TACL
Therapeutic Advances in
Childhood Leukemia and Lymphoma

Epigenetic Reprogramming Study

Epigenetic Reprogramming in Relapse AML: A Phase 1 Study of Decitabine and Vorinostat Followed by Fludarabine, Cytarabine and G-CSF (FLAG) in Children and Young Adults with Relapsed/Refractory AML

Protocol Chairperson
Michael J. Burke, MD

Protocol Vice-Chair
Deepa Bhojwani, MD

Protocol Biology Chair
Sridhar Rao, MD, PhD

TACL Member Institutions

All Children's Hospital	**Nationwide Children's Hospital**
British Columbia Children's Hospital	**National Cancer Institute, Pediatric Oncology Branch**
Children's Healthcare of Atlanta at Egleston	**Oregon Health & Science University**
Children's Hospital and Clinics of Minnesota	**Primary Children's Hospital**
Children's Hospital Los Angeles	**Rainbow Babies & Children's Hospital**
Children's Hospital New York-Presbyterian	**Royal Children's Hospital, Melbourne**
Children's Hospital Orange County	**Sainte-Justine University Hospital Center**
Children's Hospital of Philadelphia	**Seattle Children's Hospital**
Children's National Medical Center	**Sidney Kimmel Cancer Center at Johns Hopkins**
Cincinnati Children's Hospital	**St. Jude Children's Research Hospital Memphis**
Cook Children's Medical Center	**Sydney Children's Hospital**
CS Mott Children's Hospital, Ann Arbor	**Texas Children's Cancer Center, Baylor**
Dana-Farber Cancer Institute	**The Children's Hospital at Westmead**
Hospital for Sick Children, Toronto	**The Children's Hospital, University of Colorado**
Levine Children's Hospital	**UCSF School of Medicine**
Lurie Children's Hospital of Chicago	**University of Miami**
Medical College of Wisconsin	**University of Texas Southwestern Medical Center**

TACL OPERATIONS CENTER
CHLA, 4650 Sunset Blvd, MS-54, Los Angeles, CA 90027
Phone: (323) 361-8725 or (323) 361-3022 Fax: (877) 904-2166
https://tacl.chla.usc.edu

Protocol Disclaimer

TACL protocols and their content are for research purposes only and should not be copied, redistributed or used for any other purpose. The procedures in this protocol are intended only for use at TACL member institutions under the direction of TACL physicians. The most current version of the protocol should be accessed at https://tacl.chla.usc.edu

TABLE OF CONTENTS

STUDY COMMITTEE

STUDY CHAIR Michael Burke, MD Phone: 414-955-4198 Email: mmburke@mcw.edu	**STUDY VICE CHAIR** Deepa Bhojwani, MD Phone: 323-361-5992 Email: dbhojwani@chla.usc.edu
PHYSICIAN STUDY COMMITTEE MEMBER Eric Schafer, MD, MHS Phone : (832) 824-4241 Email : esschafe@txch.org	**PHYSICIAN STUDY COMMITTEE MEMBER** Nathan Gossai, MD Phone: (612) 813-6023 Email : Nathan.Gossai@childrensmn.org
STATISTICIANS Richard Sposto, PhD Phone: 323-361-8582 Email: rsposto@chla.usc.edu Jemily Malvar, MS Phone : 323-361-8720 Email : jmalvar@chla.usc.edu	**CORRELATIVE BIOLOGY CHAIR** Sridhar Rao, MD, PhD Phone: 414-955-4993 Email: sridhar.rao@mcw.edu
TACL OPERATIONS **CLINICAL RESEARCH CONSORTIA COORDINATOR** Ellynore Florendo Phone: 323-361-3022 FAX: (877) 904-2166 Email: tacl@chla.usc.edu	**SUPPORTIVE CARE COMMITTEE MEMBER** Van Thu Huynh, MD Phone: (714) 509-4592 Fax: (714) 509-8699 Email: vahuynh@choc.org

EXPERIMENTAL DESIGN SCHEMA

A total of 2 courses will be planned.

Days (Courses 1 and 2)		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**…**	**35**
Decitabine		•	•	•	•	•									
Vorinostat		•	•	•	•	•									
Fludarabine							•	•	•	•	•				
Cytarabine							•	•	•	•	•				
Filgrastim (G-CSF)						•	•	•	•	•	•	•	•	•#	
IT Cytarabine	•														
ITT Therapy*	•														
Bone Marrow Asp/Bx	**X**														**X**

*Patients who are CNS(Centeral Nervous System) positive can receive twice weekly or three times weekly IT ARA-C or ITT therapy at the discretion of the treating physician. IT therapy should continue until negative x2
G-CSF will continue until evidence of post-nadir ANC recovery (>500/µL)

1.0 GOALS AND OBJECTIVES

1.1 Primary Objectives

1.1.1 To determine the maximum tolerated dose (MTD) of decitabine when used in this combination with vorinostat, fludarabine, high dose cytarabine and G-CSF (FLAG) for children and young adults with relapsed or refractory AML.

1.1.2 To evaluate the ability to safely deliver the combination of decitabine and vorinostat followed by fludarabine, high dose cytarabine and G-CSF (FLAG) in pediatric and young adult patients with relapsed or refractory AML.

1.2 Secondary Objectives

1.2.1 To determine preliminarily the extent of hypomethylation of peripheral blood (PB) and bone marrow (BM) pre- and post- decitabine and vorinostat treatment by:

- LINE-1 methylation assay as a surrogate marker of global DNA methylation.
- Direct Comprehensive DNA methylation analysis
- Gene expression profiling to assess genetic changes

1.2.2 To analyze the correlation between DNA methylation and gene expression pre- and post-treatment with decitabine and vorinostat.

1.2.3 To examine peripheral blood mononuclear cells for immunophenotypic changes.

1.2.4 To analyze plasma for cytokine content.

1.2.5 To analyze the correlation between biological changes and clinical response.

1.2.6 To evaluate the ability to safely deliver this treatment combination in Down syndrome patients with relapse/refractory AML

.

2.0 BACKGROUND

2.1 Relapse AML in Children and Adults

Despite progress in the treatment of AML in the past few decades, the long-term survival of children and adults younger than 60[1] with AML remains poor with survival around 30-60% depending on age. The majority of treatment failure is secondary to relapse, and relapse remains the primary cause of death in these patients[2, 3]. Elimination of the residual leukemia cells that survived front line chemotherapy remains a major challenge. Once relapse occurs, leukemia is more resistant to chemotherapy, and therefore, outcomes are poor. (Table 1) In addition, about 10-20% of AML patients are refractory to therapy and report equally dismal outcomes. Therefore, there is an urgent need to incorporate new strategies to overcome drug resistance and improve survival in our patients with relapse/refractory AML.

Once patients relapse with their disease, the complete response (CR) rate after a 2nd re-Induction attempt is around 50 – 60% in children and declines rapidly when a 3rd (17% – 32%) or > 4th attempt is made (10% - 24%).[4] In an analysis of data from three European trials for the initial treatment of adult AML, Breems et al identified a small group of adults with relapsed AML whose re-induction CR rate was as high as 80%.[5] However, the average CR rate for all adults patients with relapsed AML is around 30%,[5, 6] and 12-month survival is only 29%.[5] Improving upon these relatively poor re-Induction rates will make more patients eligible for potentially curative therapy in allogeneic hematopoietic cell transplantation.

Table 1: Overall Survival Rate for Pediatric Relapsed/Refractory AML[1-6]

	MRC AML10	TACL	St. Jude	LAME89/91	CCG 2951	NOPHO
Refractory	N/A	19% at 5 yrs.	N/A	N/A	6% at 2 yrs.	N/A
CR ≤12 mo.	11% at 3 yrs.	23% at 5 yrs.	21.5% at 5 yrs.	24% at yrs.	13% at 2 yrs.	29% at 5 yrs.
CR >12 mo.	49% at 3 yrs.	49% at 5 yrs.	26.1% at 5 yrs.	54% at 2 yrs.	75% at 2 yrs.	51% at 5 yrs.
Total	24% at 3 yrs.	29% at 5 yrs.	26.6% at 5 yrs.	33% at 2 yrs.	24% at 2 yrs.	40% at 5 yrs.

Table 2: Overall Survival for Adults with Relapsed AML[7]

	1 Year	5 Year
CR ≤ 6 mo.	14%	5%
CR 7-18 mo.	36%	12%
CR >18 mo.	57%	26%

2.2 Overview of Epigenetics in AML

2.2.1 5-AZA-2'-Deoxycytidine (Decitabine)

Decitabine is a demethylating agent that has the potential to reverse the aberrant DNA methylation that takes place in a variety of human cancers including acute leukemias (ALL and AML). Epigenetic changes in AML are

common, particularly hypermethylation and silencing of tumor suppressor genes.[8] Decitabine has been previously shown to cause demethylation and gene re-expression with subsequent hematopoietic differentiation and clinical response at doses ≥ 1 log below the maximum tolerated dose (MTD).[9]

Mechanism of Action: Decitabine is a cytidine anti-metabolite analogue that acts as a demethylating agent with anti-neoplastic activity. It is incorporated into cellular DNA thereby trapping DNA methyltransferase in the form of a covalent protein DNA adduct.[10] This leads to degradation of the DNA methyltransferase and further hypomethylation of the genomic DNA during ongoing cellular DNA replication. The demethylation process is thought to re-activate genes that were previously inactivated during the oncogenic transformation that may be involved with tumor suppressor, apoptotic, DNA repair, and growth inhibitory pathways, thereby suppressing further tumor genesis.

Summary of Toxicology: Decitabine is very well tolerated at doses below its MTD with the most commonly reported toxicities being neutropenia, anemia, thrombocytopenia, fatigue, pyrexia, nausea, cough, petechiae, constipation, diarrhea and hyperglycemia.

In Vitro Studies of Decitabine: The methylation status of acute leukemia and the role of demethylating agents (e.g. decitabine) have recently been reported by several investigators.[10,11] Yang et al. described the impact of decitabine as a single agent on cell viability using the MOLT4 ALL cell line where a reduction of 65% was seen and 90% when decitabine was given in combination with the histone deacetylase inhibitor, valproic acid (VPA).[9] Similar results were shown in apoptotic assays where three consecutive days of decitabine exposure induced cell death in 26% of cells and 50% with the combination of decitabine and VPA.

