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Bridging Study

A Phase 2 Study Investigating Clofarabine, Cyclophosphamide and Etoposide for Children, Adolescents, and Young Adults (AYA) with Acute Leukemia and Minimal Residual Disease

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TRM (post-HCT)

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LIST OF ABBREVIATIONS

AE	adverse event
ALL	acute lymphoblastic leukemia
ALT	Alanine Aminotransferase
AML	acute myeloid leukemia
ANC	absolute neutrophil count
APL	acute promyelocytic leukemia
AST	aspartate aminotransferase
AUC	area under the concentration time curve
BMT	bone marrow transplant
BP	blood pressure
BUN	blood urea nitrogen
CART Therapy	chimeric antigen receptor T-cell therapy
CHW	Children's Hospital of Wisconsin
CI	confidence interval
CLL	chronic lymphocytic leukemia
CNS	central nervous system
CR	complete remission
CrCl	creatinine clearance
CRF	case report forms
CRp	complete remission without platelet recovery
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTMS	Clinical Trials Management System
CTO	Clinical Trials Office
DEHP	Bis(2-ethylhexyl) phthalate
DFS	disease free survival
DLT	dose limiting toxicity
DSMC	data safety monitoring committee
DSMP	data safety monitoring plan
ECHO	echocardiogram
EFS	event free survival
EP	European Pharmacopeia
FISH	fluorescence in situ hybridisation
GFR	glomerular filtration rate
GVHD	graft versus host disease
GVL	graft versus leukemia
HCT	hematopoietic cell transplantation
HgB	hemoglobin
HPLC	high performance liquid chromatography
HSCT	hematopoietic stem cell transplantation
HSV	herpes simplex virus
IRB	Institutional Review Board
LDH	lactate dehydrogenase
MACC Fund Center	Midwest Athletes Against Childhood Cancer Fund Center
MCW	Medical College of Wisconsin
MFC	multiparameter flow cytometry
MRD	minimal residual disease

LIST OF ABBREVIATIONS (CONTINUED)

MTD	maximum tolerated dose
MUGA	multi gated acquisition scan
NOS	not otherwise specified
NRM	non relapse mortality
NS	normal saline
ORR	overall response rate
OS	overall survival
PCP	pneumocystis carinii pneumonia
PCR	polymerase chain reaction
PI	principal investigator
PR	partial responsego
PVC	polyvinyl chloride
RFS	relapse free survival
RIC	reduced intensity conditioning
SAE	serious adverse event
SIRS	systemic inflammatory response
SRC	Scientific Review Committee
UCBT	umbilical cord blood transplantation
ULN	upper limit of normal
UPIRSO	unanticipated problems involving risks to subjects or others
USP	United States Pharmacopeia
WBC	white blood count

SYNOPSIS

Study Title: A Phase 2 Study Investigating Clofarabine, Cyclophosphamide and Etoposide for Children, Adolescents and Young Adults (AYA) with Acute Leukemia and Minimal Residual Disease

Phase 2

Number of Patients: 50 evaluable [25 adult (19 to 39 years) and 25 pediatric (0-18 years)]

Study Objectives

- **Primary Objectives**
  - The effect of Clofarabine, Cyclophosphamide, and Etoposide in eliminating the presence of persistent disease without causing a significant delay of HCT due to treatment related toxicity, defined as greater than 56 days from completion of bridging therapy to start of HCT.
  - The proportion of any toxicities associated with Clofarabine, Cyclophosphamide, and Etoposide

- **Secondary Objectives**
  - The proportion of pre-transplant chemotherapy-induced aplasia (defined as greater than 42 days after infusion of chemotherapy)
  - The proportion of infectious complications following bridging therapy and before HCT
  - Treatment related mortality at Day 100 after HCT
  - One year disease-free survival after HCT
  - The proportion of relapse within one year of HCT

Treatment Plan: Only 1 treatment course will be permitted

Day	Agent/Procedure
Baseline	Peripheral blood draw and bone marrow aspirate (within 14 days of enrollment)
Day 1	Clofarabine 20 mg/m^2 IV over 2 hours followed by Etoposide 100 mg/m^2 IV over 2 hours followed by Cyclophosphamide 300 mg/m^2 IV as a 30-60 minute infusion
Day 2	Clofarabine 20 mg/m^2 IV over 2 hours followed by Etoposide 100 mg/m^2 IV over 2 hours followed by Cyclophosphamide 300 mg/m^2 IV as a 30-60 minute infusion
Day 3	Clofarabine 20 mg/m^2 IV over 2 hours followed by Etoposide 100 mg/m^2 IV over 2 hours followed by Cyclophosphamide 300 mg/m^2 IV as a 30-60 minute infusion
Day 4	Clofarabine 20 mg/m^2 IV over 2 hours followed by Etoposide 100 mg/m^2 IV over 2 hours followed by Cyclophosphamide 300 mg/m^2 IV as a 30-60 minute infusion
Day 5	Clofarabine 20 mg/m^2 IV over 2 hours followed by Etoposide 100 mg/m^2 IV over 2 hours followed by Cyclophosphamide 300 mg/m^2 IV as a 30-60 minute infusion

Day	Agent/Procedure
Day 30*	Perform bone marrow biopsy/aspirate (*Or upon adequate blood count recover (ANC > 0.5 AND plt > 50,000) whichever comes first
Between Day 28 & 42	Proceed to HCT independent of this study

Study Population:

- Diagnosis of acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) with < 5% blasts in the bone marrow (M1) by morphology and that meets one of the following criteria:

  - Flow cytometric evidence of MRD (≥ 0.01% leukemic blasts for ALL or ≥ 0.5% leukemic blasts for AML detected in the bone marrow)

  OR

  - Molecular/cytogenetic evidence of disease (FISH or PCR methodology) performed within 14 days **And** with the intent of going on to an allogeneic hematopoietic cell transplantation (HCT) independent of this study
- Patients must have an available donor and have intention of proceeding directly to ALL-HCT after completion of 1 cycle of Bridging therapy.
- Age 0 to 39 years
- Karnofsky Performance Status ≥ 50% for patients 16 years and older and Lansky Play Score ≥ 50 for patients under 16 years of age (see Appendix 2)
- Patients must have a life expectancy ≥ 8 weeks as determined by the enrolling investigator
- Have acceptable organ function as defined within 7 days of study registration
  - Renal: creatinine clearance ≥ 60 mL/min/1.73 m^2 or serum creatinine based on age/gender as follows:

Age	Maximum Serum Creatinine (mg/dl)	
	Male	Female
1 month to < 6 months	0.4	0.4
6 months to < 1 year	0.5	0.5
1 year to < 2 years	0.6	0.6
2 years to < 6 years	0.8	0.8
6 years to < 10 years	1.0	1.0
10 years to < 13 years	1.2	1.2
13 years to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

  - Hepatic: ALT < 5 x upper limit of normal (ULN) and total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age
  - Cardiac: left ventricular ejection fraction ≥ 40% by ECHO/MUGA
- Patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study. At least 7 days must have elapsed from prior chemotherapy.
- Hematopoietic Growth Factors: At least 7 days since the completion of therapy with a growth factor and at least 14 days since pegfilgrastim (Neulasta®) administration.

- Sexually active females of child bearing potential must agree to use adequate contraception (diaphragm, birth control pills, injections, intrauterine device [IUD], surgical sterilization, subcutaneous implants, or abstinence, etc.) for the duration of treatment and for 2 months after the last dose of chemotherapy. Sexually active men must agree to use barrier contraceptive for the duration of treatment and for 2 months after the last dose of chemotherapy.
- Voluntary written consent before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care.

Exclusion Criteria:

- Acute Promyelocytic Leukemia (APL)
- Active extramedullary disease (CNS ≥ CNS2 and/or testicular leukemia) or presence of chloromatous disease
- Receiving concomitant chemotherapy, radiation therapy; immunotherapy or other anti-cancer therapy other than is specified in the protocol
- Systemic fungal, bacterial, viral, or other infection not controlled (defined as exhibiting ongoing signs/symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment)
- Pregnant or lactating. The agents used in this study are known to be teratogenic to a fetus and there is no information on the excretion of agents into breast milk. All females of childbearing potential must have a blood test or urine study within 2 weeks prior to registration to rule out pregnancy.
- Known allergy to any of the agents or their ingredients used in this study
- Participating in a concomitant Phase 1 or 2 study

Duration of Study: 2 years

PROTOCOL REVISION HISTORY

Version No.	Revision Date	Summary of Changes	Consent Revised Yes/No
2.0	02/20/15	Added Section 12.4 NCI trial designation number	Yes – Trial number has been added to the Consent as well
3.0	03/20/2015	Added Consents for Nationwide Children's and UW Madison following official deferral to MCW IRB	Yes – Added consents specific to Nationwide Children's and UW Madison
		Synopsis Primary Objectives updated to provide clarification on what constitutes a delay of HCT by adding :"defined as greater than 56 days from completion of bridging therapy to start of HCT."	No
		Section 2 Primary Objectives updated to provide clarification on what constitutes a delay of HCT by adding :"defined as greater than 56 days from completion of bridging therapy to start of HCT."	No
		Section 3.1.1 updated to clarify and correct an inconsistency regarding inclusion criteria: flow evidence of MRD or Molecular/cytogenetic FISH or PCR evidence of disease to be performed within 14 days of enrollment	No
		Section 11.1 Statistical Endpoints updated to add the definition of delay in HCT: "defined as greater than 56 days from completion of bridging therapy to start of HCT"	No
		Section 11.2 Statistical Analysis correction made to SAS 9.3 changing this to SAS 9.4	No
		Section 11.3 Sample Size Justification was revised. Previous paragraph replaced with new one to strengthen the justification.	No
		Section 11.4 Early Stopping Rule for Excessive Toxicity section – previous paragraph replaced with new paragraph to clarify and strengthen rationale.	No
4.0	09/04/2015	Page 9 Synopsis and Page 19 Section 3.1.1-Eligibility Criteria changed bone marrow to be	No

		performed within 14 days of enrollment (increased from within 7 days). This change did not appear in the protocol after the previous amendment and is being corrected with this amendment. This change now makes these 2 sections consistent with the table on page 26. Added Appendix 5 – Bridging therapy roadmap Protocol Version number and date changed in the headers and footers to reflect the current version. Table of contents updated.	 No Yes – updated footers and version number – no content changes No
5.0	02/08/2016	Headers and Footers updated with new version number and date Page 5 Performance Site Sub Investigators: Replaced Robyn Dennis, MD with Susan Vear, MD at Nationwide Children's Hospital	Yes- updated footers with new protocol version number and date. All Assents section E2 replaced Robyn Dennis, MD with Susan Vear, MD as contact for Nationwide Children's Hospital All Consents Section D5 replaced Robyn Dennis, MD with Susan Vear, MD as contact for Nationwide Children's Hospital

1. BACKGROUND

1.1 High Risk ALL and AML in Children and Adults

Acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) remain two of the most common forms of leukemia diagnosed in both children and adults, as well as two of the most common hematological diseases for which allogeneic hematopoietic cell transplantation (HCT) is currently used.[12] There is mounting evidence to support that for patients in hematological remission, the presence of minute amounts of leukemia (known as minimal residual disease (MRD)), identified immediately prior to HCT is associated with higher rates of ALL and AML relapse following HCT.[1-11] Therefore we hypothesize that transplant outcomes can be improved by reducing pre-HCT disease burden by attaining a pre-HCT MRD negative state. We designed this protocol to test the combination of clofarabine, cyclophosphamide and etoposide, which are known active agents in the treatment of lymphoid and myeloid hematologic malignancies, in their ability to both lower MRD and improve post-HCT outcomes for patients with ALL/AML who are in morphological remission but have evidence of MRD pre-HCT.

