLI would be 20%. The stopping rules for toxicity and relapse rate under sequential design of multiple endpoints are shown below:
- For patients receiving DLI, a subset analysis of incidence of Grade III-IV aGVHD and mortality secondary to GVHD will be performed when a new patient is diagnosed with Grade III-IV GVHD or chronic GVHD.
    - Reported rates of grade III-IV acute GVHD following DLI range from 20-35%; GVHD-related mortality ranges from 5-15%.
    - If rates of Grade III-IV aGVHD or GVHD-related mortality in this study exceed published rates, enrollment and further DLI administration will be suspended while further safety analysis is performed.

Patients on Study	**<14**	**15-21**	**22-28**	**29-35**	**36-42**	**43-49**	**50-56**	**57-63**
Number of patients with SAE (Toxicity)	>8	≥10	≥13	≥16	≥18	≥21	≥23	≥26
Number of patients with relapse in 3 months	>9	≥13	≥16	≥19	≥23	≥26	≥29	≥32

9.0 Risks

9.1 Graft vs host disease

- Patients undergoing withdrawal of immunosuppression or DLI are at higher risk of developing severe and potentially fatal GVHD. The risk of GVHD after withdrawal of immunosuppression and DLI is 50 – 65%, and the chance of dying from it is approximately 10%.

9.2 Azacitidine risks

Primary risks of azacitidine are complications associated with hematologic toxicity such as bleeding and infection. At the dose proposed in this study, the risk of Grade III hematologic toxicity is low (<10%). Dose reduction will be used as described above for any undue toxicity encountered. A comprehensive list of side effects that have been reported with azacitidine (including cases in which the drug is given at a higher dose) is provided below:

9.2.1 Common side effects (>10%):

- Cardiovascular: Peripheral edema (7% to 19%), chest pain (16%), pallor (16%), pitting edema (15%)
- Central nervous system: Fever (30% to 52%), fatigue (13% to 36%), headache (22%), dizziness (19%), anxiety (5% to 13%), depression (12%), insomnia (9% to 11%), malaise (11%), pain (11%)
- Dermatologic: Bruising (19% to 31%), petechiae (11% to 24%), erythema (7% to 17%), skin lesion (15%), rash (10% to 14%), pruritus (12%)
- Endocrine & metabolic: Hypokalemia (6% to 13%)
- **Gastrointestinal**: Nausea (48% to 71%), vomiting (27% to 54%), diarrhea (36%), constipation (34% to 50%), anorexia (13% to 21%), weight loss (16%), abdominal pain (11% to 16%), abdominal tenderness (12%)
- **Hematologic**: Thrombocytopenia (66% to 70%; grades 3/4: 58%), anemia (51% to 70%; grades 3/4: 14%), neutropenia (32% to 66%; grades 3/4: 61%), leukopenia (18% to 48%; grades 3/4: 15%), febrile neutropenia (14% to 16%; grades 3/4: 13%), myelosuppression (nadir: days 10-17; recovery: days 28-31)
- Local: Injection site reactions (14% to 29%): Erythema (35% to 43%; more common with I.V. administration), pain (19% to 23%; more common with I.V. administration), bruising (5% to 14%)
- Neuromuscular & skeletal: Weakness (29%), rigors (26%), arthralgia (22%), limb pain (20%), back pain (19%), myalgia (16%)
- Respiratory: Cough (11% to 30%), dyspnea (5% to 29%), pharyngitis (20%), epistaxis (16%), nasopharyngitis (15%), upper respiratory tract infection (9% to 13%), pneumonia (11%), crackles (11%)
- Miscellaneous: Diaphoresis (11%)

9.2.2 Less common:

- Cardiovascular: Cardiac murmur (10%), hypertension (≤9%), tachycardia (9%), hypotension (7%), syncope (6%), chest wall pain (5%)
- Central nervous system: Lethargy (7% to 8%), hypoesthesia (5%), post-procedural pain (5%)
- Dermatologic: Cellulitis (8%), urticaria (6%), dry skin (5%), skin nodule (5%)
- Gastrointestinal: Gingival bleeding (10%), oral mucosal petechiae (8%), stomatitis (8%), weight loss (≤8%), dyspepsia (6% to 7%), hemorrhoids (7%), abdominal distension (6%), loose stools (6%), dysphagia (5%), oral hemorrhage (5%), tongue ulceration (5%)
- Genitourinary: Dysuria (8%), urinary tract infection (8% to 9%)
- Hematologic: Hematoma (9%), post procedural hemorrhage (6%)
- Local: Injection site reactions: Pruritus (7%), hematoma (6%), rash (6%), granuloma (5%), induration (5%), pigmentation change (5%), swelling (5%)
- Neuromuscular & skeletal: Muscle cramps (6%)
- Renal: Hematuria (≤6%)
- Respiratory: Rhinorrhea (10%), rales (9%), wheezing (9%), breath sounds decreased (8%), pharyngolaryngeal pain (6%), pleural effusion (6%), postnasal drip (6%), rhinitis (6%), rhonchi (6%), nasal congestion (6%), atelectasis (5%), sinusitis (5%)

- Miscellaneous: Lymphadenopathy (10%), herpes simplex (9%), night sweats (9%), transfusion reaction (7%), mouth hemorrhage (5%)

9.2.3 Rare side effects and/or case reports:

Abscess (limb, perirectal), acute febrile neutrophilic dermatosis (Sweet's syndrome), agranulocytosis, **anaphylactic shock**, atrial fibrillation, azotemia, blastomycosis, **bone marrow depression/failure**, bone pain aggravated, cardiac failure, cardiorespiratory arrest, catheter site hemorrhage, cellulitis, **cerebral hemorrhage**, CHF, cholecystectomy, cholecystitis, congestive cardiomyopathy, dehydration, diverticulitis, eye hemorrhage, fibrosis (interstitial and alveolar), **gastrointestinal hemorrhage**, glycosuria, hemoptysis, hepatic coma, hypersensitivity reaction, hypophosphatemia, infection (bacterial), injection site infection, injection site necrosis, interstitial lung disease, **intracranial hemorrhage**, leukemia cutis, reversible liver injury, lung infiltration, melena, neutropenic sepsis, orthostatic hypotension, pancytopenia, pneumonitis, polyuria, pyoderma gangrenosum, renal failure, renal tubular acidosis, seizure, respiratory distress, sepsis, septic shock, serum bicarbonate levels decreased, **serum creatinine increased**, splenomegaly, systemic inflammatory response syndrome, toxoplasmosis, tumor lysis syndrome, veno-occlusive disease of the liver.

9.3 Risk of extramedullary relapse or late relapse

Patients undergoing immunomodulatory therapy after transplant may develop extramedullary leukemia relapse despite prevention of bone marrow disease. Although their overall risk of relapse will not be increased by using immunomodulatory therapy, it is possible that this therapy will increase the tendency to relapse outside of bone marrow. In addition, previous studies have shown that patients undergoing immunotherapy may relapse later than usual (24-36 months post-transplant). While this therapy does not increase the risk of relapse overall, it is possible that immunotherapy only delays relapse in some patients.