Clinical Studies of DNA Methyltransferase Inhibitors (DNMTi) in Acute Leukemia: Issa et al. reported Phase 1 results with decitabine as a single agent in 30 relapsed/refractory AML patients.[12] The treatment was very well tolerated among the group without a MTD achieved with doses ranging from 5 mg/m^2 to 20 mg/m^2 and reported a CR/PR rate of 14%/8% respectively. The dose which provided the greatest response (83%) among the patient group was 15 mg/m^2. Using a 10-day regimen of 20 mg/m^2/day as upfront therapy in older adults, Blum et al reported an overall CR rate of 47%.[13] In a retrospective review by Ritchie et al, the same regimen had a CR rate of 40% and 15.7% in newly diagnosed and relapsed/refractory patients respectively[14]. Based on these encouraging data, the US National Cancer Comprehensive Network and the European LeukemiaNet both support the use of decitabine for treatment of older adults with AML (>60 years) primarily due to its favorable side effect profile and the positive impact it has on overall survival for a patient group lacking in therapeutic alternatives.[15]

Studies with decitabine in combination with chemotherapy in AML are more limited. Blum et al. investigated the combination of valproic acid and decitabine in 25 AML patients (median age 70 years). They reported greater toxicity when the combination was given, with three patients acquiring encephalopathy, compared to decitabine treatment alone.[16] Scandura et al. investigated the combination of decitabine (3 dose levels: 3 days, 5 days and 7 days) followed by standard induction therapy with daunorubicin and cytarabine in an adult phase 1 study[17]. Thirty patients enrolled (median age 54.5 years). The combined therapy was tolerable, with a MTD of decitabine not being reached, although the authors noted an increase in gastrointestinal toxicity with the 7-day cycle of decitabine compared to the same dose given for 5 or 3-days prior to standard induction chemotherapy[17]. Of the 30 patients enrolled, 27 (90%) responded to the first cycle of induction therapy reporting 17 CR (57%) and an additional 10 PR (33%). Of the patients with a PR, 8 (80%) achieved a CR after their 2nd induction cycle.

Decitabine has been combined with CAG (cytarabine, aclarubicin and G-CSF) or CAG-like regimens in 53 adults with relapsed/refractory AML reporting overall survival at 1-year as high as 59%.[18] In newly diagnosed adult patients with high-risk (HR) myeloid neoplasms including (HR) myelodysplastic syndrome and AML, decitabine

has been successfully given followed by low dose idarubicin and cytarabine in 30 patients (median age 55 years) as reported by Ye et al.[19] The overall CR rate was 67% with a manageable toxicity profile and no treatment related deaths.

Given the number of adults studies in AML investigating decitabine which report good tolerability and promising response, further investigation in children with high-risk myeloid neoplasms is warranted.

Pediatric Studies: The Children's Oncology Group (COG) piloted a Phase 1 trial investigating decitabine in children with refractory/relapsed acute leukemia that closed prematurely secondary to low patient accrual. In this study, no MTD was identified, with all patients receiving the same starting dose of 10 mg/m^2/d of decitabine. There were 5/15 patients with Grade 3/4 cytopenias (anemia, thrombocytopenia and leukopenia) that were reported as possibly being related to the study drug. There were no toxicities reported in this study that were directly related to decitabine exposure.

There has been only a single clinical trial published to date in pediatric AML testing a DNMTi followed by chemotherapy. This study, operated through the Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) Consortium, investigated 5-azacytidine followed by fludarabine and cytarabine in children and young adults ages 1 to 21 years with relapsed or refractory leukemia [NCT01861002]. Fifteen patients enrolled (13 AML, 2 ALL) with 14 evaluable for treatment related toxicity and response. Five patients with AML had received a prior bone marrow transplant. Non-hematologic Grade $\geq$3 toxicities at least possibly related to 5-azacytidine included febrile neutropenia (n=6), infection (n=3) AST elevation (n=1), oral hemorrhage (n=1) and hypokalemia (n=1). There were no dose limiting toxicities reported on study and 7/12 (58%) patients with AML reported a complete response (CR). Demethylation analysis on 4 patients with adequate samples submitted observed persistent hypermethylation in 2 patients who were non-responders and successful demethylation in 2 patients who reported a CR. Overall the therapy was well tolerated and reported encouraging response rates in a very high-risk group of patients with relapse AML. Based on the feasibility of this study and encouraging response rates reported, future epigenetic trials in pediatric AML are warranted.

2.2.2 Suberoylanilide Hydroxyamic Acid (Vorinostat; SAHA)

Alterations in histone acetylation status have been implicated in carcinogenesis. The ability to acetylate histones may play a pivotal role in disrupting tumor genesis. Vorinostat is a histone deacetylase inhibitor with known clinical activity in cutaneous T-cell lymphoma and preclinical activity in leukemia. It has been used both as a single agent and in combination with demethylating agents for the treatment of both solid tumors and hematologic malignancies.

Mechanism of Action: Vorinostat's main mechanism of action is in restoring histone acetylation by inhibiting deacetylase. This action may further reactivate aberrantly silenced genes and allow gene transcription to proceed. Vorinostat has been shown to induce growth inhibition and apoptosis in cancer cell lines but not non-malignant cells via caspase-3/caspase-9 and Fas/Fas ligand pathways.[20]

Summary of Toxicology: Vorinostat has been very well tolerated with acceptable side effect profiles reported. The most common toxicities have been diarrhea, nausea, fatigue, anorexia, vomiting, low magnesium, constipation, hypokalemia, thrombocytopenia, elevated liver enzymes, hyperglycemia, peripheral edema, elevated creatinine and dyspnea.

Clinical Studies of Vorinostat in Acute Leukemia: Vorinostat, like decitabine, has been previously investigated as a single agent for the treatment of adult AML. Garcia-Manero et al. reported 7/31 (23%) patients having hematological improvement with > 50% decrease in their blast count.[21] The doses of vorinostat in this study ranged from 100 mg to 300 mg given up to three times daily and were well tolerated overall. The reported dose limiting toxicities were fatigue, nausea, vomiting and diarrhea. These were seen in 1/12 patients treated with 200 mg TID compared to 3/5 patients treated with 300 mg TID.

Vorinostat followed by chemotherapy has been tested in adults with HR AML. In an adult Phase 1 trial, increasing doses of vorinostat were given for 7 days prior to etoposide and cytarabine in 21 patients (median age 61 years) with relapsed/refractory AML. DLTs were observed at dose level 2 (vorinostat 200mg TID) and included hyperbilirubinemia/septic death (n=1) and anorexia/fatigue (n=1). Seven of 21 patients achieved a CR (33%) with a median duration of remission at 7 months. Given the relatively high CR rate observed in these patients and the overall tolerability of the regimen, vorinostat followed by chemotherapy is a promising approach to improve response rates in adults with poor-risk AML. Studies combining vorinostat with idarubicin in 41 adult patients (median age 59 years) with relapsed leukemia also reported tolerability with some clinical efficacy with 17% of patients having a response.[22] A subsequent Phase II trial combined cytarabine with idarubicin following vorinostat in 75 adult patients (median age 52 years) where no excess vorinostat related toxicity was observed and the overall response rate was 85%, including 76% CR and another 9% CR without platelet recovery. Impressively, patients with FLT3-ITD AML (n=11) had CR rates of 100%.[23]

Given the number of adults studies in AML investigating vorinostat which report good tolerability and promising response, further investigation in children with high-risk myeloid neoplasms is warranted.

Pediatric Studies: The Children's Oncology Group (COG) piloted a Phase 1 study investigating vorinostat +/- 13 *Cis*-Retinoic Acid in children with refractory/recurrent solid tumors. Vorinostat was administered starting at 180mg/m^2 with escalations planned in 30% increments. Sixty-four patients enrolled (median age 11 years). They reported 4/12 patients experiencing dose limiting toxicities (hypokalemia, neutropenia and thrombocytopenia) related to vorinostat at a dose of 300 mg/m^2/d. The MTD for vorinostat as a single agent in patients with solid tumors was defined as 230 mg/m^2/d with 1/6 patients having a DLT (hypokalemia) at this dose. The MTD for vorinostat in the leukemia cohort was 180mg/m^2 based on 2 patients reporting DLT at the starting dose (elevated AST (n=1), hyperbilirubinemia (n=1), elevated GGT (n=1) and hypokalemia (n=1).

2.2.3 Vorinostat and Decitabine in Combination

The combination of vorinostat and decitabine has been tested in *in vitro* studies using both human leukemia[24] and hepatoblastoma[25] cell lines. Both studies showed evidence of cell growth inhibition and gene silencing. *In vivo* studies have also been performed.

Two recent Phase 1 studies evaluated the tolerability of combination therapy with decitabine and vorinostat. In a study by Yee et al., 27 adult patients with AML, median age of 67 years (range, 32-82 years), were treated with escalating doses of vorinostat (100 mg BID; 200 mg BID; 200 mg TID) either sequentially or concurrently with decitabine (20 mg/m^2/d IV Days 1-5).[26] There were no Grade 3 or Grade 4 toxicities reported with the most common toxicities being nausea (71%), fatigue (54%), diarrhea (54%), vomiting (42%), anorexia (25%), constipation (13%), abdominal pain (13%), dehydration (13%), and headache (13%). Of the 25 evaluable patients, one patient achieved an incomplete CR (without neutrophil recovery), one a morphologic remission (without blood count recovery), and three partial remissions.

A second Phase 1 study investigated the sequential therapy of decitabine (10, 15, 20 and 25 mg/m^2 IV daily × 5) followed by SAHA (100 mg PO TID × 14 in the first cohort and 200 mg PO TID × 14 in all subsequent cohorts) in 31 refractory/relapsed adult leukemia patients with a median age of 62 years (range, 22-82 years).[27] 30 patients were evaluable for toxicity. The toxicities reported included syncope, neutropenic fever, diarrhea, fatigue, renal failure, rash, nausea, thrombosis, and angioedema. Of the 30 evaluable patients, 1 patient achieved a complete remission lasting 5.5 weeks, 4 had significant reductions in the bone marrow blasts, 4 had stable disease, 7 are too early for response evaluation, and 14 had no response/disease progression.