Impact of MRD Positive Disease Prior to HCT: Patients with acute leukemia and sub-morphologic disease at the time of conditioning are at high risk of relapse following HCT. Recent studies have demonstrated that even low levels of MRD (> 0.01% abnormal blasts by multi-parameter flow cytometry, MFC) after aggressive re-induction therapy portends a relatively poor outcome in relapsed ALL patients, including those who proceed to HCT.[7] Foster et al. recently reported 116 pediatric patients who were in morphologic remission and underwent MRD evaluation with multi-parameter flow cytometry (MFC) prior to ablative transplant for ALL. For the group as a whole, the EFS for MRD positive and negative patients was 11% and 58% respectively, ($p < 0.001$).[3]

Likewise, the BFM Study Group has also evaluated the impact of MRD prior to HCT for relapsed ALL using a PCR-based assay. Their multivariate Cox regression analysis revealed MRD as the only independent parameter predictive for EFS ($p < 0.006$).[7]

MRD identified prior to HCT for AML portends a poor prognosis in both pediatric and adult patients.[13-16] In a report by Walter et al. 99 pediatric and adult patients with AML receiving a HCT in CR1 were evaluated for pre-HCT MRD using MFC with any detectable MRD defined as MRD-positive.[13] The 2-year EFS for MRD-positive patients was 9% with a relapse rate of 64.9% compared to 74.8% and 17.6% in MRD-negative patients respectively. The multivariate analysis identified pre-HCT MRD as a significant risk factor for both overall mortality (HR 4.05 95% CI 1.90-8.62, $p < 0.001$) and relapse (HR 8.49 95% CI 3.67-19.65, $p < 0.001$). In another analysis reporting a larger group of pediatric and adult patients with AML (n=253) receiving HCT in CR1 or CR2, OS (HR 2.61, 95% CI 1.62-4.20, $p < 0.001$), DFS (HR 3.74, 95% CI 2.38-5.87, $p < 0.001$), and relapse (HR 4.90, 95% CI 2.87-8.37, $p < 0.001$) were all significantly worse for patients identified as MRD-positive MFC (≥ 0.1% blasts), compared to MRD-negative patients in multivariate cox regression model.[14]

Numerous monitoring techniques such as MFC, monitoring of known cytogenetic abnormalities, and molecular abnormalities such as NPM-1 and WT-1 have identified MRD in this patient population.[14,17,18] One of the larger recent studies investigating the impact of MRD on post-transplant outcomes for AML patients evaluated 88 patients with morphologic CR, 24 of which had MRD by MFC, who went on to receive a myeloablative transplant. Survival and relapse were strongly associated with absence or presence of MRD. Specifically, two year overall survival was 30.2% (range 13-49%) for the MRD-positive patients compared with 76.6% (64.4-85.1%) for those who were MRD-negative. Two year estimates of relapse also correlated with MRD status with rates of 65% in those who were MRD-positive compared with 17.6% in those MRD-negative.[13] These data highlight the significance of MRD on post-transplant relapse and the need for improved therapy.

Investigators at the University of Minnesota analyzed 97 adult AML patients; 14 had detectable MRD prior to allogeneic donor HCT. Overall survival and disease free survival were markedly worse in MRD-positive patients receiving RIC compared to MRD-negative cohort.[19] This research also investigated impact of pre-transplant MRD (assessed by MFC) in adults and children with ALL prior to umbilical cord blood transplantation. Patients with MRD-positive prior to UCBT had a greater incidence of relapse at 2-years (30% (95% CI: 4-56%) and lower 3-year disease-free survival (DFS) (30%; 95% CI 7-58%) compared to MRD-patients (relapse 16% (95% CI: 8-25%: p=0.05 and DFS 55%; 95% CI: 43-66%; p=0.02).[20] These data suggest that in the majority of patients with residual leukemia at the time of HCT, neither the preparative regimen, nor the GVL effect of the allograft is sufficient to prevent relapse. Thus, methods to eliminate persistent leukemia that have low toxicity profiles and can serve as a "bridge" to transplant are needed.

1.2 Overview of Cyclophosphamide and Etoposide

The combination of the alkylating agent cyclophosphamide with the topoisomerase II inhibitor etoposide has been a common re-induction therapy for relapse/refractory acute leukemia. These two agents have been used in combination in a number of recent studies through the Children's Oncology Group (COG) in both AALL01P2 for relapse B-Precursor ALL as well as the Infant ALL study CCG 1953 with limited toxicities.[21,22] In addition the combination has been evaluated in adult patients with relapsed/refractory AML. Of the 34 patients enrolled, 27 received etoposide 3 gm/m^2 by continuous infusion over 48-72 hours, immediately followed by cyclophosphamide 50 mg/kg iv over 3 hours daily for 3 or 4 days (150-200 mg/kg total dose). The other seven patients received a lower etoposide dose, 1.8–2.4 gm/m^2 over 48-72 hours followed by cyclophosphamide 50 mg/kg IV over 3 hours for 3 or 4 days. The complete remission rate and treatment related mortality was 32% and 18% respectively. These results compare favorably to other salvage regimens used in the treatment of patients with relapsed refractory AML.[23] Recently they have been shown to be synergistic with the relatively new purine nucleoside analogue clofarabine.[24, 25]

1.3 Overview of Clofarabine

Clofarabine (2-chloro-9-[2'-deoxy-2'-fluoro-β-D-arabinofuranosyl]adenine; Cl-F-ara-A;

CAFdA) is a rationally designed, second generation purine nucleoside analogue. Clofarabine was designed as a hybrid molecule to overcome the limitations and incorporate the best qualities of both fludarabine (F-ara-A) and cladribine (2-CdA, CdA) both of which are currently approved by various regulatory authorities for treatment of hematologic malignancies. Because clofarabine has a chloro-group at the 2-position of adenine, its chemical structure is more closely related to 2-CdA than to F-ara-A.[26] Halogenation at the 2-position of adenine renders this class of compounds resistant to intracellular degradation by the enzyme adenosine deaminase. Substitution of a fluorine at the C-2'-position of the arabinofuranosyl moiety of clofarabine increases its stability in gastric acid[27] and decreases its susceptibility to phosphorolytic cleavage by the bacterial enzyme *Escherichia coli* purine nucleoside phosphorylase in the gastrointestinal tract,[28] both of which may lead to enhanced oral bioavailability. Clofarabine was approved in December 2004 by the United States Food and Drug Administration (US FDA) for the treatment of pediatric patients with relapsed or refractory acute lymphoblastic leukemia (ALL) after at least 2 prior regimens based on the induction of complete responses.

Mechanism of Action: The precise mechanism of action of clofarabine on dividing and non-dividing cells is unknown. Like the other nucleoside analogues (cytarabine, ara-A [vidarabine], cladribine, fludarabine), clofarabine must be converted to the 5'-triphosphate form by deoxycytidine kinase (dCK) to be active within cells. Clofarabine is more efficient as a substrate for purified recombinant dCK, exceeding cladribine and the natural substrate, deoxycytidine.[29] Evidence suggests that the primary cytotoxic effect of clofarabine is due to its inhibition of DNA synthesis. The triphosphate form of clofarabine is an inhibitor of both DNA polymerase α and ribonucleotide reductase.[30] These effects lead to depletion of intracellular deoxynucleotide triphosphate pools, and inhibition of elongation of DNA strands during synthesis.[26] With respect to inhibition of ribonucleotide reductase, clofarabine and cladribine are superior to fludarabine.[30] With respect to inhibition of DNA polymerase α, clofarabine and fludarabine are similar and both are superior to cladribine.[28] Thus, in comparison to cladribine and fludarabine, clofarabine more completely inhibits both ribonucleotide reductase and DNA polymerase α, versus one or the other. Clofarabine is active in non-dividing cells and in cells with a low proliferation rate. Clofarabine has been shown to disrupt the integrity of mitochondria in primary chronic lymphocytic leukemia (CLL) cells. The damage leads to release of pro-apoptotic mitochondrial factors.[31] These effects are postulated to induce apoptosis in indolent, non-dividing CLL cells. This result was not seen with fludarabine and may explain, at least in part, the enhanced cytotoxicity of clofarabine[30] though the physiologic and clinical implications of these observations remain uncertain and under continued investigation.

Experience With Clofarabine in Pediatric Leukemia

Phase 1 Clofarabine Studies: In the initial Phase 1 study in pediatric patients with hematologic malignancies, 25 patients were treated in cohorts of escalating doses up to 70 mg/m^2, a dose at which 1 patient had grade 4 hyperbilirubinemia and grade 3 elevated transaminases, and 1 had a grade 3 skin rash. The MTD was determined to be 52 mg/m^2.[27] Of the 13 patients treated at 52 mg/m^2, grade 2 to grade 3 increases in bilirubin and liver transaminases were observed. A total of 5 patients achieved a CR and 3 achieved a PR for an overall response rate of 32%. Clofarabine plasma concentrations

were generally lower in the pediatric population than the adult population when administered the same dose, but there did not appear to be much difference in intracellular clofarabine triphosphate concentrations. Thus, the MTD and the recommended Phase 2 dose was determined to be 52 mg/m^2 and the recommended Phase 2 dose. The Phase 1 studies in pediatric patients with hematologic malignancies led to the initiation of 2 parallel Phase 2 trials in 2002 for patients with either relapsed or refractory ALL or relapsed or refractory AML.[29]

Both studies evaluated clofarabine 52 mg/m^2. In the 61 patients enrolled in the relapsed or refractory ALL study (61% males: 39% females, 1 to 20 years old), the overall remission rate (CR + CRp) was 20%; 30% (18/61) of patients showed a response (7CR, 5CRp, 6PR). Responses were noted in 15 of 50 (30%) patients with B-lineage ALL, 2 of 6 (33%) with T-cell ALL. Responders received a median of 3 prior induction regimens; 50% (9/18) had prior HSCT and 50% (9/18) were refractory to the preceding induction regimen. Response rate in refractory patients was 26% (9/35). After clofarabine treatment, 10 patients proceeded to transplant (including 8 responders). Six of 10 patients who received a transplant were alive at last follow up (survival range: 30.1+ to 145.1+ weeks). Response duration in 6 patients with CR or CRp who did not receive a transplant ranged from 4.3 to 58.6 weeks; 2 patients maintained CR for 47.9 and 58.6 weeks after clofarabine therapy. Median overall survival for the patients who achieved at least a PR was 66.6 weeks compared to 12.9 weeks for all patients.[29] In 42 pediatric patients with relapsed or refractory AML, the response rate was 26% (1 CRp, 10 PR). Responders had received a median of 2 prior induction regimens, 36% (4/11) had prior HCT and 55% (6/11) patients were refractory to the preceding induction regimen. Response rate in refractory patients was 21% (6/28). One patient who had received 5 prior induction regimens achieved CRp. 13 (31%) patients (1CRp, 6PR, 3NE, 3TF) underwent HSCT after completing clofarabine therapy, 5 of whom were alive at last follow-up (survival range: 62.7+ to 160.1+ weeks). Many patients proceeded to HSCT as soon as a donor was identified without waiting for the patient to go into remission, making remission difficult to assess. Median overall survival for patients who achieved at least a PR was 32.1 weeks compared to 23.4 weeks for all patients.[32]

Among the 113 pediatric patients with ALL and AML, the most frequently reported drug-related AEs were vomiting (66% ALL and 65% AML) and nausea (58% ALL and 70% AML).[32] Other drug-related AEs reported by at least 10% of the pediatric patients overall included febrile neutropenia (31% ALL and 28% AML), pyrexia (21% ALL and 26% AML), pruritus NOS (24% ALL and 20% AML), dermatitis NOS (24% ALL and 17% AML), headache NOS (18% ALL and 35% AML), diarrhea NOS (21% ALL and 22% AML), anxiety (16% ALL and 7% AML), fatigue (15% ALL and 13% AML), mucosal inflammation NOS (16% ALL and 15% AML), and flushing (12% ALL and 11% AML). Anorexia occurred in 12% ALL and 9% AML and palmar-plantar erythrodysesthesia syndrome in 12% ALL and 9% AML.