9.4 Chimerism testing risks

9.4.1 Bone marrow aspiration

Patients will have BM exams every other cycle or about every 3 months after transplant. It is estimated that up to 6 bone marrow exams may be required in some patients. All bone marrow exams in children are done under general anesthesia, which carries a small risk (1:10,000- 1:30,000) of adverse event due to anesthesia, including death. There is universal but minor risk of local tenderness at the site of bone marrow exam, and a small risk (<1:100) of infection of the bone marrow exam site. Bone marrow biopsies are not required in this study; however, sometimes a treating physician may request a bone marrow biopsy in addition to aspirate.

9.4.2 Blood loss due to chimerism testing

Eight ml of blood or bone marrow will be obtained with each chimerism testing (3 – 12 times over the period of one year). This amount of blood is easily replaced by bone marrow production, even in a post-transplant patient. The blood will be obtained through the central line (Broviac) or with other routine blood tests.

9.5 Risk of overtreatment

In a previous analysis, approximately 30% of patients with mixed chimerism did not develop leukemia relapse, despite mixed chimerism. Unfortunately, those patients cannot be distinguished from 70% of patients who will relapse. In a more recent subset of high-risk MRD(+) patients, 7 out of 11 high-risk patients had mixed chimerism (and thus would be treated with azacitidine plus DLI on this protocol), and of those 7, 5 relapsed. Thus 2 of 7 would be "over-treated" (although these patients also received some degree of immunotherapy that may have helped prevent relapse). Patients "over-treated" on this study may be at an increased risk of developing GVHD. These patients are expected to represent a

very small minority of patients on this study, as patients with negative MRD (Standard-Risk) will not receive DLI.

10.0 Data and Safety Monitoring Plan

10.1 Oversight and monitoring plan

The UCSF Helen Diller Family Comprehensive Cancer Center (UCSF-HDFCCC) Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and patient safety for all UCSF-HDFCCC institutional clinical studies **(see Appendix 19.6).** A summary of DSMC activities for this study includes:

- Review of subject enrollment
- Review of all serious adverse events (See section 11.0)
- Monitoring every six months (depending on study accrual)
- Minimum of a yearly audit

10.2 Monitoring and reporting guidelines

Investigators will conduct continuous review of data and patient safety at weekly meetings where the results of each patient's treatment are discussed and 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 grade 3-5 AE's and SAE's will be entered in the HDFCCC Clinical Trials Management System. All institutional Phase 2 studies are designated with a moderate risk assessment; therefore, the data is monitored every six months, with twenty percent of the subjects monitored (or at least three subjects if the calculated value is less than three).

10.3 Regulatory considerations

This study will be reviewed by the UCSF–HDFCCC Protocol Review Committee, in addition to the UCSF IRB (the Committee on Human Research, or CHR). Participating sites will submit this study to the relevant local IRB for review.

10.4 Independent ethics committees / Institutional Review Board

This protocol and the informed consent will be approved by the IRB at all sites. The Principal Investigator at each site is responsible for keeping the IRB advised of the progress of the study and of any changes made in the protocol prior to implementation. The Principal Investigator will also keep the IRB informed of any significant adverse reactions (see section 11.0), and any protocol exceptions or deviations. Records of all study review and approval documents must be kept on file by the Principal Investigator and are subject to FDA inspection during or after completion of the study. The IRB at all sites will receive notification of the termination of the study.

11.0 Adverse Events

11.1 Grading adverse events and serious adverse events

This study will use the NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v 4.0) found at the following website: http://evs.nci.nih.gov/ftp1/CTCAE/About.html for grading the severity of adverse events. The investigator is responsible for making an assessment of whether or not it is reasonable to suspect a causal relationship between the adverse event and the study treatment.

11.2 Adverse Event Review and Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as per the study schedule, and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-

up period. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

11.3 Definition of Adverse Event

An adverse event (also known as an adverse experience) is defined as any untoward medical occurrence associated with the use of the study intervention 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 study intervention, without any judgment about causality. An adverse event can arise from any use of the study intervention (e.g., off-label use, use in combination with another treatment) and from any route of administration, formulation, or dose, including an overdose.

11.4 Adverse reaction

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

11.6 Suspected

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

11.7 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 or study intervention under investigation.

11.7 Serious

An adverse event or suspected adverse reaction is considered serious if, in the view of the study PI 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, but 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.

11.8 Life-threatening

An adverse event or suspected adverse reaction is considered life-threatening if, in the view of the study PI, 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.

11.9 Evaluation of an Adverse Event

All grade 3 and above adverse events will be entered into OnCore®, whether or not the event is believed to be associated with use of the study drug.

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

11.10 NCI Common Terminology for Adverse Events (CTCAE)

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded as per study requirements.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

11.11 Follow-up of Adverse Events

All adverse events will be followed with appropriate medical management until resolved. Patients removed from study for unacceptable adverse events will be followed for 90 days or until resolution or stabilization of the adverse event. For selected adverse events for which administration of the investigational treatment was stopped, a re-challenge of the subject with the investigational treatment may be conducted if considered both safe and ethical by the

Investigator.

11.12 Adverse Events Reporting

The Study Chair will assess all adverse events and determine reportability requirements to the UCSF Data and Safety Monitoring Committee (DSMC) and UCSF's Institutional Review Board, and the Committee on Human Research (CHR). Adverse events will be reported to the DSMC via OnCore.®

All adverse events entered into OnCore® will be reviewed by the Helen Diller Family Comprehensive Cancer Center Site Committee on a monthly basis. The Site Committee will review and discuss monthly the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study treatment.

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 treatment.

In addition, all 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 weeks.

Adverse event reporting will continue until 24 months post-transplant, or until 90 days after the patient stops trial participation, if applicable.

11.14 Expedited Reporting

The following adverse events, when they occur in patients undergoing withdrawal of immunosuppression or DLI, must be reported to the Study Chair within 10 days of occurrence:

- Any evidence of acute or chronic GVHD toxicities of any organ of Grade III or higher (using CTCAE v. 4.0)
- Any change in GVHD prophylaxis or conditioning regimen outside of the planned GVHD prophylaxis taper.
- Any invasive fungal infection or disease caused by viral infections in patients treated with immunosuppression for GVHD that developed following study intervention
- Inpatient hospitalization or prolongation of existing hospitalization
- Relapse of leukemia/malignancy.

<u>Reporting to the Data and Safety Monitoring Committee</u>

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study treatment 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 Study Chair within one business day of knowledge of the event, who must then notify the DSMC Chair (or qualified alternate) within 1 business day. The contact may be by phone or e-mail.

<u>Reporting Committee on Human Research (Institutional Review Board)</u>

The Study Chair must report events meeting the UCSF CHR definition of "Unanticipated Problem" (UP) within 10 business days of his/her awareness of the event. Participating site Principal Investigators must report events to their IRB as per institutional guidelines.