A recent Phase 1 trial was completed testing two schedules of escalating vorinostat in combination with decitabine (sequential administration of decitabine followed by vorinostat and concurrent administration of decitabine and vorinostat) in 36 adult patients with relapsed/refractory or previously untreated AML.[28] MTDs were not reached on either schedule. The overall response rates were greater for patients with relapsed/refractory AML and previously untreated AML who received concurrent administration of vorinostat and decitabine (n=20) compared to sequential (n=16) (30% and 36% versus 13% and 0%; p=0.26, respectively). The authors concluded that concurrent administration of these two epigenetic agents appeared more effective than sequential dosing.

A pediatric Pilot Study investigating decitabine and vorinostat in combination with chemotherapy was studied in relapse/refractory acute lymphoblastic leukemia (ALL) (NCT02412475). In this trial, decitabine and vorinostat were given prior to (decitabine days 1-5, vorinostat days 2-7) and concurrently (decitabine days 15-19, vorinostat days 16-21) with an intensive re-induction regimen consisting of vincristine, dexamethasone, PEG-asparaginase, and mitoxantrone (ALL UKR3) days 8 through 35. This Pilot Study was operated through the TACL consortium. 23 patients with $\geq 2^{nd}$ relapse or refractory B-ALL enrolled. Median age was 12 years (range, 20 months to 21 years). 11 patients (47.8%) had a prior HSCT and 6 had refractory disease (failing $\geq$2 prior re-induction attempts). 3 patients had a DLT reported which included Grade 3 cholestasis, Grade 3 steatosis, Grade 4 bilirubin (patient #3); Grade 3 delirium, Grade 3 seizure, Grade 4 depressed level of consciousness (patient #5); and Grade 3 bilirubin (patient #6). Of the 23 patients, 15 were evaluable for response with 9 achieving a CR (60%). The remaining 6 patients reported SD (40%). 6/9 patients who achieved a CR were evaluable for MRD analysis reporting a median MRD of 0.056% (range, <0.001 to 1.6%; flow cytometry). However, the infectious toxicities reported on this study were significant with 82.6% (n=19) of patients reporting at least 1 infection including 34.8% with an invasive fungal infection.[29] Despite these toxicities, in part believed to be due to the highly pretreated patient population and the intensive chemotherapy regimen (ALL UKR3), the clinical response and in particular the depth of MRD reduction was impressive and much better than expected in comparison to historical controls.[30]

Although there have been clinical studies reporting the combination of a histone deacetylase inhibitor (suberoylanilide hydroxyamic acid, SAHA) and a demethylating agent (5-Aza-2'-deoxycytidine, decitabine) in AML patients, there has yet to be a trial reporting this combination followed by re-induction chemotherapy in children and AYA with relapsed/refractory AML. This study aims to establish feasibility of the regimen and improved efficacy in terms of greater re-induction remission rates using combination therapy of a DNMTi agent and HDACi followed by cytotoxic therapy in children and AYA with relapsed/refractory AML.

2.2.4 Re-Induction with Fludarabine, Cytarabine and G-CSF (FLAG) in Relapsed Leukemia

Fludarabine, cytarabine and G-CSF (FLAG) has previously been shown to be an effective and tolerable regimen in adult patients with relapse/refractory AML. An analysis of 65 patients in an adult Phase II trial (median age 47.5 years), reported complete remission (CR) rates of 81% (17/21) in those with a late relapse and 30% (13/44) in early relapse (<6 months from end of therapy) or refractory disease.[28] All patients experienced severe myelosuppression however the only grade $\geq$3 non-hematologic toxicity reported included nausea, vomiting, alopecia and elevated ALT. The greatest toxicity was observed in elderly patients who had on average 4 days and

8 days longer for neutrophil and platelet recovery. The effect of G-CSF on the in vitro cytotoxicity of pediatric AML samples supports its use in combination with fludarabine and cytarabine in children[29] and in a trial of 197 pediatric patients with relapse AML receiving FLAG, 59% achieved a CR, 10% suffered a toxic death, 19% relapsed, resulting in a 4-year overall survival of 36% (95% CI, 29-43).[30] Based on these encouraging results, this study will use FLAG as the backbone of therapy for which epigenetic modifying agents will be tested in children and AYA with relapsed/refractory AML.[31]

2.2.5 Study Rationale

The use of DNMTi agents (e.g. decitabine) and HDACi (e.g. vorinostat) in combination have been previously shown to have synergistic effects in altering neoplastic pathways of cancer cells[25, 39] and be well tolerated in human clinical studies.[26, 27] This study proposal is based on the hypothesis that the greatest impact of combining DNMTi and HDACi agents together for the treatment of leukemia, may rely on their further combination with cytotoxic therapy (i.e. chemotherapy). With the ability of decitabine and vorinostat to alter the abnormal cellular pathways of leukemic blasts and essentially turn off anti-apoptotic proteins, the leukemia cells have become primed for cytotoxic cell kill via chemotherapeutic agents. Therefore this study concept will ask the question as to whether or not the combination of decitabine and vorinostat followed by standard re-Induction chemotherapy is feasible and whether it can positively impact outcome.

The initial doses of decitabine and vorinostat to be investigated in this study (decitabine, 7.5 mg/m^2; vorinostat, 180 mg/m^2 for patients < 18 years and 200 mg twice daily for > 18 years) are both at or below their identified MTDs and based on their tolerable side effect profile, are not likely to add further toxicity to patients receiving the additional chemotherapy agents on this study.

Dosing Rationale:

This study has a dose escalation of decitabine at 4 dose levels (starting at 7.5mg/m^2), as well as potential for dose de-escalation to dose level 0 (5mg/m^2) based on toxicity. Patients will receive decitabine [dose levels 0-4 (5, 7.5, 10, 15, 20mg/m^2)] in combination with vorinostat days 1-5 (180mg/m^2 daily ages <18 years, 200 mg twice daily ages 18-25 years). There has been no MTD previously identified for decitabine in children and AYA. Doses of 5mg/m^2 to 20mg/m^2 of decitabine as a single agent have been well tolerated in adult leukemia studies and resulted in demethylation of blasts and clinical responses.[12] The pediatric MTD for vorinostat in patients with hematologic malignancies is 180mg/m^2 once daily based on results from the Children's Oncology Grouyp (COG) phase I study.[32] The dose of vorinostat has varied greatly in adults ranging from 100mg BID to 500mg TID.[21] The dose of 200mg BID for patients >18 years was chosen in this study based on ease of dosing/adminstration as well as this dose having good efficacy in maintaining histone acetylation.[32]

Correlative biology studies:

Determination of Baseline "functional epigenetic profile" on leukemic blasts isolated at study entry using the following triad of assays:

To quantitatively assess global changes in DNA methylation, a LINE-methylation assay will also be utilized and specific genes monitored through advanced Infinium MethylationEPIC BeadChip from Illumina;

Chromatin immunoprecipitation (ChIP) with antibodies specific for histone modifications associated with transcriptional activation (H3K4me3, H3K27ac) and repression (H3K9me3 and H3K27me3) and isotype controls, followed by DNA sequencing (ChIP-seq) and total levels will be measured by western blotting;

RNA sequencing analysis will be used to measure global transcriptome changes. Correlation between DNA methylation and gene expression using RNA seq pre/post treatment with decitabine/vorinostat will be performed and correlated with clinical response, for which it is likely that over- or under-expression is truly driven by epigenetic regulation. Profiles of CD33+ umbilical cord blood cells, whole bone marrow, or Peripheral Blood Stem Cells (PBSCs) will be used as normal controls for each sample.

Immune assessment: Peripheral blood immune cell phenotyping and serum cytokine analysis.

Assessment of the *in Vivo* Effects

Assessment of the *in vivo* effects of combined DNMTi/HDACi on the functional epigenetic profile by comparing the following in paired pre- (Day 0) and post-exposure (Day 5, Day 14 and Day 35) leukemic blasts:

2.2.5.1 Reversal of DNA promoter hypermethylation of "repressed" genes of interest using Infinium MethylationEPIC BeadChip, validated with Pyrosequencing-based methylation assay;

2.2.5.2 Increase in H3K27m3 and H3K9me3 or descrese H3K27ac and H3K4m3 in association with "repressed" genes of interest using H3K4m3, H3K27ac, H3K27m3, and H3K9m3 ChIP-seq, validated with ChIP-qPCR;

2.2.5.3 Reversal of transcriptional silencing of "repressed" genes of interest using RNA seq, validated by qRT-PCR. Since significant acute cell kill is unlikely during the 5-day "window" of DNMTi/HDACi, we will have a unique opportunity to assess the in vivo effects of epigenetic therapy with the Day 5 sample. The Day 14 peripheral blood and Day 35 marrow samples will also contribute in patients whose leukemic blasts persist at these time points.

2.2.5.4 Changes in peripheral blood immune cell subsets and serum cytokines.

Correlations of Baseline functional epigenetic phenotype and pharmacodynamic modulation of repressed genes of interest with clinical responses: While the ability to definitively correlate either baseline epigenetic phenotypes or successful reversal of epigenetic phenotypes with clinical response will be limited by small patient numbers, the descriptive data we generate will be invaluable in developing biomarkers to be validated in future trials.

2.2.6 Chemo-Sensitivity Profiling

We hypothesize that vorinostat and decitabine may influence sensitivity of leukemic cells to the standard re-induction chemotherapy. We will test blasts isolated at baseline (pre-treatment) vs. those isolated at Day 5 (post-exposure to DNMTi/HDACi) and compare cytotoxic IC50 for fludarabine and cytarabine using enzyme-based proliferation assays (WST-1, e.g.) and flow-based apoptosis assays (annexin V and 7-AAD).

2.2.7 Immune monitoring

It is now clear that the immune system can have an important role in controlling cancer progression. Epigenetic modifying drugs and many chemotherapeutic agents are known to have both positive and negative effects on the immune system. The combination of agents being used here could have either beneficial or detrimental effects on immune cell function and composition. We hypothesize that immune cell subsets and serum cytokines levels will be impacted by the treatment regimen. Plasma will be quantitatively analyzed for 65 different cytokines and chemokines in multi-plex assays. We will also immunophenotype peripheral blood mononuclear cells using multi-

color (8 parameters) flow cytometry. Cell subsets to be analyzed will include T cells (naïve, effector and regulatory), B cells, NK cells, dendritic cells and myeloid cells.