Phase 2 Clofarabine Studies: A Phase 2 trial of clofarabine in combination with cyclophosphamide and etoposide in pediatric patients with refractory/relapsed acute lymphoblastic leukemia was recently reported by Hijiya et al.[25] The efficacy and safety of intravenous clofarabine 40 mg/m^2 per day, cyclophosphamide 440 mg/m^2 per day, and etoposide 100 mg/m^2 per day for 5 consecutive days in pediatric patients with

refractory or relapsed ALL was evaluated in this Phase 2 study. The primary endpoint was overall response rate (complete remission [CR] plus CR without platelet recovery [CRp]). Among the 25 patients (median age, 14 years; pre-B cell ALL, 84%; ≥ 2 prior regimens: 84%; refractory to previous regimen: 60%), the overall response rate was 44% (7 CR, 4 CRp) with a 67.3-week median duration or remission censored at last follow-up. Most patients proceeded to alternative therapy, and 10 patients (40%) received HCT. Six patients (24%) died because of treatment-related adverse events associated with infection, hepatotoxicity, and/or multi-organ failure. The study protocol was amended to exclude patients with prior hematopoietic stem cell transplantation after 4 of the first 8 patients developed severe hepatotoxicity suggestive of veno-occlusive disease. No additional cases of veno-occlusive disease occurred.

Experience With Clofarabine in Adult Patients

Clofarabine activity has been evaluated in children and adults with acute leukemia and found to have no difference in activity between these two age groups.[33] Based on this finding as well as the known clinical activity using clofarabine in relapsed pediatric ALL and AML,[25,29] it is reasonable to further evaluate the clinical efficacy of clofarabine in combination with chemotherapy in adult patients with acute leukemia.

Phase 1 Clofarabine Studies: Clofarabine was evaluated in a Phase 1 study in patients with both hematologic malignancies and solid tumors. In patients with solid tumors, the starting dose level was 15 mg/m^2 daily for 5 days. Grade 4 myelosuppression occurred and the clofarabine dose was reduced by several dose levels with the MTD defined at 2 mg/m^2 in patients with solid tumors. In patients with leukemia, the starting dose level was 7.5 mg/m^2. This dose was escalated by several dose levels until 55 mg/m^2 was achieved. At the 55 mg/m^2 dose level, grade 3 hepatic toxicity was observed in 2 of 4 patients, which caused dose de-escalation to 40 mg/m^2 and became the recommended Phase 2 dose. This cohort was expanded to include 12 patients. Of the 12 patients treated at 40 mg/m^2, 2 patients achieved CR (1 AML and 1 ALL) suggesting some efficacy and safety at this dose level in patients with acute leukemia Based on *in vitro* data suggesting synergism between clofarabine and cyclophosphamide, Karp et al designed a Phase 1study with escalating doses of both agents[34]. Of the 18 patients enrolled, 12 and 6 had relapsed or refractory AML and ALL respectively. The median age was 51. Dose Level 1 was cyclophosphamide 200 mg/m^2 on Days 0 and 1, cyclophosphamide 400 mg/m^2 on Days 2, 3, and 8-10, and clofarabine 20 mg/m^2 Days 1-3 and 8-10. The total cyclophosphamide and clofarabine dose was 2400 mg/m^2 and 120 mg/m^2 respectively. In our study, the total cyclophosphamide and clofarabine dose is 1500 mg/m^2 and 100 mg/m^2 respectively. At that dose level, 2 of the 6 patients experienced prolonged bone marrow suppression which led to the reduction of the clofarabine dose to 10 mg/m^2. At this dose level, one death occurred at Day 14 and that cohort was expanded to include 12 patients and was defined as the DLT. Overall 4 patients died on the study, 2 in each dose cohort. The median time to bone marrow recovery was decreased from 45 days to 35 days from Dose Level 1 to Dose Level 0. Of the 12 patients enrolled at Dose Level 0, 3 patients achieved a CR suggesting activity of this combination in adult relapsed/refractory leukemia.

Phase 2 Clofarabine Studies: A Phase 2 study enrolled 116 newly diagnosed AML

patients with a median age of 71 years.[35] Unfavorable cytogenetics were present in 55%. Clofarabine at 30 mg/m^2 IV was administered daily for 5 days for induction followed by 20 mg/m^2 for 5 days as consolidation. The ORR was 46% with 38% of patients achieving a CR or CRp. The median overall survival for all patients, patients in CR/CRp and those in CR was 41, 59 and 72 weeks respectively. Overall clofarabine was well tolerated with a 60-day mortality rate of 16% (from any cause).

Study and Dose Rationale

The safety profile of clofarabine appears acceptable within the target populations studied to date in the clinical studies summarized above. Clofarabine has demonstrated anti-cancer activity through inhibition of DNA synthesis and repair, induction of apoptosis, and possibly through other mechanisms. Numerous responses have been observed after treatment with clofarabine in heavily pre-treated relapsed/refractory patients with ALL or AML. Additionally there have been responses in several adult studies of lower dose clofarabine in combination with a variety of agents.[31, 36, 37]The patient population selected, MRD positive prior to transplantation would be an ideal patient population to investigate the role of a "bridge" chemotherapy combination prior to HCT and potentially improve the event-free survival (EFS) of this group of patients who historically have had inferior outcomes but are ineligible for most current relapsed studies.

Experience With Clofarabine/Cyclophosphamide/Etoposide as Bridging Therapy in Pediatric ALL

Investigators at the University of Minnesota have been using this combination of lower dose clofarabine and cyclophosphamide with etoposide as "bridging therapy" for patients with B-precursor ALL who were in a morphologic remission (< 5% leukemia blasts in the marrow) but with evidence of MRD (> 0.01% leukemia blasts) via flow cytometry and awaiting HCT. To date, they have treated three pediatric patients with this regimen and in all three achieved MRD reduction (2/3 becoming MRD-negative) after a single course of therapy and experienced no Grade III/IV toxicities with both rapid neutrophil and platelet recovery. All three patients successfully proceeded to HCT after completion of this bridging therapy without further delay or toxicity and have experienced no increase in post-HCT toxicity.

Based on the success using this clofarabine-based regimen at the University of Minnesota, five additional patients were treated at four pediatric centers across the US, all with MRD-positive ALL (ages ranging 23 months to 20 years). All 5 patients had reduction in MRD after a single course of therapy with 4/5 achieving MRD-negativity. All 5 patients proceeded to HCT with 4/5 alive and free of disease at the time of this report and one case of TRM.

Reporting the results of all 8 patients treated with this bridging regimen across all centers provides 1-year EFS of 44% (95% CI 6-81) with two relapses occurring.[38] One relapse occurred in a patient with Philadelphia chromosome positive B-ALL who received the bridging therapy, but remained with persistent MRD prior to HCT, which incidentally was a reduced intensity conditioning regimen and the patient's 2nd HCT. The second relapse occurred in a patient with *MLL* gene rearrangement who achieved

MRD-negativity post-bridging therapy but subsequently relapsed 106 days post-HCT. This patient then received another course of the clofarabine-based bridging regimen resulting in elimination of his disease becoming MRD-negative (4% → < 0.01% by flow cytometry) and proceeded to chimeric antigen receptor T-cell (CART) therapy.

Experience With Clofarabine as Bridging Therapy in Adult Patients With AML

Clofarabine when given immediately prior to HCT was found to be safe in the setting of haploidentical HCT. In a study by Tischer et al, 18 patients with relapsed leukemia were enrolled, 15 having the diagnosis of AML.[39] All patients had active leukemia at time of HCT and received cytoreductive chemotherapy with clofarabine at a dose of 30 mg/m^2 IV over 5 days followed by 3 days of rest prior to starting their HCT conditioning regimen. Conditioning consisted of five doses of fludarabine at 30 mg/m^2/day IV and two doses of cyclophosphamide at 14.5 mg/kg/day IV. The estimated OS, RFS and NRM was 55.5%, 39% and 23% respectively. Five patients died from NRM with 4 patients dying from infectious complications and one patient dying from liver toxicity, and GVHD.

2. STUDY DESIGN

This is a Phase 2 study designed for the purpose of estimating various parameters surrounding the efficacy of Clofarabine, Cyclophosphamide and Etoposide in eliminating minimal residual disease (MRD) in acute leukemia patients otherwise in remission and without causing significant delay of HCT due to treatment related toxicity. A single course of "bridge" chemotherapy is given prior to the transplant procedure as an approach to improved disease-free survival in a patient group who historically has had inferior outcomes.

The following parameters will be monitored.

Primary Objectives

- The effect of Clofarabine, Cyclophosphamide and Etoposide in eliminating the presence of MRD without causing a significant delay of HCT due to treatment related toxicity, defined as greater than 56 days from completion of bridging therapy to start of HCT
- The proportion of any toxicities associated with Clofarabine, Cyclophosphamide and Etoposide

Secondary Objectives

- The proportion of pre-transplant chemotherapy-induced aplasia (defined as greater than 42 days after infusion of chemotherapy)
- The proportion of infectious complications following bridging therapy and before HCT
- Treatment–related mortality at Day 100 after HCT
- One year disease-free survival after HCT
- The proportion of relapse within one year of HCT

3. SELECTION OF PATIENTS

Study entry is open to patients regardless of gender or ethnic background. While there will be every effort to seek out and include females and minority patients, the patient population is expected to be no different than that of other acute leukemia studies at the Medical College of Wisconsin.

3.1 Inclusion Criteria

3.1.1 Diagnosis of ALL or AML with < 5% blasts in the bone marrow (M1) by morphology and that meets one of the following criteria:

a. Flow cytometric evidence of MRD (≥ 0.01% leukemic blasts for ALL or ≥ 0.5% leukemic blasts for AML detected in the bone marrow)

OR

b. Molecular/cytogenetic evidence of disease (FISH or PCR methodology) performed within 14 days

AND with the intent of going on to an allogeneic hematopoietic cell transplantation (HCT) independent of this study

3.1.2 Patients must have an available donor and have intention of proceeding directly to ALL-HCT after completion of 1 cycle of Bridging therapy.