<u>Reporting Requirements for participating sites</u>:

Any adverse event meeting SAE criteria must be reported to UCSF within 24 hours of the site learning about the event.

- SAEs must be reported to UCSF PI via the SAE Case Report Form:

- SAEs and Safety Reports will be submitted to IRBs as per local requirements.

11.15 Review of adverse event rates

If the study has an increase of unexpected or expected Severe Adverse Events above the rate reported in this protocol, the increased rate of AEs will be reported to the DSMC at the time of identification. The DSMC Chair and Study Chair will discuss the findings and proceed with a written course of action. If at any time the Study Chair stops enrollment or stops the study due to safety issues the DSMC Chair must be notified within 24 business hours via e-mail. The DSMC must receive a formal letter within 10 business days and the IRB must be notified.

If any of the above action occurs in multiple-institutional clinical trial, the Study Coordinator will insure that all participating sites are notified.

11.16 SAE Reports Associated with Study Stopping Rules

The following SAEs have a potential to trigger study stopping rules (see Section 8.7) and must be reported to the Study Chair within 24 hours:

11.16.1 aGVHD of grade IV

11.16.2 Severe cGVHD

11.16.3 Any organ toxicity of grade IV (using CTC Common Toxicity criteria version v4.0) that is judged to be related to withdrawal of immunosuppression, azacitidine, or DLI.

11.16.4 Any death judged to be related to fast immunosuppression withdrawal, azacitidine, or DLI.

11.16.5 Note: investigators must report any aGVHD grade IV, severe cGVHD, any organ grade IV toxicity or any death in patients undergoing immunosuppression withdrawal to the Study Chair within 24 hours. Judgment about contribution of withdrawal of immunosuppression to the reported severe event will be made by DSMC within 10 days.

11.16.6 Phone or fax reports of SAE's due within 24 hours to:

11.17 DSMC review of treatment toxicity

Judgment about contribution of withdrawal of azacitidine +/- DLI to toxicity will be made by the DSMC and based on:

11.4.1 Temporal relationship of the event to the study drug;

11.4.2 Whether an alternative etiology has been identified;

11.4.3 Biological plausibility.

11.18 Classification of GVHD

To be classified as severe cGVHD, a minimum of 3 organs should be involved with stage 2 disease, or 2 organs with stage 3 disease. Please see Appendix 2 for a list of symptoms and staging. (For example: skin: 10 – 50% skin rash; Joint: mild joint contractures; and esophagus: dysphagia or odynophagia requiring dietary changes would qualify for severe cGVHD. Multiple symptoms within the same organ system should not be counted. Rash, sclerodermatous changes and dry flaky skin would all count as 1 organ/system involvement).

11.19 Hematopoietic toxicity:

11.4.4 Grade 3: ANC >500/microliter but < 1000/microliter for a duration of > 4 weeks. Platelets < 50,000/microliter but > 20,000/microliter for a duration of > 4 weeks.

11.4.5 Grade 4: neutrophils <500/microliter and/or platelets <20,000/microliter for a duration of > 4 weeks.

11.4.6 Grade 5: death due to bacterial or fungal infection or hemorrhage associated with hematopoietic toxicity.

12.0 Benefits

Treatment on this protocol may decrease the risk of leukemia relapse.

13.0 Alternatives

13.1 Not undergoing careful chimerism monitoring.

13.2 Not receiving azacitidine as prophylactic post-transplant therapy.

13.3 Performing chimerism testing and donor lymphocyte infusion off-study.

14.0 Cost

14.1 The patient will be responsible for the cost of all clinical procedures. Post-transplant azacitidine and immunomodulatory therapy with DLI have been shown to be effective to prevent leukemia relapse in the post-transplant setting; third party payers have usually covered these costs. The frequency of bone marrow testing and DLI may be increased for some patients, based on risk factors for relapse.

15.0 Record Keeping and Record Retention

The Principal Investigator at each site is required to maintain adequate records of the disposition of the study treatment, including dates, quantity, and use by subjects. The Principal 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 study treatment. Case histories include the case report forms and supporting data (e.g., signed and dated consent forms and medical records, such as 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 CRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, CHR 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 and if applicable to the study intervention, 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.

16.0 Coordinating Center Documentation of Distribution

It is the responsibility of the Study Chair to maintain adequate files documenting the distribution of study documents as well as their receipt (when possible). The HDFCCC recommends that the Study Chair maintain a correspondence file and log for each segment of distribution (e.g., participating sites, etc.):

- Correspondence file: should contain copies (paper or electronic) of all protocol versions, cover letters, amendment outlines (summary of changes), etc., along with distribution documentation and (when available) documentation of receipt.

- Correspondence log: should be a brief list of all documents distributed including the date sent, recipient(s), and (if available) a tracking number and date received.
- At a minimum, the Study Chair must keep documentation of when and to whom the protocol, its updates and safety information are distributed.

17.0 Multicenter communication

The UCSF Coordinating Center provides administration, data management, and organizational support for the participating sites in the conduct of a multicenter clinical trial. The Coordinating Trial Center will also coordinate conference calls with participating sites at the completion of each cohort or more frequently as needed to discuss risk assessment. The following issues will be discussed as appropriate:

- Enrollment information
- Adverse events (i.e. new adverse events and updates on unresolved adverse events and new safety information)
- Protocol violations
- Other issues affecting the conduct of the study
- Record Keeping and record retention

18.0 Protection of Human Subjects

Prior to implementing this protocol at UCSF, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the UCSF Committee on Human Research (CHR). Prior to implementing this protocol at the participating sites, each site must obtain IRB approval for the UCSF CHR approved protocol. The following documents must be provided to UCSF before the participating site can be initiated and begin enrolling participants:

- Participating Site IRB approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Participating Site IRB approved consent form
- Participating Site IRB membership list
- Participating Site IRB's Federal Wide Assurance number and OHRP Registration number
- Curriculum vitae and medical license for each investigator and consenting professional
- Documentation of Human Subject Research Certification training for investigators and key staff members at the Participating Site
- Participating site laboratory certifications and normal reference ranges

UCSF will also request that all sub-sites send their Data Safety and Monitoring Plan (DSMP) to UCSF for review for approval. If a sub-site does not have its own DSMP in place, UCSF will at that time review the resources necessary to include that sub-site and determine whether the UCSF study personnel are able to manage the regulatory burden for that sub-site. Upon receipt of the required documents, UCSF will formally contact the site and grant permission to proceed with enrollment.

18.1 Protection from Unnecessary Harm

Each clinical site is responsible for protecting all subjects involved in human experimentation. This is accomplished through the CHR mechanism and the process of informed consent. The CHR reviews all proposed studies involving human experimentation and ensures that the subject's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The CHR also reviews the informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

18.2 Protection of Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign the HIPAA form and informed consent documents. The original signed document will become part of

the patient's medical records, and each patient will receive a copy of the signed document. The use and disclosure of protected health information will be limited to the individuals described in the informed consent document.