3.0 PATIENT ELIGIBILITY CRITERIA AND ENROLLMENT

3.1 Patient Reservation

Investigators should consult the Member's Section of the TACL web site to determine if the study is currently open for accrual before approaching patients for participation. Before enrolling a patient on study, a reservation must be made with the TACL Operations Center. In order to make a reservation you may call (323) 361-3022 or send an email to tacl@chla.usc.edu with the following information (if sending an email, please put "Reservation Request" in the subject line):

- Study for which you want to make a reservation:
- Name of the institution requesting reservation with contact information
- Patient Initials (Last, First)
- Patient month and year of birth

If an enrollment slot is available, you will receive an email from the TACL Operations Center to confirm your reservation. All reservations are good for 5 full calendar days starting with the next full day after the day the reservation is made.

3.2 Enrollment

An enrollment guide is available on the web site (https://tacl.chla.usc.edu). Patients must be enrolled prior to beginning treatment on this study. It is allowable to enroll a patient that has received IT ARA-C, IT MTX or triple IT therapy within 7 days of enrollment as part of their evaluation to diagnose disease relapse. Patients will be enrolled by contacting the TACL Operations Center Monday through Friday, 8:30 am – 5:00 pm Pacific Time at (323) 361-3022, except holidays. You will be asked to complete the eligibility form on the TACL Database System prior to making your call. In addition, the supporting documentation, which confirms eligibility, should be scanned and emailed to the TACL Operations Office through tacl@chla.usc.edu.

Each patient will be assigned a unique TACL registration and study subject number. An email confirming eligibility and assigned dose level will be sent to the treating facility, Study Chair, and Study Vice-Chair. Patients should begin treatment within 3 calendar days of study enrollment.

Contact Person: Clinical Research Coordinator: Ellynore Florendo
TACL Operations Center
Children's Hospital Los Angeles
4650 Sunset Blvd, MS #54
Los Angeles, CA 90027
Phone: (323) 361-3022
FAX: (877) 904-2166
Pager: (213) 209-5592
Email: tacl@chla.usc.edu

3.3 Inclusion Eligibility Criteria

The eligibility criteria listed below are interpreted literally and cannot be waived.

3.3.1 Age

Patients must be ≥ 1 and ≤25 years of age.

3.3.2 Diagnosis

- Patients with relapse or refractory AML must have measurable disease (≥M1 marrow)
  - 1st or greater relapse, OR
  - Failed to go into remission after 1st or greater relapse, OR
  - Failed to go into remission from original diagnosis after 2 or more induction attempts

a) Eligibility for patients with an M1 marrow; defined as ≥0.1% by flow or molecular testing (e.g. PCR).
   - must include two serial marrows (at least 1-week apart) demonstrating stable or rising minimal residual disease (MRD) (i.e. not declining).

b) Patients may have CNS or other sites of extramedullary disease. No cranial irradiation is allowed during the protocol therapy.

c) Patients with secondary AML are eligible. Of note, these patients will be eligible even without prior therapy for AML.

d) Patients with Down syndrome are eligible.

e) Patients with DNA fragility syndromes (such as Fanconi anemia, Bloom syndrome) are excluded.

3.3.3 Performance Level

Karnofsky > 50% for patients > 16 years of age and Lansky > 50% for patients ≤ 16 years of age. (See Appendix II for Performance Scales)

3.3.4 Prior Therapy

Patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study.

a) **Cytoreduction with hydroxyurea** Hydroxyurea can be initiated and continued for up to 24 hours prior to the start of decitabine/vorinostat. It is recommended to use hydroxyurea in patients with significant leukocytosis (WBC >50,000/L) to control blast count before initiation of systemic protocol therapy.

b) **Patients who relapsed while they are receiving cytotoxic therapy** At least 14 days must have elapsed since the completion of the cytotoxic therapy,except Intrathecal chemotherapy.

A. Hematopoietic stem cell transplant(HSCT): Patients who have experienced their relapse after a HSCT are eligible, provided they have no evidence of acute or chronic Graft-versus-Host Disease (GVHD) and are off all transplant immune suppression therapy for at least 7-days (e.g. steroids, cyclosporine, tacrolimus). Steroid therapy for non-GVHD and/or non-leukemia therapy is acceptable.

B. Hematopoietic growth factors: It must have been at least 7 days since the completion of therapy with GCSF or other growth factors at the time of enrollment. It must have been at least 14 days since the completion of therapy with pegfilgrastim (Neulasta®).

C. Biologic (anti-neoplastic agent): At least 7 days after the last dose of a biologic agent. For agents that have known adverse events occurring beyond 7 days after administration, this period must be extended beyond the time during which adverse events are known to occur. The duration of this interval must be discussed with the study chair.

D. Monoclonal antibodies: At least 3 half-lives of the antibody must have elapsed after the last dose of monoclonal antibody. (i.e. Gemtuzumab = 36 days)

E. Immunotherapy: At least 42 days after the completion of any type of immunotherapy, e.g. tumor vaccines or CAR T-cell therapy.

F. XRT: Cranio or craniospinal XRT is prohibited during protocol therapy. No washout period is necessary for radiation given to non-CNS chloromas; ≥90 days must have elapsed if prior TBI, cranio or craniospinal XRT.

G. Prior Demethylating and/or HDAC Inhibitor Therapy: Patients who have received prior DNMTi (e.g. decitabine) and/or HDACi (e.g. vorinostat) therapy are eligible to participate in this Phase 1 study. At least 7 days must have passed from prior DNMTi or HDACi as a washout period.

3.3.5 Renal and hepatic function

Patients must have adequate renal and hepatic functions as indicated by the following laboratory values:

A. Adequate renal function defined as: Patient must have a calculated creatinine clearance or radioisotope GFR ≥ 70ml/min/1.73m^2 OR a normal serum creatinine based on age/gender in the chart below:

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 to < 2 years	0.6	0.6

2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

B. Adequate Liver Function Defined as: Direct bilirubin < 1.5 x upper limit of normal (ULN) for age or normal, AND alanine transaminase (ALT) < 5 x ULN for age. The hepatic requirements are waived for patients with known or suspected liver involvement by leukemia. This must be reviewed by and approved by the study chair or vice chair.

3.3.6 Adequate Cardiac Function Defined as: Shortening fraction of ≥ 27% by echocardiogram, OR ejection fraction of ≥ 50% by radionuclide angiogram (MUGA).

3.3.7 Reproductive Function

A. Female patients of childbearing potential must have a negative urine or serum pregnancy test confirmed within 1 week prior to enrollment.

B. Female patients with infants must agree not to breastfeed their infants while on this study.

C. Male and female patients of child-bearing potential must agree to use an effective method of contraception approved by the investigator during the study and for a minimum of 6 months after study treatment.

3.3.8 Informed Consent

Patients and/or their parents or legal guardians must be capable of understanding the investigational nature, potential risks and benefits of the study. All patients and/or their parents or legal guardians must sign a written informed consent. Age appropriate assent will be obtained per institutional guidelines. To allow non-English speaking patients to participate in this study, bilingual health services will be provided in the appropriate language when feasible.

3.3.9 Protocol Approval

All institutional, FDA, and OHRP requirements for human studies must be met.

3.4 Exclusion Eligibility Criteria

Patients will be excluded if they meet any of the following criteria

3.4.1 No NG or G-Tube administration of Vorinostat is allowed. Capsule must be swallowed whole or given as oral suspension.

3.4.2 They are currently receiving other investigational drugs.

3.4.3 There is a plan to administer non-protocol chemotherapy, radiation therapy, or immunotherapy during the study period.

3.4.4 They have significant concurrent disease, illness, psychiatric disorder or social issue that would compromise patient safety or compliance, interfere with consent, study participation, follow up, or interpretation of study results.

3.4.5 They have a known allergy to any of the drugs used in the study.

3.4.6 Patients with DNA fragility syndromes are excluded (e.g. Fanconi Anemia, Bloom Syndrome)

3.4.7 They are receiving valproic acid (VPA) therapy.

3.4.8 Patients with Acute Promyelocytic Leukemia (APL, APML) are excluded

3.4.9 Patients with documented active and uncontrolled infection at the time of study entry are not eligible

3.4.10 Patients will be excluded if they have had any positive fungal culture within 30 days prior to enrollment.

4.0 TREATMENT PROGRAM

The following sections detail the treatment plan for each course of therapy. Please refer to the Drug Information section for additional administration guidelines. Treatment should begin within 3 calendar days of enrollment.

4.1 Course 1 and 2

Table 3: Overview of Treatment Plan – Courses 1 and 2

Drug	Dose	Route of Administration	Days
Decitabine [A]	***See Table 6*** Starting at Hour 0	IV over 1 hour	1-5
Vorinostat[B]	Age < 18 years 180 mg/m^2/day once daily Give after the Decitabine infusion	PO	1-5
	Age ≥ 18 years 200 mg twice daily Give after the Decitabine infusion	PO	1-5
Filgrastim [C] **(G-CSF)**	5 µ/kg/dose (Starting at Hour 0)	IV or SQ	5-12[C]
Fludarabine[F]	30 mg/m^2/day (Starting at Hour 0 – **Immediately after G-CSF**)	IV over 30 minutes	6-10
Cytarabine	2000 mg/m^2/day (Starting at Hour 4.0)	IV over 3 hours	6-10
Cytarabine [D,E]	***See Table 4 – Age Specific Dosing***	IT	0 (or -1 [D])
ITT Therapy[D]	***See Table 5-Age Specific Dosing***	IT	See [E]

A Decitabine will be assigned according to Protocol Dose Escalation Schedule. Refer to Section 6.2 and 12 for further information.

B A compounded liquid formulation of Vorinostat is available.

C G-CSF will begin on Day 5 prior to FLAG and continue until evidence of post-nadir ANC recovery (>500/µL). Biosimilars are permitted

D Refer to Table 4: Patient Age Specifications for Intrathecal Cytarabine

E IT Cytarabine can be given up to 72 hours prior to initial dose of decitabine/vorinostat for patient convenience. Patients who are CNS positive can receive twice weekly or three times weekly IT ARA-C or IT ITT therapy at the discretion of the treating physician. IT therapy should continue until negative x2.