3.1.3 Age 0 to 39 years

3.1.4 Karnofsky Performance Status ≥ 50% for patients 16 years and older and Lansky Play Score ≥ 50 for patients under 16 years of age (see Appendix 2)

3.1.5 Patients must have a life expectancy ≥ 8 weeks as determined by the enrolling investigator

3.1.6 Have acceptable organ function as defined within 7 days of study registration:

Renal: creatinine clearance ≥ 60 mL/min/1.73m^2 or serum creatinine based on age/gender as follows:

Age	Maximum Serum Creatinine (mg/dl)	
	Male	Female
1 month to < 6 months	0.4	0.4
6 months to < 1 year	0.5	0.5
1 year to < 2 years	0.6	0.6
2 years to < 6 years	0.8	0.8
6 years to < 10 years	1.0	1.0
10 years to < 13 years	1.2	1.2

Age	Maximum Serum Creatinine (mg/dl)	
13 years to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

Hepatic: ALT < 5 x upper limit of normal (ULN) and total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age

Cardiac: left ventricular ejection fraction ≥ 40% by ECHO/MUGA

3.1.7 Patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study. At least 7 days must have elapsed from prior chemotherapy.

Hematopoietic Growth Factors: At least 7 days since the completion of therapy with a growth factor and at least 14 days since pegfilgrastim (Neulasta®) administration.

3.1.8 Sexually active females of child bearing potential must agree to use adequate contraception (diaphragm, birth control pills, injections, intrauterine device [IUD], surgical sterilization, subcutaneous implants, or abstinence, etc.) for the duration of treatment and for 2 months after the last dose of chemotherapy. Sexually active men must agree to use barrier contraceptive for the duration of treatment and for 2 months after the last dose of chemotherapy.

3.1.9 Voluntary written consent before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care.

3.2 Exclusion Criteria

3.2.1 Acute Promyelocytic Leukemia (APL)

3.2.2 Active extramedullary disease (CNS ≥ CNS2 and/or testicular leukemia) or presence of chloromatous disease

3.2.3 Receiving concomitant chemotherapy, radiation therapy; immunotherapy or other anti-cancer therapy other than is specified in the protocol

3.2.4 Systemic fungal, bacterial, viral, or other infection not controlled (defined as exhibiting ongoing signs/symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment)

3.2.5 Pregnant or lactating. The agents used in this study are known to be teratogenic to a fetus and there is no information on the excretion of agents into breast milk. All females of childbearing potential must have a blood test or urine study within 2 weeks prior to registration to rule out pregnancy.

3.2.6 Known allergy to any of the agents or their ingredients used in this study

3.2.7 Participating in a concomitant Phase 1 or 2 study

4. REGISTRATION PROCEDURES

Registration will occur after eligibility is confirmed and the patient/parent has signed the subject consent, but before any treatment has been administered.

The eligibility checklist will be completed at the time of study enrollment.

4.1 Registration

Upon completion of the screening evaluation, eligibility confirmation and obtaining written consent, the patient will be registered in the study file by the Department of Pediatrics Division of Hematology/Oncology/BMT MACC Fund Center Clinical Trials Office (CTO). When the Research Electronic Data Capture (REDCap) Database is fully functioning for this study, the MARCH Consortium will use this program for patient registration.

4.2 Patients Who Do Not Begin Study Treatment

If a patient is registered on the study, and is later found not able to begin the planned study treatment, for whatever reason, the patient will be removed from study and treated at the physician's discretion. Study data will be collected until the time of study removal. The reason for removal from study will be clearly indicated on the case report forms.

If a patient begins treatment, and then is discontinued for whatever reason, the patient must be followed per Section 5.4.

5. TREATMENT PLAN

The administration of the study drugs will follow institutional drug and supportive care guidelines. Refer to Section 5.2 for additional therapy based on specific disease features.

Only 1 treatment course is permitted.

5.1 Treatment Plan Regardless of Diagnosis

Day	Agent/Procedure
Baseline	Peripheral blood draw and bone marrow aspirate (within 14 days of enrollment)
Day 1	Clofarabine 20 mg/m^2 IV over 2 hours followed by Etoposide 100 mg/m^2 IV over 2 hours followed by Cyclophosphamide 300 mg/m^2 IV as a 30-60 minute infusion
Day 2	Clofarabine 20 mg/m^2 IV over 2 hours followed by Etoposide 100 mg/m^2 IV over 2 hours followed by Cyclophosphamide 300 mg/m^2 IV as a 30-60 minute infusion

Day	Agent/Procedure
Day 3	Clofarabine 20 mg/m^2 IV over 2 hours followed by Etoposide 100 mg/m^2 IV over 2 hours followed by Cyclophosphamide 300 mg/m^2 IV as a 30-60 minute infusion
Day 4	Clofarabine 20 mg/m^2 IV over 2 hours followed by Etoposide 100 mg/m^2 IV over 2 hours followed by Cyclophosphamide 300 mg/m^2 IV as a 30-60 minute infusion
Day 5	Clofarabine 20 mg/m^2 IV over 2 hours followed by Etoposide 100 mg/m^2 IV over 2 hours followed by Cyclophosphamide 300 mg/m^2 IV as a 30-60 minute infusion
Day 30*	Perform bone marrow biopsy/aspirate (*Or upon adequate blood count recover (ANC > 0.5 AND plt > 50,000) whichever comes first
Between Day 28 & 42	Proceed to HCT independent of this study

Pretreatment stress doses of steroids (i.e. hydrocortisone 50-100 mg/m^2/dose IV daily) during clofarabine administration for prophylaxis against SIRS is allowed on study.

A bone marrow evaluation to determine study response and remission status will be performed on study Day 30 or upon adequate blood count recovery (ANC > 0.50 **and** platelet > 50,000), whichever occurs first. If the marrow is hypocellular and without evidence of normal tri-lineage hematopoiesis the marrow should be repeated at Day 42.

5.2 Supportive Care Guidelines

All supportive measures consistent with optimal patient care will be given throughout the study.

Venous Access: A central venous access device, preferably a double lumen, is strongly recommended for this study.

Anti-emetics will be used according to standard medical practice. Growth factor use is allowed on this study.

Antimicrobial Prophylaxis: Patients should be placed on standard of care prophylaxis during this time period at the discretion of the treating physician according to institutional guidelines for PCP, HSV, bacterial and fungus.

5.3 Duration of Therapy

Treatment will be limited to one course. Patients will receive protocol therapy unless:

- Patient withdraws consent or is non-compliant
- Disease progression
- Inter-current illness that prevents further administration of treatment
- Unacceptable toxicity

5.4 Follow-Up

All patients, including those who discontinue protocol therapy early, will be followed

for 60 days from the first dose of study drug or until the start of the transplant prep regimen (or other therapy), whichever occurs earlier.

For patients who go on to transplant (the intent of this study), routine outcomes (treatment related mortality, disease free survival) will be abstracted from the Blood and Marrow Transplantation (BMT) database through the 3rd year post-transplant follow-up.

6. DOSE MODIFICATION

The intent of this study design is for all patients to receive and complete one course of therapy. Patients who exhibit signs of disease progression or experience an unacceptable toxicity will be discontinued from treatment.

There will be no dose delays or dose reductions of study drugs for hematologic toxicity during Consolidation "Bridging" therapy (Day 1 through Day 30); however, prolonged hematopoietic recovery or bone marrow aplasia during the first 42 days may meet a study stopping rule per Section 11.4.

6.1 Clofarabine

Cardiac Dysfunction (Grade 3-4): Discontinue clofarabine administration for Grade 3-4 left ventricular systolic dysfunction.

Hyperbilirubinemia (Grade 3-4): Hold clofarabine administration if Grade 3 or higher increase in bilirubin is noted. If the patient's condition stabilizes and organ function has returned to baseline, clofarabine administration may be continued at a 25% dose reduction.

Pancreatitis (Grade 3-4): Discontinue clofarabine in the presence of hemorrhagic pancreatitis or severe pancreatitis. In the case of mild pancreatitis, clofarabine should be held until symptoms and signs subside, and amylase levels return to normal and then resumed at a 25% dose reduction.

Renal Dysfunction (Grade 3-4): Hold clofarabine administration if Grade 3 or higher increase in creatinine is noted. Adjust clofarabine as follows:

- GFR/CrCl 30 < 60 mL/min/1.73 m^2: Decrease dose by 50%
- GFR/CrCl < 30 mL/min/1.73 m^2: Do **NOT** administer clofarabine. Resume only when ≥ 30 mL/min/1.73 m^2

Systemic Inflammatory Response (SIRS)/Capillary Leak Syndrome: Hold clofarabine administration if signs of SIRS develops (tachypnea or other evidence of respiratory distress, unexplained hypotension and/or unexplained tachycardia). Although clofarabine has been associated with systemic inflammatory response, other causes of SIRS, such as infection, should be investigated. If the patient's condition stabilizes or improves, clofarabine administration may be continued at a 25% dose reduction with the addition of pretreatment stress doses of steroids (e.g. hydrocortisone 50-100 mg/m^2/dose IV daily during clofarabine administration).

6.2 Cyclophosphamide

Hematuria: Omit in the presence of macroscopic hematuria. If there is a history of previous significant hematuria, hydrate before cyclophosphamide until specific gravity is < 1.010 and hydrate at 125 mL/m^2/hr for 24 hours after dose. Monitor for adequate urine output as per institution guidelines. Give IV mesna at a total dose that is 60% of the cyclophosphamide dose divided to 3 doses (i.e. if the cyclophosphamide dose is 1000 mg/m^2, the total mesna dose is 600 mg/m^2 or 200 mg/m^2/dose). Give the first mesna dose 15 minutes before or at the same time as the cyclophosphamide dose and repeat 4 and 8 hours after the start of cyclophosphamide. This total daily dose of mesna can also be administered as IV continuous infusion. The continuous infusion should be started 15-30 minutes before or at the same time as cyclophosphamide and finished no sooner than 8 hours after the end of cyclophosphamide infusion.

Renal Dysfunction: If creatinine clearance or radioisotope GFR is < 10 mL/min/1.73 m^2, reduce dose of cyclophosphamide by 50%. Prior to dose adjustment of cyclophosphamide, the creatinine clearance should be repeated with good hydration.

6.3 Etoposide (VP-16)

Allergic Reaction: Pre-medicate with diphenhydramine (1-2 mg/kg slow IV push, maximum dose is 50 mg). If symptoms persist, add hydrocortisone 100-300 mg/m^2. Continue to use premedication before etoposide in future. Also consider substituting an equimolar amount of etoposide phosphate, in the face of significant allergy and/or hypotension. Etoposide phosphate is a water soluble prodrug that does not contain polysorbate 80 and polyethyleneglycol, the solubilizing agent in etoposide that may induce allergic reactions and hypotension. Etoposide phosphate is rapidly converted to etoposide *in vivo* and provides total drug exposure, as represented by AUC (0-infinity) that is statistically indistinguishable from that measured for etoposide at equimolar doses.