19.1 Appendix 1: Chimerism analysis

Chimerism will be determined using a semi-quantitative PCR-based method involving amplification of genes containing short tandem repeats. For each donor-recipient pair, informative alleles will be determined with a panel of short tandem repeat loci (VWF, D21S11, D18S51, D16S539, PENTA D, D3S1358, FGA, D7S820, D2S1338, D10S2325, D12S391, SE33, PENTA E). If multiple loci were informative, two loci will be selected for post-transplant testing for each donor-recipient pair. Peripheral blood mononuclear cells will be prepared for all patients using a Ficoll-Hypaque gradient. Cell subsets will be isolated using Miltenyi magnetic particles (,) to select for desired subset from the peripheral blood mononuclear cells. Purity will be determined for every subset. In order to consider the result valid, the purity of the subset should be ≥90%. For peripheral blood specimens cells will be selected based upon CD3+, CD14/15+ and CD19+ expression. For BM specimens, CD33+ and CD34+ subsets will also be analyzed. The DNA will be isolated from all specimens using spin columns containing a silica gel membrane ( ). The size of the amplified fragments will be determined using an automated nucleotide sequencer (Applied Biosystems, Foster City, CA, USA) and the quantity for each PCR product will be determined using peak areas. Sensitivity controls consisting of mixtures containing donor DNA mixed with recipient DNA are included in every assay. For most donor-recipient pairs, the level of sensitivity for detecting donor cells is ≥1%, depending upon the relative efficiency of amplification and detection of donor alleles. During validation, inter-assay variation was ±1% when the minority population represented 1-5% of specimen; inter-assay variation was ±3% when the minority population represented 6-10% of specimen; and inter-assay variation was 6% when the minority population represented ≥ 11% of the specimen.

In this study the goal will be to achieve 100% donor cells in all subsets.

19.2 Appendix 2: Assessment of Chronic GVHD

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritus ☐ Hair involvement ☐ Nail involvement **% BSA involved** ☐	☐ No Symptoms	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
EYES Mean tear test (mm): ☐ >10 ☐ 6-10 ☐ ≤5 ☐ Not done	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI TRACT	☐ No symptoms	☐ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	☐ Bilirubin or enzymes > 5 x ULN

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
LUNGS† **FEV1** ☐ **DLCO** ☐	☐ No symptoms ☐ FEV1 > 80% **OR** LFS=2	☐ Mild symptoms (shortness of breath after climbing one flight of steps) ☐ FEV1 60-79% **OR** LFS 3-5	☐ Moderate symptoms (shortness of breath after walking on flat ground) ☐ FEV1 40-59% **OR** LFS 6-9	☐ Severe symptoms (shortness of breath at rest; requiring 0_2) ☐ FEV1 ≤39% **OR** LFS 10-12
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

Other indicators, clinical manifestations or complications related to chronic GVHD (check all that apply and assign a score to its severity (0-3) based on its functional impact where applicable (none – 0,mild -1, moderate -2, severe – 3)

Esophageal stricture or web___	Pericardial Effusion___	Pleural Effusion(s)___
Ascites (serositis)___	Nephrotic syndrome___	Peripheral Neuropathy___
M yasthenia Gravis___	Cardiomyopathy___	Eosinophilia > 500µl___
Polymyositis___	Cardiac conduction defects___	Coronary artery involvement___
Platelets <100,000/µl ___	Progressive onset___	

OTHERS: Specify:_____

19.3 Appendix 3: Use of Donor Lymphocyte Infusions or Hematopoietic Progenitor Cell Boosts after Hematopoietic Progenitor Cell Transplant (HSCT)

Implementation date: [redacted] Obsolete date: _____

Review dates: [redacted] -new [redacted]

Revision dates: [redacted]

1.0 Objective:

1.1 To provide guidelines for use of Donor Lymphocyte Infusions (DLI) and hematopoietic progenitor cell boosts after transplant for patients in whom DLI is not performed on an investigational study.

2.0 Scope:

2.1 PBMT attending physicians, fellows, nurse specialists, and PBMT laboratory staff.

3.0 Materials/Equipment:

None

4.0 Definitions:

4.1 **"Additional donor cells"**: Donor lymphocyte infusion (DLI) and/or hematopoietic progenitor cell boost.

4.2 **aGvHD**: Acute graft vs. host disease

4.3 **DLI: Donor lymphocyte infusion:** Infusion of additional donor's cells into bone marrow transplant recipients with the goal to enhance graft-versus-leukemia effect or speed up immune reconstitution in recipients of T-cell depleted (TCD) transplants.

4.4 Therapeutic DLI – DLI used for the treatment of relapse (in malignancies) or treatment of infection (in malignancies or non-malignant disorders).

4.5 Prophylactic DLI – DLI used in a setting of mixed chimerism and without documentation of disease relapse (in malignancies), or used in order to enhance immune reconstitution (usually in T-cell depleted transplants).

4.4 **Hematopoietic progenitor cell boost**: Additional infusion of progenitor cells (autologous or allogeneic) in order to enhance engraftment in patients who are cytokine or transfusion dependent post transplant.

4.5 **PBMT**: Pediatric bone marrow transplant

4.6 **TCD**: T-cell depleted

5.0 Procedure:

5.1 Indications for DLI in leukemia patients:

5.1.1 A patient may undergo post-transplant DLI if:
Patient with malignancy has evidence of increasing host chimerism (defined as increase in host chimerism that is outside of laboratory error, obtained in the absence of acute infection), OR a patient has stable mixed chimerism that has not converted to full donor chimerism 4-8 weeks following discontinuation of immunosuppression, OR DLI can be given at any time post transplant if a patient has leukemia relapse post transplant measured by molecular, cytogenetic, immunoflow or bone marrow morphology findings. The amount of leukemic cells in the bone marrow should not exceed 5%. Patients with full donor chimerism who are at a very high risk of relapse (positive MRD prior to transplant, previous relapse after transplant, primary induction failure) may require DLI to speed up immune reconstitution post transplant and prevent relapse **AND:**

5.1.2 There is no evidence of active GvHD in a patient who is off GvHD prophylaxis and there is no history of acute GVHD of grade >II, **AND:**

5.1.3 Patient/family has understood risks and benefits of the procedure as outlined in main transplant consent..

5.1.4. Patients with a history of peri-engraftment syndrome or acute GVHD may be at a higher risk for GVHD following DLI than patients who never had peri-engraftment syndrome or GVHD and caution should be taken with using DLI in these patients.

5.2. Indications for DLI in patients undergoing TCD transplants:

5.2.1 At 90 days post transplant, there is evidence of engraftment but the absolute CD4 count is <100/microliter, or any time post-transplant if a patient has an infection (CMV, EBV, etc.) that has not responded to an appropriate trial of standard therapy (such as antivirals or Rituximab) **AND:**

5.2.2 There is no active GvHD or history of Grade II-IV, **AND:**

5.2.3 Patient/family has understood risks and benefits of the procedure as outlined in main transplant consent.

5.3. Indications for progenitor cell boost:

5.3.1. Progenitor cell boost is typically used in autologous transplant recipients or in TCD depleted transplant recipients. Rarely, progenitor cell boost shall be required, or available in allogeneic non-modified transplants. If, at 3 months post transplant, the patient has engrafted, but requires cytokines in order to keep ANC >500, or if patient requires platelet (PLT) transfusions in order to keep PLT >20,000 and packed red blood cells (PRBC) transfusions in order to keep hemoglobin >7 g/dl, and alloimmunization has been ruled out, additional infusion of stem cells, if available, may be done.