F Dosing for Fludarabine applies only when creatinine clearance <u>>60mL/min/1.73m</u>

A bone marrow evaluation to determine study response and remission status will be performed on study Day 35-42 or upon adequate blood count recovery (ANC > 500/µL AND platelet > 50,000/µL), whichever occurs first. If the marrow is hypocellular and without evidence of normal tri-lineage hematopoiesis the marrow should be repeated at Day 42 – 49 (7 days post the last evaluation or when ANC/Plt counts recover whichever is sooner).

Table 4: Patient Age Specifications for Intrathecal Cytarabine

Patient Age (years)	IT Cytarabine Dose
1 to 1.99	30 mg
2 to 2.99	50 mg
≥ 3	70 mg

Table 5: Patient Age Specific Dosing for Triple Intrathecal Therapy

Patient Age (years)	Methotrexate	Hydrocortisone	ARA-C
1 to 1.99	8mg	8mg	16mg
2 to 2.99	10mg	10mg	20mg
3 to 8.99	12mg	12mg	24mg
≥ 9	15mg	15mg	30mg

This study will follow a 3+3 dose escalation design, as described in section 10.1, starting at dose level 1 (7.5 mg/m^2/dose) with possible escalation to dose levels 2 through 3 and possible de-escalation to dose level 0.

Table 6: Decitabine Dose Levels

Decitabine Dose Level	Dose	Days Given
0	5 mg/m^2	1-5
1	7.5 mg/m^2	1-5
2	10 mg/m^2	1-5
3	15 mg/m^2	1-5

Table 7: Fludarabine Dose based on Creatinine Clearance

Creatinine Clearance mL/min	Daily Fludarabine Dose (mg/m^2)
> 60	30
46-60	24
31-45	22.5
21-30	Withhold Fludarabine
< 20	Withhold Fludarabine

4.2 Disease Evaluation During Course 1 and 2

A bone marrow aspiration/biopsy to assess remission status should be performed approximately day **35-42** when blood counts recover (ANC > 500/uL and PLT > 50,000/uL).

If the marrow is hypoplastic and/or there is little or no evidence of normal hematopoiesis, a repeat marrow should be performed after a further 7-21 days (based upon peripheral blood count recovery and the clinician's judgment)

and remission status assessed at this later time point. If the marrow examination is still hypoplastic and no peripheral blood counts recovery (ANC >500 and platelets >50,000) without evidence of malignant infiltration beyond day 63, the patient will be considered to have experienced a DLT.

4.2.1 Criteria to begin course 2

Patients who achieve CR, CRi, PR, or SD may receive a second course of chemotherapy no earlier than 35 days after the start of course 1, as soon as clinically acceptable to the treating institution and the following criteria are met:

- All grade 3 and 4 non-hematologic toxicities have resolved to grade 2 or less
- It is recommended but not required that an ANC>750/ul and platelet count >50,000/ul to be achieved before proceeding with a second course of therapy **EXCEPT** patients with CRi or SD. These patients, if in good enough clinical condition (based on treating physician's clinical judgment), and at least 42 days have passed from start of Course 1, may begin Course 2 irrespective of blood counts.
- Administration of course 2 intrathecal therapy at the time of the bone marrow evaluations is permitted to avoid additional sedation procedures.

4.3 Dose-limiting Toxicity (DLT)

Toxicity will be graded according to the NCI Common Toxicity Criteria (CTC) version 4.0. A copy of the CTCAE can be downloaded from the CTEP home page (http://ctep.cancer.gov). Any DLT should be reported immediately through the TACL Operation Center to the Study Chair.

DLT will be assessed in the first course only. DLT will be defined as any of the following events that are related to DECITABINE with further explanation as follows:

a) Any grade 3 or higher non-hematologic toxicity persisting for >48 hours without resolution to ≤ grade 2, with the exception of
   - Alopecia
   - Anorexia
   - Nausea
   - Grade 3 mucositis that resolves to ≤ Grade 2 within 14 days
   - Grade 3 vomiting or diarrhea that resolves to ≤ Grade 2 within 7 days
   - Grade 3 elevation in amylase, lipase, or direct or total bilirubin that is asymptomatic and that returns to ≤ Grade 2 elevation or baseline within 14 days
   - Grade 3 elevation in hepatic transaminases (AST, ALT or GGT) or alkaline phosphatase that returns to ≤ Grade 2 elevation or baseline within 14 days
   - Grade 3 or 4 fever with neutropenia, with or without infection
   - Grade 3 or 4 infection
   - Grade 3 electrolyte abnormalities that are not associated with clinical sequelae
   - Grade 3 or 4 hypotension that can be explained by sepsis

– Grade 3 injection site reaction

b) Failure to recover a peripheral ANC > 500/ul and platelet count > 20,000/ul due to documented bone marrow hypoplasia within 56 days without evidence of active disease or active infection (such as persistent fever or positive culture) by bone marrow aspiration. Bone marrow hypoplasia is defined as overall marrow cellularity less than 10%. Patients with CRi or SD after course 1 are excluded from this since they may proceed to course 2 regardless of blood counts.

No other hematologic grade 3 or 4 adverse events will be considered dose-limiting.

4.4 Dose Modifications for Toxicity

The intent of this study design is for all patients to receive and complete at least 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 study therapy (Day 1 through Day 35); however, prolonged hematopoietic recovery or bone marrow aplasia during the first 63 days may meet a study stopping rule.

4.4.1 Fludarabine

Fludarabine dose is based on estimated creatinine clearance (**Table 7**)

4.4.1.2 Fludarabine Modification for Obesity: No dose modifications will be made for obesity. Obese patients should be dosed based on actual height and weight and not based on ideal or adjusted body weight.

4.4.2 Cytarabine

4.4.2.1 Cytarabine Dose Modification for Changes in Renal Function

Cytarabine is not nephrotoxic, although cytarabine clearance is impaired in the setting of decreased renal function. Patients whose renal function does not meet the criteria given in Section 3.3.5 at the time they are due to start a cycle of cytarabine should have the cytarabine dose reduced by 50%.

With subsequent cycles, if renal function has returned to meet the criteria given in Section 3.3.5., then the cytarabine dose should be increased back to assigned dose.

4.4.2.2 Cytarabine Dose Modification for Cerebellar Toxicity

Patients who develop cerebellar symptoms (including, but not limited to, nystagmus, ataxia, dysmetria, dysarthria, somnolence, or seizure) of any grade attributable to cytarabine will be removed from protocol therapy. No dose reductions will be made for suspected cerebellar toxicity.

4.4.2.3 Dose Modification for Infections Requiring Ongoing Systemic Therapy

Patients who develop a new infection that requires ongoing systemic therapy resulting in a treatment delay of > 14 days will be removed from protocol therapy. Patients who complete their planned antibiotic therapy within 14 days of the scheduled start of the next cycle of cytarabine will continue on protocol therapy with cytarabine dose reduced by 25%.

4.4.2.4 Cytarabine Dose Modification for Obesity

No dose modifications will be made for obesity. Obese patients should be dosed based on actual height and weight and not based on ideal or adjusted body weight.

5.0 SUPPORTIVE CARE

Best supportive care and treatment will be given as appropriate to each patient (antiemetics, antibiotics, transfusions, oxygen therapy, nutritional support, palliative treatment for pain or cough, etc.). Patients may experience profound myelosuppression and immune suppression during this time. Caregivers must also be made aware that patients may experience very rapid clinical deterioration. This suggests the need for a supportive care network that can recognize and respond to sudden changes in a patient's condition.

Hospitalization during each course of chemotherapy is strongly recommended until the absolute phagocyte count (sum of the neutrophils, bands and monocytes) is rising for two successive days, and the patient is afebrile and clinically stable. An additional discharge criterion of an absolute neutrophil count (ANC) of at least 200/µL is also suggested.

Aggressive supportive care improves outcome. The following guidelines are intended to give general health direction for optimal patient care and to encourage uniformity in the treatment of this patient population. Notify the Study Chair of any unexpected or unusually severe complications.

5.1 Concurrent Therapy

5.1.1 Chemotherapy or Investigational Therapy
Patients must not receive any non-protocol chemotherapy, investigational therapy, or immune modulating agents.

5.1.2 Cranial Irradiation for CNS disease is NOT allowed during protocol therapy.

5.1.3 Radiotherapy to Chloroma is acceptable. No irradiated lesion may be used to assess tumor response.

5.1.4 Administer Steroid Eye Drops or other eye drops per institutional practice prior to the first dose of cytarabine and continuing for 24 hours after the last dose.

5.1.5 Appropriate Antibiotics, Blood Products, Antiemetics, Fluids, Electrolytes and general supportive care are to be used as necessary. See section 5.3 for additional information regarding infection control

5.1.6 Patients May Have Received Hydroxyurea to keep WBC below 50,000/µL prior to beginning protocol therapy, but hydroxyurea **must** be discontinued 24 hours prior to starting systemic protocol therapy.

5.1.7 Alternative Medications: Use of alternative medications (eg, herbal or botanical for anticancer purposes) is not permitted during the entire study period.

5.1.8 Drug interactions: Prolonged QT interval is a possible risk with vorinostat. Use caution if additional supportive care drugs that prolong QT interval are necessary. Updated list of agents that can prolong QT interval is available at https://crediblemeds.org/index.php/drugsearch
Also avoid coumarin-derivative anticoagulants to minimize drug interactions with vorinostat.
Also avoid coumarin-derivative anticoagulants to avoid drug interactions.

5.2 Blood Products

Investigators should follow institutional guidelines regarding the administration of blood products.

5.3 Infection Control

5.3.1 Pneumocystis Prophylaxis

All patients are to receive prophylaxis with Trimethoprim/Sulfamethoxazole (TMP-Sulfa) for Pneumocystis for two sequential days each week at a dose of 2.5 mg/kg/dose TMP-Sulfa (maximum dose of 160 mg) twice daily (BID) or according to institutional policy, unless they have a documented sulfa allergy. Sulfa-allergic patients should receive dapsone 2 mg/kg orally (PO) q day with a maximum dose of 100 mg/day, or monthly pentamidine (IV or aerosolized). Pneumocystis prophylaxis should be continued throughout the entire study period.