Hypotension: If diastolic or systolic blood pressure (BP) falls 20 mmHg during infusion, reduce infusion rate by 50%. Start a simultaneous infusion of NS 10 mL/kg if BP fails to recover or falls further. Stop infusion if BP does not recover, continue NS. If the patient has had any episode of hypotension, prehydrate with 0.9% NaCl at 10 mL/kg/hr for 2 hours prior to any subsequent infusion.

Renal Insufficiency: If renal function decreases, adjust etoposide as follows: CrCl 10-50 mL/min/1.73 m^2, decrease dose by 25%; if CrCl < 10 mL/min/1.73 m^2, decrease dose by 50%.

Hyperbilirubinemia: If direct bilirubin is > 2 mg/dL, decrease dose by 50%. If direct bilirubin is > 5 mg/dL, hold etoposide.

7. Study Calendar

7.1 Standard of Care

Evaluations	Pre-Study	Day 1-5	Day 6-29	Day 30-60[4]
Clinical history	X			
Physical exam	X	X	Weekly	Weekly
Vital exam	X	X	Weekly	Weekly
Weight	X		Weekly	Weekly
Height	X			
Performance status	X		Weekly	Weekly
Adverse event, toxicity notation	X	X	Weekly	Weekly
Laboratory				
WBC, Hgb, platelets, diff	X	X	Twice Weekly	Twice Weekly
Electrolytes including Ca++, PO4, Glucose	X	X	Weekly	Weekly
BUN, Creatinine, ALT, AST, LDH, Alkaline Phosphatase, bilirubin	X	X	Weekly	Weekly
Albumin and Total Protein	X			
Serum amylase and lipase	X			
Urinalysis	X			
Creatinine clearance[1]	X			
Echocardiogram/MUGA	X			
Pregnancy test[2]	X			
Bone marrow aspirate (within 14 days of enrollment for pre-study sample)[6]	X			X[3]
Lumbar puncture[5]	X			
Treatment				
Clofarabine		Daily		
Etoposide		Daily		
Cyclophosphamide		Daily		

[1] Optional for eligibility, see Section 3.5

[2] For females of childbearing potential only

[3] A bone marrow evaluation to determine study response and remission status will be performed on study Day 30 or upon adequate blood count recovery (ANC > 0. 5 **and** plt > 50,000), whichever occurs first. If the marrow is hypocellular and without evidence of normal tri-lineage hematopoiesis the marrow should be repeated at Day 42

[4] Follow-up for safety continues until the start of a new therapy (i.e. transplant preparative regimen) or Day 60 whichever occurs earlier (transplant related endpoints will be abstracted from the BMT database through the 1 year follow-up)

[5] Intrathecal chemotherapy prophylaxis is allowed at the discretion of the treating physician.

[6] Bone marrow must be sent for morphology, flow cytometry, standard cytogenetics, FISH and molecular testing for routine AML analysis.

8. DRUG FORMULATION AND PROCUREMENT

8.1 Clofarabine

8.1.1 Other Names: CLOLAR, clofarabine; CAFdA; Cl-F-ara-A

8.1.2 Classification: Antineoplastic

8.1.3 Mode of Action: Clofarabine is sequentially metabolized intracellularly to the 5'-monophosphate metabolite by deoxycytidine kinase and mono- and di-phosphokinases to the active 5'-triphosphate metabolite. Clofarabine has high affinity for the activating phosphorylating enzyme, deoxycytidine kinase, equal to or greater than that of the natural substrate, deoxycytidine. Clofarabine inhibits DNA synthesis by decreasing cellular deoxynucleotide triphosphate pools through an inhibitory action on ribonucleotide reductase, and by terminating DNA chain elongation and inhibiting repair through incorporation into the DNA chain by competitive inhibition of DNA polymerases. The affinity of clofarabine triphosphate for these enzymes is similar to or greater than that of deoxyadenosine triphosphate. In preclinical models, clofarabine has demonstrated the ability to inhibit DNA repair by incorporation into the DNA chain during the repair process. Clofarabine 5'-triphosphate also disrupts the integrity of mitochondrial membrane, leading to the release of the pro-apoptotic mitochondrial proteins, cytochrome C and apoptosis-inducing factor, leading to programmed cell death. Clofarabine is cytotoxic to rapidly proliferating and quiescent cancer cell types in vitro. The population pharmacokinetics of clofarabine were studied in 40 pediatric patients aged 2 to 19 years (21 males/19 females) with relapsed or refractory ALL or AML. At the given 52 mg/m^2 dose, similar concentrations were obtained over a wide range of BSAs. Clofarabine was 47% bound to plasma proteins, predominantly to albumin. Based on non-compartmental analysis, systemic clearance and volume of distribution at steady-state were estimated to be 28.8 $L/h/m^2$ and 172 L/m^2, respectively. The terminal half-life was estimated to be 5.2 hours. No apparent difference in pharmacokinetics was observed between patients with ALL and AML or between males and females. Based on 24-hour urine collections in the pediatric studies, 49-60% of the dose is excreted in the urine unchanged. *In vitro* studies using isolated human hepatocytes indicate very limited metabolism (0.2%), therefore the pathways of non-renal elimination remain unknown. Although no clinical drug-drug interaction studies have been conducted to date, on the basis of the *in vitro* studies, cytochrome p450 inhibitors and inducers are unlikely to affect the metabolism of clofarabine. The effect of clofarabine on the metabolism of cytochrome p450 substrates has not been studied. The pharmacokinetics of clofarabine have not been evaluated in patients with renal or hepatic dysfunction.

8.1.4 Storage and Stability: Clofarabine is a white to off-white solid with a melting point of 228°C to 230°C and a molecular weight of 303.5. The drug substance is very stable in the dry state, and aqueous solutions are stable to heat treatment.

Clofarabine is freely soluble in water (1.5 mg/mL) or buffered solutions at room temperature. Clofarabine is not less than 97% pure on a dried basis by high performance liquid chromatography (HPLC) analysis. Clofarabine is formulated at a concentration of 1 mg/mL. Clofarabine is supplied in 1 vial size: a 20-mL clear, glass vial with gray stopper and blue flip-off seal. The 20-mL vials contain 20 mL (20 mg) of sterile solution. The pH range of the solution is 4.5 to 7.5. The solution is clear and practically colorless, preservative free, and free from foreign matter. Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).

Vials containing undiluted clofarabine for injection should be stored at controlled room temperature.

Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F). The commercial expiry period for clofarabine is 24 months at room temperature. Ongoing stability studies will continue to confirm the appropriate quality of drug product used for clinical trials beyond 24 months. Clofarabine for injection should be diluted with 0.9% sodium chloride injection USP or European Pharmacopeia (EP) normal saline (NS) or 5% dextrose injection (D5W) USP or EP prior to IV infusion. The resulting admixture may be stored at room temperature, but must be used within 24 hours of preparation.

8.1.5 Dose Specifics: For the purposes of this study, clofarabine will be administered at the dose of 30 mg/m^2 for patients 0-30 years (20 mg/m^2 for patients > 30 years) IV over 2 hours (given at Hours 0 to 2) on Days 1 through 5.

8.1.6 Administration: USP or 0.9% sodium chloride injection USP to a convenient volume such as the maximum final concentration should be between 0.15 mg/mL and 0.4 mg/mL, and infuse over 2 hours. The resulting admixture may be stored at room temperature, but must be used within 24 hours of preparation. To prevent drug incompatibilities, no other medications should be infused concurrently through the same IV lines as clofarabine. Also, no blood products should be administered at the same time as clofarabine.

To reduce the effects of tumor lysis and other adverse events it is recommended that continuous IV fluids be given throughout the 5 days of clofarabine administration. Since clofarabine is primarily excreted through the kidneys, drugs with known renal toxicity should be avoided during the 5 days of clofarabine administration. In addition, since the liver is a known target organ for clofarabine toxicity, concomitant use of medications known to induce hepatic toxicity should be avoided.

Pretreatment stress doses of steroids with hydrocortisone (50-100 mg/m^2/dose IV daily) during clofarabine administration is allowed on this study.

8.1.7 Availability: Commercially available by prescription.

8.1.8 Side Effects: Refer to Appendix 4.

Pregnancy Category D: Clofarabine may cause fetal harm when administered to

a pregnant woman. Women of childbearing potential should be advised to avoid becoming pregnant while using Clofarabine. Men should be advised not to father a child while receiving treatment with Clofarabine, and for 2 months afterwards.

8.2 Cyclophosphamide

8.2.1 Other Names: Cytoxan, CTX

8.2.2 Classification: Alkylating agent

8.2.3 Mode of Action: Cyclophosphamide is an alkylating agent related to nitrogen mustard. Cyclophosphamide is inactive until it is metabolized by P450 isoenzymes (CYP_2B_6, CYP_2C_9, and CYP_3A_4) in the liver to active compounds. The initial product is 4-hydroxycyclophosphamide (4-HC) which is in equilibrium with aldophosphamide which spontaneously releases acrolein to produce phosphoramide mustard. Phosphoramide mustard, which is an active bifunctional alkylating species, is 10 times more potent *in vitro* than is 4-HC and has been shown to produce interstrand DNA cross-link analogous to those produced by mechlorethamine. Approximately 70% of a dose of cyclophosphamide is excreted in the urine as the inactive carboxyphosphamide and 5-25% as unchanged drug. The plasma half-life ranges from 4.1 to 16 hours after IV administration.

8.2.4 Storage and Stability: Cyclophosphamide for injection is available as powder for injection or lyophilized powder for injection in 500 mg, 1 g, and 2 g vials. The powder for injection contains 82 mg sodium bicarbonate/100 mg cyclophosphamide and the lyophilized powder for injection contains 75 mg mannitol/100 mg cyclophosphamide. Storage at or below 25ºC (77ºF) is recommended. The product will withstand brief exposures to temperatures up to 30ºC (86ºF).

8.2.5 Dose Specifics: For the purposes of this study, cyclophosphamide 300 mg/m^2 will be administered intravenously over 30 to 60 minutes on Days 1 through 5.

8.2.6 Administration Cyclophosphamide for Injection: Reconstitute with Sterile Water or Bacteriostatic Water for Injection to a concentration of 20 mg/mL. Solutions reconstituted with preservative should be used within 24 hours if stored at room temperature or within 6 days if stored under refrigeration. If administered as undiluted drug at the 20 mg/mL concentration, reconstitute with NS only to avoid a hypotonic solution. Cyclophosphamide may be further diluted in dextrose or saline containing solutions for IV use.

8.2.7 Availability: Cyclophosphamide is commercially available by prescription. Cyclophosphamide for Injection, USP contains cyclophosphamide monohydrate and is supplied in vials for single dose use.

8.2.8 Side Effects: Refer to Appendix 4.

Pregnancy Category D: Cyclophosphamide can cause fetal harm when administered to a pregnant woman.