5.4 Frequency of chimerism testing and evaluation before and after giving additional donor's cells:

5.4.1 In patients with leukemia peripheral blood engraftment studies should be obtained at day 30 post transplant and then repeated monthly until full donor chimerism is achieved. Once full donor chimerism is confirmed, testing should be done every 3 months until 3 years post transplant. In patients with acute or cGVHD, chimerism should be obtained annually, or as clinically indicated. More sensitive testing for leukemia, such as deep sequencing (ClonoSight) may replace some or all chimerism tests.

In patients with non-malignancies, if not dictated by the protocol, engraftment should be obtained monthly until 1 year post transplant if there is evidence of ”unstable” mixed chimerism (defined as whole blood chimerism <70% and/or CD3+ chimerism <50%), and every 3 months if mixed chimerism is stable (documented by at least 2 monthly tests). During the second year post transplant chimerism will be tested every 3 months in patients with unstable chimerism and every 6 months in patients with stable chimerism. After that chimerism will be tested as clinically indicated.

5.4.2 In patients with leukemia, bone marrow examination should be done at approximately Day 30. Bone marrow should be evaluated for the presence of residual leukemia by the most sensitive and specific test available. Engraftment studies from the bone marrow should be obtained as well.

5.4.3. A donor shall undergo an infectious disease evaluation within 7 days of each allogeneic DLI donation (30 days for European donors) as outlined in CL 200: Autologous/Allogeneic Donor Evaluation.

5.4.4 A recipient shall be evaluated clinically and by performing liver function tests for infection and evidence of GVHD prior to each infusion of additional donor cells. If there is suspicion of infection, further work-up shall be done by ordering an appropriate infectious disease antigen tests. Infection is not an absolute contraindication for additional donor cells; in some instances an infection can be treated by the infusion of additional donor T-cells.

5.4.5 Patients who undergo additional donor cell infusions should be followed in the BMT clinic for evidence of GvHD. The frequency of visits may vary from once a week to once every 3 months, depending on patient's condition.

5.5. Timing of additional donor cell infusions:

5.5.1. Prophylactic DLI in patients with leukemia: please see SOP CL 246: Strategies for the Reduction of Relapse Risk in Patients With Acute Leukemias Undergoing Allogeneic Blood and Marrow Transplantation.

5.5.2. Prophylactic DLI after TCD transplants:

5.5.2.1 Prophylactic DLI can be given in patients undergoing TCD transplants if the absolute CD4 count is <100/microliter at 90 days post transplant. DLI can be repeated at 4-8 week intervals until the CD4+ count is >100/microliter as long as there is no evidence of aGvHD.

5.5.3. Therapeutic DLI in patients with leukemia:
DLI can be given at any time post-transplant if there is cytogenetic or molecular evidence of relapse (bone marrow <5% blasts), patient is off immunosuppression and there is no evidence of active GVHD .

5.5.4 Therapeutic DLI for treatment of infections:
DLI can be used for treatment of infections. DLI are used only if the patient has not responded to appropriate anti-microbial therapy and if specific T-cells cannot be obtained. In order to receive DLI the patient should not, have active GVHD,. or history of GVHD of ≥grade II.

5.5.5. Progenitor cell boost in autologous transplants:

5.5.3.1 Additional cells can be given any time past day +28 in autologous transplant recipients.

5.6 Sources of additional donor cells:

5.6.1 If a donor is undergoing a peripheral blood progenitor cell collection, all cells remaining after transplant may be stored for future DLI or progenitor cell boosts. These cells are G-CSF mobilized. In TCD transplants after G- CSF mobilized progenitor cell collection, T-cells are separated and stored for future DLI, and CD34+ enriched cells are saved for progenitor cell boost. Less commonly, additional progenitor cells are available after the allogeneic bone marrow transplant. If available, additional bone marrow may be stored for future progenitor cell boost. T-cells may be obtained by apheresis or peripheral blood draw.

5.7. Cell dose for DLI and progenitor cell boost:
DLI dose depends on purpose of DLI (therapeutic vs. prophylactic), donor match, and whether cells were obtained following G-CSF mobilization.

5.7.1. CD3+ cell dose used for prophylactic DLI in fully matched related transplants, if progenitor cells were obtained **with G-CSF mobilization**:

1^{st} DLI – 5 x 10^6/kg of recipient's weight

2^{nd} DLI – 1 x 10^7/kg of recipient's weight

3^{rd} DLI – 5 x 10^7/kg of recipient's weight

4^{th} DLI – 1 x 10^8/kg of recipient's weight

5.7.2. CD3+ cell dose used for prophylactic DLI in fully matched related transplants if DLI are used without G-CSF mobilization, or if DLI are obtained from unrelated or one antigen mismatched related transplants **with G-CSF mobilization:**

1^{st} DLI – 1 x 106/kg of recipient's weight,

2^{nd} DLI – 5 x 10^6/kg of recipient's weight,

3^{rd} DLI – 1 x 10^7/kg of recipient's weight

4^{th} DLI – 5 x 10^7/kg of recipient's weight

5.7.3. CD3+ cell dose used for prophylactic DLI in fully matched unrelated transplants or one antigen mismatched related transplants when DLI are collected **without G-CSF mobilization**:

1st DLI - 1-5 x 10^5/kg of recipient's weight

2nd DLI - 1 x 10^6/kg of recipient's weight

3rd DLI - 5 x 10^6/kg of recipient's weight

4th DLI - 1 x 10^7/kg of recipient's weight

5.7.4. When DLIs are used for treatment of molecular or cytogenetic relapse, one may start with the 2nd dose level, or skip dose levels.

5.7.5. CD3+ cell dose used for DLI for patients with >1 antigen mismatch or for patients who underwent TCD transplants:

5.7.5.1 Dose is 3 x 10^4/kg of recipient's weight; subsequent DLI is the same as the first dos or it could be increased to 6 x 10^4/kg. CD3+ cells.

5.7.6. Cell dose for progenitor cell boost:

5.7.6.1 Autologous progenitor cell boost - No limitations to cell dose, unless a DMSO limit is reached (DMSO should not exceed 10cc/kg/day of unwashed cryopreserved cells).

5.7.6.2 Note: Progenitor cell boost in allogeneic transplants: The amount of progenitor cells infused will be limited by CD3+ content of the graft. The dose should not exceed 1x10^8 CD3+ cells/kg of recipient's weight. If more than 1x10^8 CD3+ cells/kg of recipient's weight are infused, GvHD prophylaxis is recommended.