5.3.2 Bacterial, Fungal and Viral Prophylaxis

Prophylaxis for bacterial infections is not universally accepted; therefore, it is not mandatory. However, treating physicians should be aware that a high incidence of serious bacterial and fungal infections has been observed in patients receiving this combination of cytarabine and fludarabine. The use of prophylactic antibacterial and antiviral agents is strongly recommended according to the following parameters:

Treating physicians may use institutional guidelines but should strongly consider antibacterial prophylaxis during each course of therapy until the post-nadir ANC is >750/µL. Avoid using vancomycin or other nephrotoxic antibiotics during high dose cytarabine administration for prophylaxis.

Given the high risk of fungal infections in patients with FLAG-based therapy, it is mandated that all patients receive antifungal prophylaxis during each course of therapy until the post-nadir ANC is >750/µL. It is strongly encouraged to use the drug with anti-mold and anti-yeast activity such as liposomal amphotericin B (e.g. AmBisome), an echinocandin (caspofungin, micafungin,

anidulafungin**) or an extended-spectrim azole (voriconazole, posazonazole). The choice of antifungal therapy can be selected based on local fungal prevalence and sensitivity patterns, and patient's age and availability of appropriate formulation/route**.**The doses used should be in the treatment range more so than prophylactic as that is when colonization and infection may occur with patients at high risk for fungal infection. Close hepatic and renal monitoring with dose adjustments is recommended, particularly for those receiving concurrent hepatic and nephrotoxic agents.**

Patients who have HSV antibodies are recommended to receive prophylactic acyclovir at 750 mg/m2/day divided BID or valacyclovir

5.3.3 Fever and Neutropenia

All patients with a fever ≥38.5°C on a single occasion, or ≥38°C on 2 occasions within one hour , and an ANC <0.50 x 10^9/L are to be hospitalized and treated immediately with intravenous broad-spectrum antibiotics after obtaining appropriate blood cultures. Selection of empiric therapy should be determined by institutional pathogen prevalence and susceptibility patterns and should include *Pseudomonas* coverage, (e.g. such as meropenem, imipenem, cefepime, ceftazidine, or piperacillin-tazobactam). Duration of therapy should be determined by site of infection, culture results, and response to treatment. For the the persistence of fever, or emergence of a new fever in neutropenic patients after 3 days of broad-spectrum antibiotic coverage without an identified source/site, patients on prophylactic dosing of antifunal therapy should have their therapy escalated to treatment dosing. Additionally, radiographic imaging surveillance for sites of infection should also be performed as clinically indicated and for persistent fevers. These scans would include CT sinus, chest, abdomen and pelvis. Additional recommendations for initial diagnostic work-up for invasive fungal infection (IFI) include serum fungal markers galactomannan (GMN) and beta-d-glucan along with blood fungal cultures.

When severe mucositis or a sepsis syndrome is present in patients with febrile neutropenia, or a patient has a history of prior alpha hemolytic sepsis, consider inclusion of Vancomycin in the empiric antibiotic regimen.

5.3.4 Empiric Management of Respiratory Symptoms and Pulmonary Infiltrates

Patients with respiratory symptoms should have a viral film array or PCR sent to evaluate for respiratory viral infection. CT chest imaging is warranted for patients with hypoxemia and/or pulmonary infiltrates on CXR. Pulmonary infiltrates should be evaluated in the context of the patient's clinical and laboratory profile as well as institutional infection patterns. If the patient is not neutropenic, and the pulmonary lesions on CT scan are not particularly suggestive of a fungal infection (Aspergillus, mucor), consider using broad spectrum antibiotics. If the patient develops progressively worsening clinical or laboratory features, or if, the pulmonary lesions on CT scan are suggestive of a fungal infection (Aspergillus, mucor), then more aggressive diagnostic measures should be undertaken. Pulmonary infiltrates may be evaluated with bronchoscopy and biopsy, lavage or open lung biopsy. BAL/biopsy diagnostics should include routine culture (acid-fast bacilli, bacterial, and fungal culture), cytology, and GMN and PCR (AFB, bacterial, fungal including *Pneumocystis jiroveci*, and viral).If a procedure cannot be tolerated, and/or if

there is high clinical suspicion consider beginning empiric treatment with amphotericin B given the high likelihood of fungal disease. It is advisable to seek an infectious disease consult under these circumstances. Empiric coverage may include treatment of gram-negative and positive bacteria, Legionella (erythromycin), Pneumocystis (TMP/SMX), and fungi (amphotericin) pending culture results.

If fungal pulmonary disease is documented, surveillance radiographic imaging studies of the sinuses, abdomen/pelvis are indicated. Surgical excision of pulmonary lesions should be considered at the discretion of the treating physician. Treatment of fungal infections with amphotericin B and/or other antifungal agents **with anti-mold and anti-yeast activity such as an echinocandin (caspofungin, micafungin, anidulafungin) or an extended-spectrim azole (voriconazole, posazonazole)** will be at the discretion of the treating physician.

5.3.5 Management of Mucositis/Perirectal Cellulitis

Mucositis should be managed with IV hydration and hyperalimentation (if indicated), effective analgesia, broad-spectrum gram-positive and gram-negative antibiotic therapy and empiric antiviral and antifungal therapy as indicated. Management of perirectal cellulitis should include broad-spectrum antibiotic therapy with dual gram-negative coverage as well as anaerobic coverage (i.e. ceftazidime + aminoglycoside + metronidazole; or piperacillin-tazobactam + aminoglycoside), Sitz baths, a strong barrier technique and effective analgesia.

5.3.6 Use of G-CSF

The routine use of G-CSF outside of protocol therapy is not generally recommended, but may be used at the discretion of the investigator in situations such as serious infection with neutropenia.

6.0 DRUG INFORMATION

6.1 Decitabine (Dacogen™)

6.1.1 Nomenclature and Molecular Structure Dacogen™ (decitabine) for Injection contains decitabine (5-aza-2'-deoxycytidine), an analogue of the natural nucleoside 2'-deoxycytidine. Decitabine is a fine, white to almost white powder with the molecular formula of $C_8H_{12}N_4O_4$ and a molecular weight of 228.21. Its chemical name is 4-amino-1-(2-deoxy-b-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1*H*)-one and it has the following structural formula:

6.1.2 Mode of Action and Pharmacology Decitabine is believed to exert its antineoplastic effects after phosphorylation and direct incorporation 16 into DNA and inhibition of DNA methyltransferase, causing hypomethylation of DNA and cellular 17 differentiation or apoptosis. Decitabine inhibits DNA methylation in vitro, which is achieved at concentrations that do not cause major suppression of DNA synthesis. Decitabine-induced hypomethylation in neoplastic cells may restore normal function to genes that are critical for the control of cellular differentiation and proliferation. In rapidly dividing cells, the cytotoxicity of

decitabine may also be attributed to the formation of covalent adducts between DNA methyltransferase and decitabine incorporated into DNA. Non-proliferating cells are relatively insensitive to decitabine.

Patients with advanced solid tumors received a 72-hour infusion of decitabine at 20 to 30 mg/m^2/day. Decitabine pharmacokinetics were characterized by a biphasic disposition. The total body clearance (mean ± SD) was 124 ± 19 L/hr/m^2, and the terminal phase elimination half-life was 0.51 ± 0.31 hr. Plasma protein binding of decitabine is negligible (< 1%). The exact route of elimination and metabolic fate of decitabine is not known in humans. One of the pathways of elimination of decitabine appears to be deamination by cytidine deaminase found principally in the liver but also in granulocytes, intestinal epithelium and whole blood.

6.1.3 Toxicity/Adverse Events

Decitabine		
System Organ Class	**Adverse Events Reported in ≥ 10% of Patients who received decitabine in a Phase 3 MDS trial**	**Adverse Events Reported in ≥ 5% but < 10% of Patients who received decitabine in a Phase 3 MDS trial**
Blood and Lymphatic System	▪ Neutropenia ▪ Thrombocytopenia ▪ Anemia ▪ Febrile neutropenia ▪ Leukopenia ▪ Lymphadenopathy	▪ Thrombocythemia
Gastrointestinal	▪ Nausea ▪ Constipation ▪ Diarrhea ▪ Vomiting ▪ Abdominal pain ▪ Decreased appetite ▪ Anorexia ▪ Oral Mucosal petechia ▪ Stomatitis ▪ Dyspepsia ▪ Ascites	▪ Gingival bleeding ▪ Hemorrhoids ▪ Tongue ulceration ▪ Dysphagia ▪ Oral soft tissue disorders ▪ Lip ulceration ▪ Abdominal distention ▪ Upper abdominal pain ▪ Gastro-esophogeal reflux disease ▪ Glossodynia
General Disorders	▪ Pyrexia ▪ Peripheral edema ▪ Rigors ▪ Edema NOS ▪ Pain NOS ▪ Lethargy	▪ Falls ▪ Chest discomfort ▪ Intermittent pyrexia ▪ Malasise ▪ Crepitations ▪ Catheter site erythema ▪ Catheter site pain ▪ Injection site swelling

Decitabine		
System Organ Class	**Adverse Events Reported in ≥ 10% of Patients who received decitabine in a Phase 3 MDS trial**	**Adverse Events Reported in ≥ 5% but < 10% of Patients who received decitabine in a Phase 3 MDS trial**
Infection	▪ Pneumonia NOS ▪ Cellulitis ▪ Candidal infection NOS	▪ Catheter related infection ▪ Urinary tract infection ▪ Staphylococcal infection ▪ Oral candidiasis ▪ Sinusitis ▪ Bacteremia
Metabolic/Laboratory	▪ Hyperbilirubinemia ▪ Elevated alkaline phophatase ▪ Elevated AST ▪ Elevated BUN ▪ Hyperglycemia ▪ Hypoalbuminemia ▪ Hypomagnesemia ▪ Hypokalemia ▪ Hyponatremia ▪ Hyperkalemia	▪ Dehydration ▪ Lactate dehydrogenase increased ▪ Increased blood bicarboanate ▪ Decreased blood bicaroboate ▪ Hypochloremia ▪ Blood protein decreased ▪ Blood bilirubin decreased
Musculoskeletal	▪ Arthralgia ▪ Limb pain ▪ Back pain	▪ Chest wall pain ▪ Musculoskeletal discomfort ▪ Myalgia
Neurological	▪ Headache ▪ Dizziness ▪ Hypoesthesia ▪ Insomnia ▪ Confusional state ▪ Anxiety	
Pulmonary	▪ Cough ▪ Pharyngitis ▪ Crackles lung ▪ Breath sounds decreased ▪ Hypoxia	▪ Rales ▪ Postnasal drip
Renal		▪ Dysuria ▪ Urinary frequency
Skin	▪ Ecchymosis ▪ Rash ▪ Erythema ▪ Skin lesions NOS ▪ Pruritus ▪ Petechiae ▪ Pallor	▪ Alopecia ▪ Urticaria ▪ Facial swelling
Cardiac	▪ Cardiac murmur NOS	▪ Hypotension ▪ Hematoma

6.1.4 **Pregnancy and Nursing Mothers** Decitabine may cause fetal harm when administered to a pregnant woman. Women of childbearing potential should be advised to avoid becoming pregnant while using Decitabine. Men should be advised not to father a child while receiving treatment with Decitabine, and for 2 months afterwards. It is not known whether decitabine or its metabolites are excreted in human milk. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions from decitabine in nursing infants, breast feeding must be avoided.