8.3 Etoposide

8.3.1 Other Names: VP-16, Etopophos; Toposar; VePesid.

8.3.2 Classification: A semisynthetic derivative of podophyllotoxin that forms a complex with topoisomerase II and DNA which results in single and double strand DNA breaks. Its main effect appears to be in the S and G2 phase of the cell cycle. The initial t½ is 1.5 hours and the mean terminal half-life is 4 to 11 hours. It is primarily excreted in the urine. In children, approximately 55% of the dose is excreted in the urine as etoposide in 24 hours. The mean renal clearance of etoposide is 7 to 10 mL/min/m² or about 35% of the total body clearance over a dose range of 80 to 600 mg/m². Etoposide, therefore, is cleared by both renal and non-renal processes, i.e., metabolism and biliary excretion. The effect of renal disease on plasma Etoposide clearance is not known. Biliary excretion appears to be a minor route of etoposide elimination. Only 6% or less of an intravenous dose is recovered in the bile as etoposide. Metabolism accounts for most of the non-renal clearance of etoposide. The maximum plasma concentration and area under the concentration time curve (AUC) exhibit a high degree of patient variability. Etoposide is highly bound to plasma proteins (~ 94%), primarily serum albumin. Pharmacodynamic studies have shown that etoposide systemic exposure is related to toxicity. Preliminary data suggests that systemic exposure for unbound etoposide correlates better than total (bound and unbound) etoposide. There is poor diffusion into the CSF < 5%. Cmax and AUC values for orally administered etoposide capsules consistently fall in the same range as the Cmax and AUC values for an intravenous dose of one-half the size of the oral dose. The overall mean value of oral capsule bioavailability is approximately 50% (range 25-75%). Etoposide phosphate is a water soluble ester of etoposide which is rapidly and completely converted to etoposide in plasma. Pharmacokinetic and pharmacodynamic data indicate that etoposide phosphate is bioequivalent to etoposide when it is administered in molar equivalent doses.

8.3.3 Dose Specifics: For the purposes of this study, etoposide 100 mg/m^2 will be administered intravenously over 2 hours on Day 1 through 5.

8.3.4 Administration: Dilute etoposide to a final concentration ≤ 0.4 mg/mL in Dextrose or Normal Saline containing IV solutions. Etoposide infusions are stable at room temperature for 96 hours when diluted to concentrations of 0.2 mg/mL; stability is 24 hours at room temperature with concentrations of 0.4 mg/mL. The time to precipitation is highly unpredictable at concentrations > 0.4 mg/mL. Administer over 30 to 60 minutes; slow rate of administration if hypotension occurs. **Do not administer etoposide by rapid intravenous injection.** Use in-line filter during infusion secondary to precipitate formation risk. To avoid leaching of DEHP from PVC bags and tubing, prepare the etoposide solution as close as possible preferably within 4 hours to the time of administration or alternatively as per institutional policy, non-PVC containers and tubing may be used.

8.3.5 Availability: Commercially available by prescription.

8.3.6 Storage and Stability: Etoposide for Injection is available in sterile multiple dose vials. The pH of the clear, nearly colorless to yellow liquid is 3 to 4. Each mL contains 20 mg etoposide, 2 mg citric acid, 30 mg benzyl alcohol, 80 mg modified polysorbate 80/tween 80, 650 mg polyethylene glycol 300, and 30.5 percent (v/v) alcohol. Vial headspace contains nitrogen. Unopened vials of etoposide are stable until expiration date on package at room temperature (25°C). Etoposide phosphate for injection is available for intravenous infusion as a sterile lyophilized powder in single-dose vials containing etoposide phosphate equivalent to 100 mg etoposide, 32.7 mg sodium citrate *USP*, and 300 mg dextran 40. Etoposide phosphate must be stored under refrigeration 2°-8°C (36°-46°F). Unopened vials of Etoposide phosphate are stable until the expiration date on the package.

8.3.7 Side Effects: Refer to Appendix 4.

Pregnancy Category D: Etoposide may cause fetal harm when administered to a pregnant woman. Women of childbearing potential should be advised to avoid becoming pregnant while using Etoposide. Men should be advised not to father a child while receiving treatment with Etoposide, and for 2 months afterwards.

9. ADVERSE EVENT MONITORING, DOCUMENTATION, AND REPORTING

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 4.0 (CTCAE) and reported on the schedule below. A copy of the CTCAE can be downloaded from the CTEP home page: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

9.1 Definitions

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21 CFR 312.32(a)).

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Life-Threatening Adverse Event Or Life-Threatening Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death.

Event Attribution Categories: CTCAE does not define an AE as necessarily 'caused by a therapeutic intervention.' The clinical investigator must assign attribution for an adverse

event after naming and grading of the event.

Attribution	Description
Unrelated	The AE is clearly NOT related to the intervention
Unlikely	The AE is doubtfully related to the intervention
Possible	The AE may be related to the intervention
Probable	The AE is likely related to the intervention
Definite	The AE is clearly related to the intervention

Unanticipated (unexpected) adverse event or unexpected suspected adverse reaction as defined by the Medical College of Wisconsin IRB are those that are *not* already described as potential risks in the consent form, *not* listed in the Investigator's Brochure or *not* part of an underlying disease.

Expedited (Rapid) Reporting: Certain events may require rapid notification to entities providing patient safety oversight (i.e. IRB) as detailed in Section 9.3.

9.2 Adverse Event Documentation

Adverse events occurring after the initiation of any study treatment must be documented. Refer to Appendix 4 for a list of expected toxicities.

Since a key endpoint of this study is to characterize the toxicities of clofarabine, cyclophosphamide and etoposide when used in combination, adverse event documentation requirements will be determined based on grade, expectedness and relationship to study therapy as follows:

	Grade 1	Grade 2		Grade 3		Grade 4 and 5
	Expected or Unexpected	**Expected**	**Unexpected**	**Expected**	**Unexpected**	**Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	Not required	Not Required	**Required**	**Required**
Possible Probable Definite	Not required	Not required	Not Required	Not Required	**Required** **(Non hematologic only)**	**Required**

Stopping Rule Events: The following events count toward a study stopping rule per Section 11.4 and must be reported to the MCW Study Coordinator and Dr. Burke using the SAE Form found in REDCap which is also immediately reported (within 24 hours of PI notification) to the MCW DSMC:

- Any Grade 4 non-hematologic treatment related event
- Any death, regardless of cause during the reporting period for this study (from initiation of study treatment through the start of a new treatment (i.e. transplant preparative regimen), or Day 60, whichever occurs earlier)

All patients will be monitored until the start of a new treatment (i.e. transplant preparative regimen) or Day 60, whichever occurs earlier, as it is expected that most treatment related adverse events will occur during this period.

For patients who go on to transplant, transplant related endpoints will be collected by record review.

9.3 Adverse Event Reporting Requirements

The reporting period for this study is from initiation of study treatment through the start of a new treatment (i.e. transplant preparative regimen), or Day 60, whichever occurs earlier; however after this time point, the investigator must report upon knowledge any study treatment related event meeting the expedited reporting criteria listed in the following table.

Agency	Criteria for Reporting	Timeframe	Form to Use	Copy AE To:
MCW IRB	UPIRSO: Any event which is unanticipated, involved new or increased risk to subjects, and was at least possibly related to study procedures through Day 60 or the start of a new treatment whichever is earlier	5 calendar days after PI Notification	SAE Found in REDCap	Dr. Michael Burke & MCW Study Coordinator
	Other Problems or Events meeting the definition of UPIRSO			
MCW DSMC	Any event that counts toward a study stopping rule (see Section 11.4)	Within 24 hours of PI notification	SAE Found in REDCap	Dr. Michael Burke & MCW Study Coordinator

Serious adverse events are reported directly to Dr. Michael Burke and the MCW Study Coordinator within 72 hours of the event via the REDCap database (Section 10.1). The adverse event report is reviewed by Dr. Michael Burke and Causality/Attribution assigned, as applicable.

MCW CC DSMC Reporting: The MCW Cancer Center DSMC is responsible for the global oversight and review of patient safety on this clinical trial. The DSMC meets twice a year (at a minimum) and all submitted outcome data is reviewed including all adverse events reported to the MCW IRB. The DSMC reserves the right to meet more frequently on an as needed basis based on the events submitted.

The DSMC semi-annual review confirms that the trial has not met any stopping rules and reviews any patient safety problems necessitating discontinuation of the trial. A report from the DSMC is submitted to the MCW IRB as well as the trial coordinators/local PIs of this protocol.

Grade 3 Unexpected (non-hematological only), and all Grade 4 and 5 events will be submitted to the DSMC within 24 hours of PI (Dr. Michael Burke) Notification. The DSMC will acknowledge the submission with a responding email.

MCW IRB Reporting: Serious adverse events are reported directly to the PI and the MCW Study Coordinator within 72 hours of the event via the REDCap database (Section 10.1). The adverse event report is reviewed by Dr. Michael Burke and Causality/Attribution assigned, as applicable. If the adverse event meets the MCW IRB criteria for expedited reporting, an official signed report is submitted to the MCW Institutional Review Board. All deaths, regardless of the cause, are reported to the MCW IRB.

An official report of a serious adverse event is faxed or emailed to the MARCH coordinating center within five days of occurrence.

9.4 Event Notification, Deviations, or Patient Complaints

Any event, protocol deviation, or patient complaint, not meeting the criteria for serious adverse event reporting, should be reported to the MCW Study Coordinator via the REDCap database (Section 10.1). The report is reviewed by the PI and any further action, if deemed necessary, is discussed with the reporting site.

10. STUDY DATA COLLECTION AND MONITORING

10.1 Data Management

Data management will be performed utilizing an electronic data capture system called REDCap. REDCap (Research Electronic Data Capture) is a secure, web-based application designed exclusively to support data capture for research studies.

Security is provided through a table based authentication system where users are added to a project and are granted specific rights within the project(s) they have access to. These specific access rights consist of access to the study calendar, 3 levels (no access, de-identified, full data set) of access to the Data Export Tool, access to the Data Import Tool, the Data Comparison tool (used when double-data entry is utilized), access to the complete logging records, the File Repository, User Rights (ability to add users and grant rights), access Data Access Groups, access to reports and the report builder, access for project setup and design, and a few more. Users can only access the specific projects they have been granted access to.

The CTSI of Southeast Wisconsin's REDCap installation is housed on a CTSI-owned server located in the TBRC Data Center at the Medical College of Wisconsin, and all traffic between the user's browser and the server is encrypted with a 128-bit Digicert SSL certificate. The server OS is incrementally backed up nightly and rotated off-site. The database (MySQL) is backed up 4 times daily and is also part of the incremental nightly OS back up.

The Data Access Groups feature within REDCap will be enabled for this project. This feature allows users to be designated into groups (sites), after which any user in the Data Access Group may only see records created by another person in that group. Thus, sites may enter patient data but not be allowed to see other site's data/records. Only the CDCC will be able to view and analyze data entered from all site records. The data export function is included as part of the website. Once data has been successfully entered into

the database, the investigator and his/her staff can extract this data for different analysis software programs.

Once an institution consents a patient, they will enter the patient demographics into the REDCap system which will assign a study ID. Centers will be required to upload the signed informed consent to the CDCC office via the REDCap database. Subsequent data will be entered via REDCap for review by the CDCC in the same manner. The study coordinator will review the data reports with Dr. Burke within 24-72 hours The overall study coordinator will follow-up with the institution if there are missing or delinquent data forms, and request submission within a timely manner.