5.7.6.3 In TCD transplants, progenitor cell boost is limited by CD3+ cell content. The infusion is typically 10-20 x10^6 of CD34+ cells/kg of recipient weight, and up to 3 x 10^4 /kg of CD3+ of recipient's weight.

5.7.7. Discontinuation of DLI or progenitor cell boosts.

5.7.7.1 If GvHD or any other side effects such as pancytopenia or pulmonary complications develop after DLI, no more DLI infusions are given. If patient reaches 100% donor engraftment, DLIs are discontinued. If a patient undergoing TCD transplant reaches CD4+ count of >100/microliter, DLIs are discontinued.

5.7.7.2 Progenitor cell boosts are discontinued if a complication related to boost occurs (such as GvHD), or if a patient achieves transfusion independence.

6.0 Outcomes:

6.1 The number of additional donor cell infusions is recorded in the Pediatric BMT database and reported to the CIBMTR/NMDP.

6.2 The incidence of grade 3 and 4 complications related to the infusions are reported in the PBMT database and analyzed annually.

7.0 References:

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19.4: Appendix 4 Azacitidine Administration

Name: _____ MRN: _____

Height:_____cm Weight:_____kg BSA: _____ m^2 Cycle #: _____

- Cytopenias prior to Cycle 1 require dose reductions as outlined below. Cytopenias following subsequent cycles require dose adjustments based on nadir counts as outlined in appendix 5.
    - For platelets <30,000/uL, 50% dose reduction is recommended.
    - If platelet transfusion dependent, hold therapy until platelets >30,000/uL.
    - For neutrophils <750 or requiring GCSF support, hold therapy until toxicity improves.
- Renal insufficiency:
    - Creatinine <2X baseline: No dose adjustment is required.
    - Creatinine 2-3X above baseline: Hold therapy until AKI resolves. Restart therapy at 50% dose reduction. If well-tolerated, full dose may be administered.
- Hepatotoxicity:
    - AST/ALT <5x ULN: No dose adjustment is required.
    - AST/ALT 5-20x ULN: Hold therapy until transaminitis resolves. May consider 50% dose reduction and close monitoring.
    - AST/ALT >20x ULN: Hold therapy until transaminitis resolves
    - Total bilirubin 3-10x ULN: Hold therapy until resolution; may consider 50% dose reduction and close monitoring
    - Total bilirubin >10x ULN: Hold therapy until resolution.

Drug	**Route**	**Dosage**	**Days**	**Notes**	**Observations**
Azacitidine (Aza)	IV over 30-60 minutes or SQ	40mg/m²/dose x 4 doses	Days 1-4	Note prior nadir and adjust dose if indicated.	a Hx, PE, Wt, Ht, CBC, CMP b CBC, CMP *q 1 week labs during cycle 1-2 *q 2 week labs during cycle 3-7 if no previous dose reductions required.

Day	Date	Drug	Dosing	Dose	Route	Duration	Time	Studies	Notes
1	_____	Aza	40mg/m²/dose	_____	_____	_____	_____	a	<2 weeks prior to cycle
2	_____	Aza	40mg/m²/dose	_____	_____	_____	_____		
3	_____	Aza	40mg/m²/dose	_____	_____	_____	_____		
4	_____	Aza	40mg/m²/dose	_____	_____	_____	_____		
5-11									DLI if indicated
8								b*	
15								b*	
22								b*	
29								b*	
35-42								a	

- Patients will receive azacitidine at a dose of 40mg/m2 IV or SC x 4 days every 6 weeks.
- A total of 7 cycles may be administered as tolerated.
- Administration of cycle #2 azacitidine following withdrawal of immunosuppression will commence (typically on a Monday) 2-8 weeks following completion of SWI/FWI (see section 6.2 for dose adjustments).
- Escalating doses of DLI, if planned, will be administered (typically on a Friday) within 7 days following the last dose of azacitidine every cycle (see section 5.8 for DLI schedule).

19.5 Appendix 5: Azacitidine dose adjustments

A) Decrease in Serum Bicarbonate

1. Unexplained reductions of serum bicarbonate to less than 20 mEq/L occurring during therapy: decrease dose by 50% on the next treatment course

B) Myelosuppression

1. For patients with baseline WBC of 3 x 10(9)/L or greater, absolute neutrophil count (ANC) of 1.5 x 10(9)/L or greater, and platelets of 75 x 10(9)/L or greater, adjust dose using nadir counts for any given cycle as follows:

Nadir Counts		
ANC (x 10^9/L)	**Platelets (x 10^9/L)**	**% Dose in the Next Course**
less than 0.5	less than 25	50%
0.5 to 1.5	25 to 50	67%
greater than 1.5	greater than 50	100%

2. For patients with baseline WBC less than 3 x 10(9)/L, absolute neutrophil count (ANC) less than 1.5 x 10(9)/L, or platelets less than 75 x 10(9)/L, adjust dose based on nadir counts and bone marrow biopsy cellularity at time of nadir as shown in the table below, **unless there is a clear improvement with differentiation at the time of the next cycle, in which case the dose of the current treatment should be continued** :

WBC or Platelet Nadir % Decrease in Counts from Baseline	**Bone Marrow Biopsy Cellularity at Nadir**		
↓	30 to 60	15 to 30	less than 15
	% Dose in the Next Course		
50 to 75	100%	50%	33%
more than 75	75%	50%	33%

3. If a nadir as defined in the table has occurred and both the WBC and platelet counts are more than 25% above the nadir and rising, the next course should be given at least 28 days after the start of the preceding course. If the WBC and platelet counts are not at least 25% above the nadir by day 28, counts should be reassessed every 7 days. If a 25% increase has not occurred by day 42, the patient should be treated with 50% of the scheduled dose.

19.6 Appendix 6: Procedure for AML gene expression and mutation panel testing

AML gene expression and mutation panels to NIH (Hourigan Lab) for AML patients only

At the time of routine laboratory evaluation (venipuncture or bone marrow examination) additional patient samples will be obtained for research purposes if in the judgment of the principal investigator this can be accomplished safely and within national and institutional guidelines regarding blood sampling in children involved in clinical research.

1.0 Required research samples (see Table 1):

1.1 Bone marrow aspirate samples

1.1.1 An additional 2ml of bone marrow aspirate sample for research will be collected during scheduled clinically indicated bone marrow examination at day +30, +90 and +180 (+/- 14 days) using commonly used anticoagulants and transferred to a PAXgene Bone Marrow RNA tube (Qiagen/BD, Catalog: 764114).

1.2 Peripheral blood samples

1.2.1 An additional 2.5ml of peripheral blood will be collected for research at days +30, +90 and +180 (+/- 14 days) during scheduled venipuncture for clinical indications and transferred to a PAXgene Blood RNA tube (Qiagen/BD, Catalog: 762165)

2.0 Storage of PAXgene tubes

PAXgene tubes should be used in accordance with manufacturer instructions. Generally, following sample collection tubes should be inverted 8-10 times, stored at room temperature for between 2 and 72 hours before transfer to -20C freezer. Samples may be stored in a -20C freezer for up to five years.