6.1.5 **Formulation and Stability** Dacogen™ (decitabine) for Injection is supplied as a sterile lyophilized white to almost white powder, in a single-dose vial, packaged in cartons of 1 vial. Each vial contains 50 mg of decitabine. Store vials at 25°C (77°F); excursions permitted to 15 - 30°C (59 - 86°F).

Unless used within 15 minutes of reconstitution, the diluted solution must be prepared using cold (2°C - 8°C) infusion fluids and stored at 2°C - 8°C (36°F - 46°F) for up to a maximum of 7 hours until administration.

6.1.6 **Preparation and Administration** Dacogen should be aseptically reconstituted with 10 mL of Sterile Water for Injection (USP); upon reconstitution, each mL contains approximately 5.0 mg of decitabine at pH 6.7-7.3. Immediately after reconstitution, the solution should be further diluted with 0.9% Sodium Chloride Injection, 5% Dextrose Injection, or Lactated Ringer's Injection to a final drug concentration of 0.1 - 1.0 mg/mL. Unless used within 15 minutes of reconstitution, the diluted solution must be prepared using cold (2°C - 8°C) infusion fluids and stored at 2°C - 8°C (36°F - 46°F) for up to a maximum of 7 hours until administration.

6.1.7 **Supplier** Decitabine is commercially available. See package insert and prescriber information for further information.

6.1.8 **Decitabine Modification for Obesity** No dose modifications will be made for obesity. Obese patients should be dosed based on actual height and weight and not based on ideal or adjusted body weight.

6.2 Vorinostat (Zolinza®, SAHA, suberoylanilide acid)

6.2.1 Nomenclature and Molecular Structure: ZOLINZA contains vorinostat, which is described chemically as *N*-hydroxy-*N′*-phenyloctanediamide. The empirical formula is $C_{14}H_{20}N_2O_3$. The molecular weight is 264.32.

6.2.2 **Mode of Action and Pharmacology:** Vorinostat inhibits the enzymatic activity of histone deacetylases HDAC1, HDAC2 and HDAC3 (Class I) and HDAC6 (Class II) at nanomolar concentrations (IC50 < 86 nM). These enzymes catalyze the removal of acetyl groups from the lysine residues of proteins, including histones and transcription factors. In some cancer cells, there is an overexpression of HDACs, or an aberrant recruitment of HDACs to oncogenic transcription factors causing hypoacetylation of core nucleosomal histones. Hypoacetylation of histones is associated with a condensed chromatin structure and repression of gene transcription. Inhibition of HDAC activity allows for the accumulation of acetyl groups on the histone lysine residues resulting in an open chromatin structure and transcriptional activation. In vitro, vorinostat causes the accumulation of acetylated histones and induces cell cycle arrest and/or apoptosis of some transformed cells. The mechanism of the antineoplastic effect of vorinostat has not been fully characterized.

Vorinostat is approximately 71% bound to human plasma proteins over the range of concentrations of 0.5 to 50 µg/mL. The major pathways of vorinostat metabolism involve glucuronidation and hydrolysis followed by β-oxidation. Vorinostat is eliminated predominantly through metabolism with less than 1% of the dose recovered as unchanged drug in urine, indicating that renal excretion does not play a role in the elimination of vorinostat.

6.2.3 Toxicity/Adverse Events: The most common drug-related adverse reactions can be classified into 4 symptom complexes:

1. Gastrointestinal symptoms (diarrhea, nausea, anorexia, weight decrease, vomiting, constipation)
2. Constitutional symptoms (fatigue, chills)
3. Hematologic abnormalities (thrombocytopenia, anemia)
4. Taste disorders (dysgeusia, dry mouth)

The most common serious drug-related adverse reactions were pulmonary embolism and anemia.

Adverse Events Reported in ≥ 10% of Patients With Cutaneous T-cell Lymphoma Who Were Receiving Vorinostat (Regardless of Causality)	
▪ Fatigue ▪ Diarrhea ▪ Nausea ▪ Dysguesia ▪ Thrombocytopenia ▪ Anorexia ▪ Weight decrease ▪ Muscle spasms ▪ Alopecia ▪ Dry mouth ▪ Blood creatinine increased ▪ Chills	▪ Vomiting ▪ Constipation ▪ Dizziness ▪ Anemia ▪ Decreased appetite ▪ Peripheral edema ▪ Headache ▪ Pruritus ▪ Cough ▪ Upper respiratory infection ▪ Pyrexia
Adverse Events Reported in ≥ 5% but < 10% of Patients With Cutaneous T-cell Lymphoma Who Were Receiving Vorinostat (Regardless of Causality)	
▪ Pulmonary embolism	▪ Hyperglycemia
Rare Adverse Events Reported in Other Patients Who Were Receiving Vorinostat (Regardless of Causality)	
▪ Deep vein thrombosis ▪ Abnormal electrolytes (including blood creatinine, magnesium, potassium and calcium) ▪ Ischemic stroke ▪ Pelviureteric obstruction ▪ Spinal cord injury ▪ Syncope ▪ Ureteric obstruction	▪ Squamous cell carcinoma ▪ Cholecystitis ▪ Death ▪ Blood stream infections (including specifically, for example: enterococcal, streptococcal) ▪ Exfoliative dermatitis ▪ Gastrointestinal hemorrhage ▪ Lobar pneumonia ▪ Myocardial infarction

	▪ T-cell lymphoma ▪ Prolonged QT interval

6.2.4 Pregnancy and Breast Feeding: Vorinostat may cause fetal harm when administered to a pregnant woman. Women of childbearing potential should be advised to avoid becoming pregnant while using vorinostat. Men should be advised not to father a child while receiving treatment with vorinostat, and for 2 months afterwards. It is not known whether vorinostat or its metabolites are excreted in human milk. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions from vorinostat in nursing infants, breast feeding must be avoided.

6.2.5 Formulation and Stability: 100 mg white, opaque, hard gelatin capsules with "568" over "100 mg" printed within radial bar in black ink on the capsule body. Store at 20-25°C (68-77°F), excursions permitted between 15-30°C (59-86°F).

6.2.6 Preparation and Administration: Swallow the capsules whole, do not open, chew or crush them. Take with food the same time each day. If dose is vomited within 30 minutes of administration, the patient may be re-dosed. If patient vomits dose beyond 30 minutes of administration, contact the Study Chair. If dose is forgotten and/or missed, contact the Study Chair.

6.2.7 Supplier: Vorinostat is commercially available. See package insert and prescriber information for further information.

6.2.8 Vorinostat Modification for Obesity: No dose modifications will be made for obesity. Obese patients should be dosed based on actual height and weight and not based on ideal or adjusted body weight.

6.3 FLUDARABINE

6.3.1 Nomenclature and Molecular Structure: FLUDARA FOR INJECTION contains fludarabine phosphate, a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-β-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination by adenosine deaminase. Each vial of sterile lyophilized solid cake contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Reconstitution with 2 mL of Sterile Watr for Injection USP results in a solution containing 25 mg/mL of fludarabine phosphate intended for intravenous administration.

The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2-fluoro-9 (5-0-phosphono-B-D-arabino furanosyl fludarabine phosphate) (2-fluoro-ara-AMP). The molecular formula of fludarabine phosphate is $C_{10}H_{13}FN_5O_7P$ (MW 365.2).

6.3.2 Mode of Action and Pharmacology: Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

Phase 1 studies in humans have demonstrated that fludarabine phosphate is rapidly converted to the active metabolite, 2-fluoro-ara-A, within minutes after intravenous infusion. Consequently, clinical pharmacology studies have focused on 2-fluoro-ara-A pharmacokinetics. After the five daily doses of 25 mg 2-fluoro-ara-AMP/m2 to cancer patients infused over 30 minutes, 2-fluoro-ara-A concentrations show a moderate accumulation. During a 5-day treatment schedule, 2-fluoro-ara-A plasma trough levels increased by a factor of about 2. The terminal half-life of 2-fluoro-ara-A was estimated at approximately 20 hours. In vitro, plasma protein binding of fludarabine ranged between 19% and 29%.

A correlation was noted between the degree of absolute granulocyte count nadir and increased area under the concentration × time curve (AUC).

6.3.3 Toxicity/Adverse Events: The most common adverse events include myelosuppression, fever and chills, infection, and nausea and vomiting. Other commonly reported events include malaise, fatigue, anorexia, and weakness. Serious opportunistic infections have occurred in CLL patients treated with FLUDARA FOR INJECTION. The most frequently reported adverse events and those reactions which are more clearly related to the drug are arranged below according to body system.