Any paper data (i.e. safety reports, reports generated from REDCap) will be kept in a locked and secure location within the Clinical Trials Office of the Pediatric Hematology/Oncology and Bone Marrow Transplant Program. The Clinical Trials Office has secure, electronic employee badge access only. Any paper data that is generated and pertinent to the conduct of study, will be kept for 10 years after the study file is closed with the IRB.

REDCap training will be provided by the MCW Clinical and Translational Science Institute (CTSI) to each participating site.

For patients going on to transplant, the transplant related endpoints for this study will be collected by record review for 3 years post-transplant.

10.2 Case Report Forms

Initial Plans: Participant data will be collected using protocol specific case report forms developed by the MACC Fund Center CTO. The CRFs will be approved by the study's Principal Investigator and the Biostatistician prior to release for use. The Study Coordinator or designee will be responsible for registering the patient at time of study entry, completing CRFs based on the patient specific calendar or roadmap, and updating the patient record until the end of required study participation.

Implementation of REDCap: Participant data will be collected using protocol specific electronic case report forms (e-CRFs) developed within REDCap based on its library of standardized forms. The e-CRF will be approved by the study's Principal Investigator and the Biostatistician prior to release for use. The Study Coordinator or designee will be responsible for registering the patient into REDCap at time of study entry, completing e-CRFs based on the patient specific calendar, and updating the patient record until the end of required study participation.

10.3 Data and Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with the Medical College of Wisconsin Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://www.mcw.edu/FileLibrary/Groups/HumanResearchProtectionOffice/IRB/Newand DraftPolicies/IRBSOPDSMP.pdf

This study will be reviewed by the Medical College of Wisconsin Cancer Center Data Safety Monitoring Committee (MCW CC DSMC). A summary of the MCW CC DSMC activities are as follows:

- Review the clinical trial for data integrity and safety
- Review all Grade 3 unexpected adverse events, all Grade 4 and 5 adverse events, and all events requiring expedited reporting as defined per protocol
- Review all DSM reports
- Submit a summary of any recommendations related to study conduct
- Terminate the study if deemed unsafe for patients

A copy of the MCW CC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI twice annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary.

Any available DSMC letters will be submitted to the IRB of record as required.

For the purposes of data and safety monitoring, this study is classified as moderate risk. Therefore the following requirements will be fulfilled:

- The PI will complete and submit a semi-annual Trial Progress Report to the MCW Cancer Center Data and Safety Monitoring Committee (DSMC) with the understanding the MCW DSMC may require more frequent reporting.
- The PI will oversee the submission of all reportable events per the definition of reportable in Section 9.3 to the MCW Cancer Center's DSMC and the MCW IRB.

10.4 Monitoring

The PI at each participating center is responsible for monitoring this study for accuracy of data and protocol compliance at their institution. Patient eligibility will be confirmed by Dr. Michael Burke before each patient is enrolled.

The PI, data coordinators, and research nurses are responsible for review and maintenance of all patient records at their individual institution to ensure data integrity and protocol adherence.

The site PI will permit study-related monitoring, audits, and inspections by the MCW compliance groups, as necessary. The investigator will make available all study related documents (i.e. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (i.e. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

10.5 Record Retention

The investigator will retain study records including source data, copies of case report forms, consent forms, HIPAA authorizations, and all study correspondence in a secured

facility for 10 years after the study file is closed with the IRB.

In addition, the Clinical Trials Office (CTO) will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient. Please contact the CTO before destroying any study related records.

11. STATISTICAL CONSIDERATIONS

This is a Phase 2 study designed for the purpose of estimating the key parameters for use in future studies and for estimating whether there is a difference between adult and pediatric response.

11.1 Statistic Endpoints

The following parameters will be monitored and used to estimate the effect sizes with their 95% confidence intervals.

- The effect of Clofarabine, Cyclophosphamide, and Etoposide in eliminating the presence of persistent disease without causing a significant delay of HCT due to treatment related toxicity, defined as greater than 56 days from completion of bridging therapy to start of HCT. The proportion of any toxicities associated with Clofarabine, Cyclophosphamide, and Etoposide
- The proportion of pre-transplant chemotherapy-induced aplasia (defined as greater than 42 days after infusion of chemotherapy)
- The proportion of infectious complications following bridging therapy and before HCT
- The proportion of patients who do not proceed to HCT after receiving bridging therapy due to disease status or treatment related morbidity/mortality
- Treatment–related mortality at Day 100 after HCT
- One year disease-free survival after HCT
- One year overall survival after HCT
- The proportion of relapse within one year of HCT

11.2 Statistical Analysis

SAS 9.4 (Cary, NC, USA) will be used for the statistical analyses. The summary statistics including 95% confidence intervals for MRD negative conversion rate will be calculated. All toxicity and safety assessments will be presented in the data listings and summarized with point estimates and 95% confidence intervals. Proportions will use exact binomial confidence intervals. Overall survival and disease free survival estimates after HCT will use the Kaplan-Meier method. The Nelson-Aalen counting process method will be used to estimate cumulative survival/relapse and the intensity of the process. Relapse and treatment-related mortality after HCT will be estimated by competing risk analysis using the counting process approach.

11.3 Sample Size Justification

This is a Phase 2 study designed for the purpose of estimating the following key

parameters for use in future studies and for estimating whether there is a difference between young adult and pediatric response: 1) The effect of CCE in eliminating the presence of MRD; 2) Whether CCE will significantly delay time to HCT due to treatment related toxicity; 3) The proportion of any toxicities associated with CCE; 4) The proportion of pre-transplant chemotherapy-induced aplasia (defined as greater than 42 days after infusion of chemotherapy); 5) The proportion of infectious complications following bridging therapy and before HCT; 6) The proportion of patients who do not proceed to HCT after receiving bridging therapy due to disease status or treatment related morbidity/mortality; 7) Treatment–related mortality at Day 100 after HCT; 6) One year DFS after HCT; 7) One year OS after HCT; and 8) The proportion of relapse within 1-year of HCT. Post-HCT outcomes (OS, EFS and Relapse) will be compared to historical controls for children, adolescent and young adults (separated by age; 0-18 and 19-39 years) receiving a HCT for ALL or AML with pre-HCT MRD, using national and international data.

11.4 Early Stopping Rule for Excessive Toxicity (pre-HCT) and TRM (post-HCT)

The complications and adverse events will be closely monitored in this study. Rates of grade 3 or greater non hematologic toxicity that does not resolve by day 56 following bridging therapy, such that patients are not able to proceed to HCT on time, that exceed 10% will be considered unacceptable. The trial will be stopped for review if the Grade 3 non-hematologic toxicity rate statistically exceeds 10%. Similarly, a pre-HCT toxic death rate attributed to the bridging therapy that exceeds p0=5% will be considered unacceptable. No formal statistical rule will be employed. Rather, if the crude proportion of patients experiencing a toxic death exceed 5% at any time (using the Kaplan-Meier curve and log-rank comparisons) prior to HCT, the cause and circumstances of these deaths will be reviewed with the study committee and with the Data and Safety Monitoring Committee to determine whether modifications to or termination of the study is warranted.. As well, if we see a lack of efficacy in the ability of this regimen to eliminate MRD at 1/3 or 2/3 the total number of patients, the study will be suspended to consider dose escalation of clofarabine.

Nth Patient	Stopping Number Mortality	Stopping Number Toxicity	Nth Patient	Stopping Number Mortality	Stopping Number Toxicity
1	-	-	14	3	4
2	-	-	15	3	5
3	2	2	16	3	5
4	2	2	17	4	5
5	2	3	18	4	5
6	2	3	19	4	5
7	2	3	20	4	6
8	3	3	21	4	6
9	3	3	22	4	6
10	3	4	23	4	6
11	3	4	24	4	6

12	3	4	25	4	7
13	3	4			

12. CONDUCT OF THE STUDY

12.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). The investigator will be thoroughly familiar with the appropriate use of the drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

12.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, informed consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

12.3 Informed Consent

All potential study participants will be given a copy of the MCW IRB approved consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant (or parents/guardians for a minor) decides to participate in the study, he/she will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

12.4 Clinical Trial Registration

A description of this clinical trial will be available at: http://www.ClinicalTrials.gov, as required by U.S. Law. This web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time. NCI Number: NCT02349178

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APPENDIX 1
Eligibility Checklist

Eligibility Checklist – Page 1 of 2
Patient Initials ☐☐☐☐ **Patient ID** ☐☐

Date Consent Form Signed: ☐☐/☐☐/☐☐☐☐ Diagnosis ☐ **ALL** or ☐ **AML**

Inclusion Criteria
A "NO" response to any of the following disqualifies the patient from study entry.

<table>
<tr><th></th><th></th><th>Yes</th><th>No</th></tr>
<tr><td>1</td><td>Diagnosis of acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) with < 5% blasts in the bone marrow (M1) by morphology and that meets the following criteria:
☐ Flow cytometric evidence of PD (≥ 0.01% leukemic blasts for ALL or ≥ 0.5% leukemic blasts for AML detected in the bone marrow)
OR
☐ Molecular/cytogenetic evidence of disease (FISH or PCR methodology) performed within 7 days and with the intent of going on to an allogeneic hematopoietic cell transplantation (HCT) independent of this study</td><td></td><td></td></tr>
<tr><td>2</td><td>Age 0 to 39 years</td><td></td><td></td></tr>
<tr><td>3</td><td>Karnofsky ≥ 50% for patients 16 years and older and Lansky status ≥ 50 for patients under 16 years of age
Score - ☐☐☐</td><td></td><td></td></tr>
<tr><td>4</td><td>Patients must have a life expectancy ≥ 8 weeks as determined by the enrolling investigator</td><td></td><td></td></tr>
<tr><td>5</td><td>Adequate organ function defined as within 7 days of the start of study treatment, unless otherwise noted
<table>
<tr><th>Test</th><th>Required Value</th><th>Patient's Result</th><th>Date Performed</th></tr>
<tr><td>Creatinine</td><td>Creatinine clearance ≥ 60 mL/min/1.73m^2
OR serum creatinine based on age:
<table>
<tr><th>Age (Yrs)</th><th>Maximum Serum Creatinine (mg/dl)</th></tr>
<tr><td>2 to < 6</td><td>0.8 0.8</td></tr>
<tr><td>6 to < 10</td><td>1.0 1.0</td></tr>
<tr><td>10 to < 13</td><td>1.2 1.2</td></tr>
<tr><td>13 to < 16</td><td>1.5 1.4</td></tr>
<tr><td>≥ 16</td><td>1.7 1.4</td></tr>
</table></td><td>☐☐.☐ mL/min/1.73 m^2
OR
☐.☐ mg/dl</td><td>☐☐/☐☐/☐☐</td></tr>
<tr><td>Total Bilirubin</td><td>≤ 1.5 x ULN for age</td><td>☐.☐</td><td>☐☐/☐☐/☐☐</td></tr>
<tr><td>ALT</td><td>< 5 X ULN</td><td>☐☐☐☐</td><td>☐☐/☐☐/☐☐</td></tr>
<tr><td>Cardiac</td><td>Left ventricular ejection fraction ≥ 40% by ECHO/MUGA</td><td>☐☐%</td><td>☐☐/☐☐/☐☐</td></tr>
</table></td><td></td><td></td></tr>
<tr><td>6</td><td>Patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study. At least 7 days must have elapsed from prior chemotherapy.
Hematopoietic growth factors: At least 7 days since the completion of therapy with a growth factor and at least 14 days since pegfilgrastim (Neulasta®) administration.</td><td></td><td></td></tr>
<tr><td>7</td><td>Sexually active females of child bearing potential must agree to use adequate contraception (diaphragm, birth control pills, injections, intrauterine device [IUD], surgical sterilization, subcutaneous implants, or abstinence, etc.) for the duration of treatment and for 2 months after the last dose of chemotherapy. Sexually active men must agree to use barrier contraceptive for the duration of treatment and for 2 months after the last dose of chemotherapy.</td><td></td><td></td></tr>
</table>

8	Voluntary written consent before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the subject/guardian at any time without prejudice to future medical care.		