3.0 Sample de-identification/coding

All human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability will be stored securely at University of California San Francisco in a secure electronic database designed for this purpose. Enrolled patients will be assigned a unique identification. Patient samples will be de-identified and labeled with an identification code and the date the sample was drawn. The Study Chair will supervise the creation of de-identified reports, using the patient unique identification code, containing the following information to disclose with scientific and statistical collaborators at the National Institute of Health:

- Date of enrolment in this study
- Dates and kinds of interventions received on study.
- Dates and volumes of study samples collected
- Details of donor and recipient chimerism analysis.
- AML morphological subtype
- AML karyotype (Cytogenetics, FISH)
- AML molecular mutation status (e.g.: FLT3, NPM1)
- Current AML remission status and duration (e.g.: CR1 since 09/01/12)

4.0 Sample Transportation, Storage and Tracking: Cryopreserved, de-identified samples will be transferred on dry-ice to the National Institutes of Health in batches. Samples will be received, stored and tracked in existing password protected database under the supervision of:

[redacted]

Samples will be stored until they are no longer of scientific value or until the volunteer withdraws consent, at which time they will be destroyed.

5.0 Proposed laboratory studies

5.1 Research samples will be analyzed by investigators of the National Institutes of Health intramural program. They will not be submitted for pathology review or used for diagnostic purposes rather they will be used strictly for laboratory research studies designed to characterize the levels of disease burden in patients with AML.

5.2 We will utilize multiple molecular biology technologies for this research including (but not limited to) microarrays, single nucleotide polymorphism arrays, gene-expression arrays using qRT-PCR, mutational arrays using qRT-PCR, digital PCR, targeted re-sequencing for common AML somatic mutations and RNA sequencing.

19.7: Multicenter Institutional Studies

Data and Safety Monitoring Plan for Multicenter Institutional Study (Phase 2 or 3 Institutional Study)

The UCSF Helen Diller Family Comprehensive Cancer Center (HDFCCC) Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and subject safety for all HDFCCC institutional clinical studies. A summary of DSMC activities for this study includes:

- Review of subject data
- Review of suspected adverse reactions considered "serious"
- Monthly monitoring (depending on study accrual)
- Minimum of a yearly regulatory audit

Monitoring and Reporting Guidelines

All institutional Phase 2 or 3 therapeutic studies are designated with a moderate risk assessment. The data is monitored every six months, with twenty percent of the subjects monitored (or at least three subjects if the calculated value is less than three).

The UCSF Coordinating Center provides administration, data management, and organizational support for the participating sites in the conduct of a multicenter clinical trial. The UCSF Coordinating Center will also coordinate quarterly conference calls with the participating sites to communicate the review of adverse events, safety data, and other study matters.

The Principal Investigator at the UCSF Coordinating Center will hold the role of Study Chair. The Study Chair is responsible for the overall conduct of the study and for monitoring its safety and progress at all participating sites. The Study Chair will conduct continuous review of data and subject safety and discuss each subject's treatment at monthly UCSF Site Committee meetings. The discussions are documented in the UCSF Site Committee meeting minutes.

<u>Multicenter communication</u>

The UCSF Coordinating Center provides administration, data management, and organizational support for the participating sites in the conduct of a multicenter clinical trial. The UCSF Coordinating Center will also coordinate conference calls with the participating sites at the completion of each cohort or as frequently as needed to discuss risk assessment. The following issues will be discussed as appropriate:

- Enrollment information
- Adverse events (i.e. new adverse events and updates on unresolved adverse events and new safety information)
- Protocol violations
- Other issues affecting the conduct of the study

Adverse events reporting to the DSMC will include reports from the UCSF Coordinating Center as well as the participating sites. The DSMC will be responsible for monitoring all data entered in OnCore® at the UCSF Coordinating Center and the participating sites. The data (i.e. copies of source documents) from the participating sites will be sent electronically or faxed over to the UCSF Coordinating Center prior to the

monitoring visits in order for the DSMC to monitor the participating site's compliance with the protocol, patient safety, and to verify data entry.

Adverse Event Review and Monitoring

Adverse Event Monitoring

All reported events entered into OnCore® will be reviewed on a monthly basis at the UCSF Site Committee meetings. All clinically significant adverse events must be reported to the UCSF Coordinating Center by the participating sites within 10 business days of becoming aware of the event or during the next scheduled quarterly conference call, whichever is sooner. The UCSF 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 treatment from the UCSF Coordinating Center and the participating sites.

In addition, all suspected adverse reactions considered "serious" must be entered in OnCore® and reported to the UCSF Coordinating Center within 1 business day of the site learning of the event. The suspected adverse reactions considered "serious" will be reviewed and monitored by the Data and Safety Monitoring Committee on an ongoing basis and discussed at the DSMC meeting, 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 treatment and is determined to be related either to the investigational drug or any research related procedure, the Study Chair at the UCSF Coordinating Center or the assigned designee, must be notified within 1 business day of the participating site learning of the event and the Study Chair must then notify the DSMC Chair or qualified alternate within 1 business day of this notification. The contact may be by phone or e-mail.

Increase in Adverse Event Rates

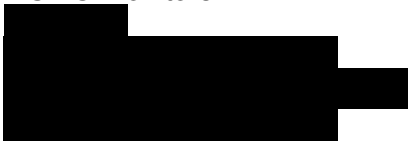
If an increase in the frequency of Grade 3 or 4 adverse events (above the rate reported in the Investigator Brochure or package insert), the Study Chair at the UCSF Coordinating Center is responsible for notifying the DSMC at the time the increased rate is identified. The report will indicate if the incidence of adverse events observed in the study is above the range stated in the Investigator Brochure or package insert.

If at any time the Study Chair stops enrollment or stops the study due to safety issues, the DSMC Chair and DSMC Manager 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:
Phone:
Email:
Address:

DSMC Monitors



* DSMP approved by NCI 09/February2012

19.8: UCSF Policy/Procedure for Required Regulatory Documents for Single Site and Multicenter Investigator-Initiated Oncology Clinical Trials

Purpose

This policy defines the required Regulatory Documents for Single Site and Multicenter Investigator Initiated Oncology Clinical Trials at the Helen Diller Family Comprehensive Cancer Center (HDFCCC) for both IND and IND-exempt trials.

Background

The International Conference on Harmonization (ICH) Good Clinical Practices (GCP) Guidelines define Essential Regulatory Documents as those documents which individually and collectively permit evaluation of the conduct of a trial and the quality of data produced. These documents serve to demonstrate compliance with standards of GCP and with all applicable regulatory requirements. Filing essential documents in a timely manner can greatly assist in the successful management of a clinical trial.

The Regulatory Documents will consist of electronic files in both iRIS and OnCore®, as well as paper files in the Regulatory Binders for both the Coordinating Site and the Participating Site(s) in the HDFCCC Investigator Initiated Oncology Clinical Trials.

Procedures

1. Single Site (HDFCCC) Therapeutic Essential Regulatory Documents:

Documents Filed in iRIS:

- Current and prior versions of the Informed Consent Form(s) (ICFs).
- IRB approvals for initial submission of application, all modifications, and continuing annual renewals.
- Current and prior approved protocol versions.
- IRB roster
- Current and prior versions of the Investigator Brochure (IB).
- Serious Adverse Event (SAE) Reports.
- Subject diary and handouts (if applicable).
- Single Patient Exception (SPE) Report(s) to IRB with Approval Letter(s) from IRB.
- Protocol Violation (PV) Reports with acknowledgement from the IRB.

Documents Filed in OnCore®:

- Package Insert (if the study drug is commercial).
- Protocol signature page(s) with PI signature(s) for all protocol versions.
- Protocol Review Committee (PRC) approved protocols, protocol amendments and Summary of Changes (SOC) document.
- Screening/enrollment log.
- Data and Safety Monitoring Committee (DSMC) monitoring reports.
- DSMC dose escalation approvals with study status summary forms.
- Case Report Form (CRF) completion manual.
- Drug Destruction Standard Operating Procedure (SOP).
- As applicable, approvals for Biosafety Committee, Radiation Committee, and Infusion Center.
- Serious Adverse Event (SAE) reports to IRB.
- Drug Destruction Standard Operating Procedure (SOP).

Documents Filed in Regulatory Binder:

- Delegation of Authority Log with signatures (to be scanned in OnCore once the trial is complete).

2. Additional Essential Documents for Therapeutic Multicenter Trials for the Coordinating Center (filed in OnCore or Zip Drive):

- Institutional Review Board (IRB) approval letters, IRB roster, Informed Consent Form (ICF), and Health Insurance Portability and Accountability Act {HIPAA) Consent Form for the Participating Site(s).
- For all Principal Investigators and Sub-Investigators listed on the 1572 at the Participating Site(s), will need Financial Disclosure Forms, CVs, MD Licenses, and Staff Training documents (i.e. Collaborative Institute Training Initiative (CITI), etc.) (for investigational New Drug Application).
- Site Initiation Visit (SIV) minutes and correspondence with the Participating Site(s).
- As applicable, approvals for Biosafety Committee, Radiation Committee, and Infusion Center for the Participating Site(s).
- Protocol Violations (PV) Reports to IRB with acknowledgement from IRB for Participating Site(s).
- Single Patient Exception (SPE) Reports to IRB with IRB Approval Letters for Participating Site(s).
- Drug Destruction Standard Operating Procedure (SOP) for the Participating Site(s).
- Data and Safety Monitoring Committee (DSMC) monitoring reports for the Participating Site(s).
- Copy of the Data and Safety Monitoring Plan (DSMP) Monitoring Plan for all participating site(s) in Multicenter studies or Contract Research Organization (CRO) Monitoring Plan (if an outside CRO is used for the study).
- Serious Adverse Event (SAE) forms submitted to the IRB for the Participating Site(s).

3. *Required Multicenter Essential Regulatory Document Checklist for Therapeutic and Non-Therapeutic Trials (For Start-Up Only):*

- See attached checklist(s).

4. Required Essential Regulatory Documents for Single Site and Multicenter Therapeutic IND-Exempt Studies (filed in OnCore):

- For IND Exempt studies, the Essential Regulatory Documents for UCSF would include all documents in Section #1 of this policy. The Essential Regulatory Documents from the participating site(s) for Multicenter Trials when UCSF is the Coordinating Center would only include the signed protocol signature page, CV of the PI, and the IRB approval letters. All other documents in Section #2 of this policy would be the responsibility of the Participating Site(s).

5. Required Essential Regulatory Documents for Single Site Non-Therapeutic Studies (filed in OnCore):

- For Single Site non-therapeutic trials, all Regulatory Documents in Section #1 of this policy are required except for: current and prior versions of the Investigator Brochure (IB), package insert (if the study drug is commercial), DSMC dose escalation approvals with study status summary forms, approvals for Biosafety Committee, Radiation Committee, and Infusion Center, and drug destruction SOPs.

***6.* Alternate Procedures**

There are no alternate procedures to the HDFCCC policy for requirements for Essential Regulatory Documents for Multicenter Investigator-Initiated Oncology Clinical Trials.

References

- ICH Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance (current version).
- International Conference on Harmonization: Good Clinical Practice: Consolidated Guideline (current version).
- International Conference on Harmonization: Essential Documents for the Conduct of a Clinical Trial (current version).
- 21CFR50
- 21 CFR56.11
- 45CFR46
- 21 CFR312

Required Regulatory Documents for Sub-sites Participating in Therapeutic UCSF Investigator Initiated Multicenter trial

Directions: Scan the documents in a zip drive and upload to OnCore.

1572

PI and Sub investigators:

- CV and Medical license
- Financial disclosure form
- NIH or CITI human subject protection training certification

Laboratories:

- CLIA &CAP and Lab Licenses
- CV and Medical License of Lab Director
- Laboratory reference ranges

Local Institutional Review Board

IRB Approval letter

Reviewed/Approved documents

- Protocol version date: _____
- Informed consent version date: _____
- Investigator Brochure version date:_____
- HIPAA

Current IRB Roster

Other

Delegation of Authority Log

- Include NIH or CITI human subject protection training certificates or GCP training certification

Pharmacy

- Drug destruction SOP and Policy

Protocol signature page

Executed sub contract

20.0 References

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2. J. Tracey, M. J. Zhang, E. Thiel, K. A. Sobocinski, M. Eapen, Transplantation conditioning regimens and outcomes after allogeneic hematopoietic cell transplantation in children and adolescents with acute lymphoblastic leukemia. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* **19**, 255 (Feb, 2013).
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8. A. Balduzzi *et al.*, Minimal residual disease before and after transplantation for childhood acute lymphoblastic leukaemia: is there any room for intervention? *Br J Haematol* **164**, 396 (Feb, 2014).
9. R. B. Walter *et al.*, Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. *Blood* **122**, 1813 (Sep 5, 2013).
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12. A. Bacigalupo *et al.*, Increased risk of leukemia relapse with high-dose cyclosporine A after allogeneic marrow transplantation for acute leukemia. *Blood* **77**, 1423 (Apr 1, 1991).
13. T. L. Andrea Bacigalupo, Francesca Gualandi, Stefania Bregante, AnnaMaria Raiola, Carmen di Grazia, Alida Dominietto, Chiara Romagnani, Domenico Occhini, Francesco Frassoni, and Maria Teresa van Lint, Increased risk of leukemia relapse with high dose cyclosporine after allogeneic marrow transplantation for acute leukemia: 10 year follow-up of a randomized study. *Blood* **98**, (November 15, 2001, 2001).
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15. R. Storb *et al.*, Methotrexate and cyclosporine for graft-vs.-host disease prevention: what length of therapy with cyclosporine? *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* **3**, 194 (Oct, 1997).
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