APPENDIX 1
Eligibility Checklist - Continued

Eligibility Checklist – Page 2 of 2
Patient Initials ☐☐☐☐

Exclusion Criteria
A "Yes" response to any of the following disqualifies the patient from study entry.

		Yes	No
9	Acute Promyelocytic Leukemia (APL)		
10	Active CNS leukemia or presence of chloromatous disease		
11	Receiving concomitant chemotherapy, radiation therapy; immunotherapy or other anti-cancer therapy other than is specified in the protocol		
12	Systemic fungal, bacterial, viral, or other infection not controlled (defined as exhibiting ongoing signs/symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment)		
13	Pregnant or lactating. The agents used in this study are known to be teratogenic to a fetus and there is no information on the excretion of agents into breast milk. All females of childbearing potential must have a blood test or urine study within 2 weeks prior to registration to rule out pregnancy.		
14	Known allergy to any of the agents or their ingredients used in this study.		

Patient Given Copy of Consent: ☐ **Yes, Date** _____ ☐ **No**

Having obtained consent and reviewed each of the inclusion/exclusion criteria, I verify that this patient is:

_____ **Eligible** ____ **Ineligible** **Date Registered** _____

_____
Signature of Person Completing This Form

APPENDIX 2
Performance Status Criteria

For patients 16 years of age and older:

Karnofsky Performance Scale	
Percent	**Description**
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead

For patients less than 16 years of age:

Lansky Performance Scale	
Lansky Score	**Play Score**
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restriction of and less time spent in play activity
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed but lies around much of the day, no active play but able to participate in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	No play; does not get out of bed
0	Unresponsive

APPENDIX 3
Response Criteria

Modified RECIST

Complete Remission (CR)
CR requires that all of the following be present:

- Peripheral Blood Counts
  - ANC count > 1,000/mm^3
  - Platelet count > 100,000/mm^3
  - Reduced hemoglobin concentration or hematocrit has no bearing on remission status
  - Leukemic blasts must not be present in the peripheral blood
- Marrow Aspirate and Biopsy
  - Bone marrow biopsy must demonstrate tri-lineage hematopoiesis with maturation of all cell lines.
  - < 5% blasts
- Extramedullary leukemia, such as CNS or soft tissue involvement, must not be present.

Morphological Remission (MR)
MR requires that the following be present:

- Patient meets all peripheral blood and bone marrow criteria for CR, except that platelet count is < 100,000/mm^3 but > 50,000/mm^3

Minimal Residual Disease (MRD) Negativity

- MRD negativity for ALL requires the following be present:
  - < 0.01% leukemic blasts by flow cytometry
  - Absence of cytogenetic and/or molecular evidence of disease if applicable

- MRD negativity for AML requires the following be present:
  - < 0.5% leukemic blasts by flow cytometry
  - Absence of cytogenetic and/or molecular evidence of disease if applicable

Relapse
Relapse following CR is defined as:

- Peripheral Blood Counts
  - Presence of peripheral blasts. A bone marrow examination must be performed to confirm relapse. However, please note that the date of relapse is the first date at which the relapsed patient had: leukemic blasts in the peripheral blood smear, or >5% blasts in the bone marrow.
- Bone Marrow Aspirate or Biopsy
  - Presence of more than 5% blasts, not attributable to another cause (i.e. bone marrow regeneration).

APPENDIX 4
Known and Expected Toxicities of Study Treatment

Known Risks and Side Effects Related to the Clofarabine Include Those Which Are:		
Likely	**Less Likely**	**Rare But Serious**
• A fast heartbeat which may cause pain in the chest • A feeling of extreme tiredness not relieved by sleep • A decrease in blood pressure • Pain in the abdomen (belly) • Constipation • Diarrhea • Nausea and/or vomiting • Headache • Fever which may also come with shaking chills • Anxiety • Fever with a low white blood cell count which could mean that you have an infection and might require hospitalization and treatment with antibiotics • Loss of appetite • Elevation in the blood of certain enzymes found in the liver which may mean the liver is not working as well as normal • Damage to the sac around the heart which can lead a build-up of fluid around the heart which may be painful and affect the ability of the heart to work normally but in most cases is only mild and temporary • Temporary change to the heart such that it does not pump the blood as well which may make you tired, weak, feel short of breath, and retain fluid • Skin rash with itching and redness or inflammation • Infections including those caused by bacteria, virus, and fungus and can be found in the lung, the blood, the skin and	• Fluid build-up in the tissues • Pain or burning at the site of the injection • Dizziness • Sleepiness • Tremor (shakiness usually of the hands) • Changes to your emotions such that you feel depressed, anxious, irritable or confused • High blood pressure • Cough or shortness of breath • Reddening of the face with feelings of warmth when the drug is infusing • Sore throat • Inflammation and/or sores in the mouth (and/or throat and /or esophagus, the tube that leads from the mouth to the stomach) that may make swallowing difficult and are painful (painful mouth sores) • Pain including back or arm or leg pain • Enlarged liver which could lead to an elevation in the blood of bilirubin which is found in the liver and if present in large amounts can lead to a yellow appearing skin (jaundice) • Weight loss • Aches and pains in the muscles and joints • Bleeding from the bladder or gums • Fluid build-up in the lungs	• The rapid death of large numbers of tumor cells which can cause the potassium and phosphate salts and the uric acid in the blood to rise quickly and this could lead to a life-threatening irregular heart beat or damage to the kidneys. • Severe loss of water from the body (dehydration) which if untreated may cause low blood pressure and severe loss of salts such as sodium and potassium from the body and could lead to the kidneys failing which could be life-threatening • Capillary leak syndrome: A condition in which fluid and proteins leak out of tiny blood vessels and flow into surrounding tissues, resulting in dangerously low blood pressure. Capillary leak syndrome may lead to multiple organ failure such as kidney, heart or liver failure and shock. • Inflammation of the pancreas (an organ in the abdomen which produces insulin and certain digestive chemicals) which may affect the function of the

Known Risks and Side Effects Related to the Clofarabine Include Those Which Are:		
Likely	**Less Likely**	**Rare But Serious**
other places in the body • Bloody nose • Fewer white blood cells, red blood cells and platelets in the blood ○ a low number of white blood cells can make it easier to get infections ○ a low number of red blood cells can make you feel tired and weak ○ a low number of platelets causes you to bruise and bleed more easily	that can make you feel short of breath • Severe rash with redness and pain on the palms of the hand and soles of the feet • Red spots on the skin from low platelets • High levels of uric acid in the blood which could damage the kidneys • Increased levels of a chemical (creatinine) in the blood which could mean kidney damage	pancreas and which may cause pain in the abdomen (belly) which can be severe and may increase the blood sugar

Known Risks and Side Effects Related to Cyclophosphamide Include Those Which Are:		
Likely	**Less Likely**	**Rare But Serious**
• Loss of appetite • Nausea • Vomiting • Fewer white blood cells in the blood. ○ a low number of white blood cells may make it easier to get infections. • Hair loss • Decreased ability of the body to fight infection • Absence or decrease in the number of sperm which may be temporary or permanent which may decrease the ability to have children	• Abnormal hormone function which may lower the level of salt in the blood • Abdominal pain • Diarrhea • Fewer red blood cells and platelets in the blood ○ a low number of red blood cells may make you feel tired and weak. ○ a low number of platelets may cause you to bruise and bleed more easily. • Bleeding and inflammation of the urinary bladder • Absence or decrease monthly periods which may be temporary or permanent and which may decrease the ability to have children • Temporary blurred vision • Nasal stuffiness with IV infusions • Skin rash • Darkening of areas of the skin and finger nails • Slow healing of wounds • Infections	• Heart muscle damage which may occur with very high doses and which may be fatal • Abnormal heart rhythms • Damage and scarring of lung tissue which may make you short of breath • A new cancer or leukemia resulting from this treatment. • Damage or scarring of urinary bladder tissue • Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever • Infertility which is the inability to have children

Known Risks and Side Effects Related to Etoposide Include Those Which Are:		
Likely	**Less Likely**	**Rare But Serious**
• Nausea and vomiting	• Loss of appetite	• Damage to the liver

Known Risks and Side Effects Related to Etoposide Include Those Which Are:		
Likely	**Less Likely**	**Rare But Serious**

• Hair loss
• A feeling of weakness or tiredness
• Fewer red and white blood cells and platelets in the blood
  ○ a low number of red blood cells can make you feel tired and weak
  ○ a low number of white blood cells can make it easier to get infections
  ○ a low number of platelets causes you to bruise and bleed more easily
	• Decreased blood pressure during the infusion which may require treatment
	• Rashes
	• Diarrhea
	• Pain in the abdomen
	• Mouth sores
	• Tingling sensation or loss of sensation in fingers or toes
	• A feeling of extreme tiredness or weakness
	• The finger or toe nails may loosen from their nail beds
	• Inflammation of the vein through which the medication was given
	• Chest pain
		• Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate, chills and fever
		• A new cancer or leukemia resulting from this treatment
		• Severe rashes which can result in loss of skin and damage to mucous membranes
		• Absence or decrease monthly periods which may be temporary or permanent and which may decrease the ability to have children
		• Damage to the heart muscle which may make you feel tired, weak, feel short of breath, and retain fluid

There also may be other side effects that we cannot predict. Many side effects go away shortly after the drugs are stopped, but in some cases side effects can be serious, long-lasting, or permanent. Other drugs may be given to make side effects less serious and uncomfortable.

APPENDIX 5

Bridging Therapy Roadmap

Drug	Route	Dosage	Days
Clofarabine	IV over 2 hours	20 mg/m^2	1-5
Etoposide (ETOP)	IV over 2 hours	100 mg/m^2	1-5
Cyclophosphamide (CYCLO)	IV over 30 to 60 mins	300 mg/m^2	1-5

Drugs Given In This Order:

Clofarabine 20 mg/m^2 IV over 2 hours followed by

Etoposide 100 mg/m^2 IV over 2 hours followed by

Cyclophosphamide 300 mg/m^2 IV as a 30-60 minute infusion

Ht _____ Wt. _____ BSA _____

Date Due	Date Given	Day	Clofarabine _____mg	ETOP _____mg	CYCLO _____mg
		1	_____mg	_____mg	_____mg
		2	_____mg	_____mg	_____mg
		3	_____mg	_____mg	_____mg
		4	_____mg	_____mg	_____mg
		5	_____mg	_____mg	_____mg
		6-28	No Chemotherapy		