Fludarabine	
Frequency of Adverse Event:	**Common: Happens to 21-100 children out of every 100**
Timing of AE Onset in Relation to Drug Administration:	**Adverse Events**
Immediate: Within 1-2 days of receiving drug	Fever, fatigue, weakness, pain, nausea, vomiting, anorexia, cough, dyspnea
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (anemia, neutropenia, thrombocytopenia), Infection (urinary tract infection, herpes simplex infection, pneumonia, upper respiratory)
Delayed: Any time later during therapy, excluding the above conditions	
Late: Any time after completion of treatment	
Frequency of Adverse Event:	**Occasional: Happens to 5-20 children out of every 100**
Timing of AE Onset in Relation to Drug Administration:	**Adverse Events**
Immediate: Within 1-2 days of receiving drug	Edema including peripheral edema, chills, rash, diarrhea, rhinitis, diaphoresis, malaise, abdominal pain, headache, back pain, myalgia, stomatitis, flu-like syndrome
Prompt: Within 2-3 weeks, prior to the next course	Weight loss, gastrointestinal bleeding, hemoptysis, paresthesia, allergic pneumonitis, bronchitis, pharyngitis, visual disturbance, hearing loss, hyperglycemia
Delayed: Any time later during therapy, excluding the above conditions	
Late: Any time after completion of treatment	

Fludarabine	
Frequency of Adverse Event:	**Rare: Happens to < 5 children out of every 100**
Timing of AE Onset in Relation to Drug Administration:	**Adverse Events**
Immediate: Within 1-2 days of receiving drug	Anaphylaxis, tumor lysis syndrome, dehydration*

Fludarabine	
Frequency of Adverse Event:	**Rare: Happens to < 5 children out of every 100**
Prompt: Within 2-3 weeks, prior to the next course	Sinusitis, dysuria, opportunistic infections and reactivation of latent viral infections like Epstein-Barr virus (EBV), herpes zoster and John Cunningham (JC) virus (progressive multifocal leukoencephalopathy [PML])L, EBV associated lymphoproliferative disorder, panycytopenia (can be prolonged), pulmonary hypersensitivitya (dyspnea, cough, hypoxia, interstitial pulmonary infiltrate), pulmonary toxicity (acute respiratory distress syndrome [ARDS], pulmonary fibrosis, pulmonary hemorrhage, respiratory distress, respiratory failure), pericardial effusion, skin toxicity (erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, pemphigus), liver failure, renal failure, hemorrhage, transfusion-associated graft-versus-host disease has occurred following transfusion of non-irradiated blood products, phlebitis*, sleep disorder*, cerebellar syndrome*, depression*, mentation impaired*, alopecia*, pruritus*, seborrhea*, esophagitis*, constipation*, mucositis*, dysphagia*, hesitancy*, cholelithiasis*, abnormal liver function tests *, osteoporosis*, arthralgia*, abnormal renal function test*, proteinuria*, epistaxis*, hemorrhagic cystitis*, eosinophilia*

<table>
<tr><th colspan="3"><u>Fludarabine</u></th></tr>
<tr><th colspan="2">Frequency of Adverse Event:</th><th>Rare: Happens to < 5 children out of every 100</th></tr>
<tr><td colspan="2">Delayed: Any time later during therapy, excluding the above conditions</td><td rowspan="8">Neurotoxicity (increased with high doses): seizures, agitation, confusion, weakness, visual disturbances, optic neuritis, optic neuropathy, photophobia, blindness, paralysis, coma, death, peripheral neuropathya); autoimmune phenomena: thrombocytopenia/thrombocytopenic purpura (ITP), Evans syndrome, hemolytic anemia, acquired hemophilia</td></tr>
<tr><td colspan="2"><u>Fludarabine</u></td></tr>
<tr><td>System Organ Class</td><td>Most Frequently Reported Adverse Events</td></tr>
<tr><td>Hematopoietic System</td><td>▪ Neutropenia
▪ Thrombocytopenia
▪ Anemia
▪ Autoimmune hemolytic anemia</td></tr>
<tr><td>Metabolic</td><td>▪ Tumor lysis syndrome</td></tr>
<tr><td>Nervous System</td><td>▪ Weakness
▪ Agitation
▪ Confusion
▪ Visual disturbance
▪ Coma
▪ Peripheral neuropathy</td></tr>
<tr><td>Pulmonary System</td><td>▪ Pneumonia
▪ Pulmonary sensitivity reactions (dyspnea, cough, interstitial pulmonary ARDS)
▪ Pulmonary hemorrhage
▪ Pulmonary fibrosis</td></tr>
<tr><td>Gastrointestinal System</td><td>▪ Nausea
▪ Vomiting
▪ Anorexia
▪ Diarrhea
▪ Stomatitis
▪ Gastrointestinal bleeding</td></tr>
<tr><td>Cardiovascular</td><td>▪ Edema</td></tr>
<tr><td>Genitourinary System</td><td>▪ Hemorrhagic cystitis</td></tr>
<tr><td>Skin</td><td>▪ Rash</td></tr>
<tr><td colspan="2">Late: Any time after completion of treatment</td><td>Myelodysplastic syndrome/acute myeloid leukemia (mainly associated with prior or concomitant or subsequent treatment with other anticancer treatments), skin cancer (new onset or exacerbation)</td></tr>
<tr><td colspan="2">Frequency of Adverse Event:</td><td>Unknown: Insufficient Data Available</td></tr>
<tr><td colspan="2">Timing of AE Onset in Relation to Drug Administration:</td><td>Adverse Events</td></tr>
<tr><td colspan="2">Unknown: Insufficient Data Available</td><td>Fetal toxicities and teratogenic</td></tr>
</table>

Fludarabine	
Frequency of Adverse Event:	**Rare: Happens to < 5 children out of every 100**
	effects of cytarabine have been noted in humans. It is unknown whether the drug is excreted in breast milk
* Reported in ≤ 3% of subjects. Since these are not considered life threatening they are not included in the consent.	

6.3.4 Drug Interactions: The use of FLUDARA FOR INJECTION in combination with pentostatin is not recommended due to the risk of severe pulmonary toxicity.

6.3.5 Pregnancy and Breast Feeding: Pregnancy Category D: Fludara for Injection may cause fetal harm when administered to a pregnant woman. Fludarabine phosphate was teratogenic in rats and in rabbits. Dose-related teratogenic effects manifested by external deformities and skeletal malformations were observed in the rabbits at 5 and 8 mg/kg/day. Drug-related deaths or toxic effects on maternal and fetal weights were not observed. There are no adequate and well-controlled studies in pregnant women. If Fludara for Injection is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Fludara for Injection, a decision should be made to discontinue nursing or discontinue the drug, taking into account the importance of the drug for the mother.

6.3.6 Formulation and Stability: Fludara for Injection is supplied as a white, lyophilized solid cake. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Store under refrigeration, between 2-8 °C (36-46 °F).

Fludara for Injection is supplied in a clear glass single dose vial (6 mL capacity) and packaged in a single dose vial carton in a shelf pack of five.

6.3.7 Preparation and Administration: Fludara for Injection should be prepared for parenteral use by aseptically adding Sterile Water for Injection USP. When reconstituted with 2 mL of Sterile Water for Injection, USP, the solid cake should fully dissolve in 15 seconds or less; each mL of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The pH range for the final product is 7.2-8.2. In clinical studies, the product has been diluted in 100 cc or 125 cc of 5% Dextrose Injection USP or 0.9% Sodium Chloride USP. Reconstituted Fludara for Injection contains no antimicrobial preservative and thus should be used within 8 hours of reconstitution. Care must be taken to assure the sterility of prepared solutions. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

Procedures for proper handling and disposal should be considered. Consideration should be given to handling and disposal according to guidelines issued for cytotoxic drugs. The use of latex gloves and

safety glasses is recommended to avoid exposure in case of breakage of the vial or other accidental spillage. If the solution contacts the skin or mucous membranes, wash thoroughly with soap and water; rinse eyes thoroughly with plain water. Avoid exposure by inhalation or by direct contact of the skin or mucous membranes.

6.3.8 Supplier: Fludarabine is commercially available. See package insert and prescriber information for further information.

6.4 Cytarabine (Cytosine arabinoside, Ara-C, Cytosar®)

6.4.1 Source and Pharmacology: Cytarabine appears to act through the inhibition of DNA polymerase. A limited, but significant, incorporation of cytarabine into both DNA and RNA has also been reported. It exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and under certain conditions blocking the progression of cells from the G1 phase to the S-phase. Cytarabine is metabolized by deoxycytidine kinase and other nucleotide kinases to the nucleotide triphosphate (Ara-CTP), an effective inhibitor of DNA polymerase. Ara-CTP is inactivated by a pyrimidine nucleoside deaminase, which converts it to the nontoxic uracil derivative (Ara-U). It appears that the balance of kinase and deaminase levels may be an important factor in determining sensitivity or resistance of the cell to cytarabine. It has an initial distributive phase $t_{½}$ of about 10 minutes, with a secondary elimination phase $t_{½}$ of about 1 to 3 hours. Peak levels after intramuscular or subcutaneous administration of cytarabine occur about 20 to 60 minutes after injection and are lower than IV administration. Intrathecally administered doses are metabolized and eliminated more slowly with a $t_{½}$ of about 2 hours.

6.4.2 Toxicity/Adverse Event: (Intravenous,)

Cytarabine Intravenous			
	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, anorexia	Flu-like symptoms with fever, rash	Ara-C syndrome (fever, myalgia, bone pain, occasionally chest pain, maculopapular rash, malaise, conjunctivitis), anaphylaxis
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (anemia, thrombocytopenia, leukopenia, megaloblastosis, reticulocytopenia), stomatitis, alopecia	Diarrhea, hypokalemia, hypocalcemia, hyperuricemia	Hepatotoxicity, sinusoidal obstruction syndrome (SOS, formerly VOD), urinary retention, renal dysfunction, pain and erythema of the palms and soles
Delayed: Any time later during therapy, excluding the above conditions			Asymptomatic nonoliguric rhabdomyolysis
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of cytarabine have been noted in humans. It is unknown whether the drug is excreted in breast milk.		

6.4.3 Toxicity/Adverse Event: (Intrathecal)

Cytarabine Intrathecal			
	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, fever, headache	Arachnoiditis	Rash, somnolence, meningismus, convulsions, paresis
Prompt: Within 2-3 weeks, prior to the next course			Myelosuppression, ataxia
Delayed: Any time later during therapy, excluding the above conditions			Necrotizing leukoencephalopathy, paraplegia, blindness (in combination with XRT & systemic therapy)

6.4.4 Formulation and Stability: Cytarabine for Injection is available in vials of 100 mg, 500 mg, 1 g, and 2 g containing a sterile powder for reconstitution. It is also available at a 20 mg/mL concentration with benzyl alcohol (25 mL per vial) or as a preservative free solution (5 mL, 50 mL per vial), and at a 100 mg/mL concentration with benzyl alcohol (20 mL vial) or as preservative free solution (20 mL vial). Hydrochloric acid and/or sodium hydroxide may be added to adjust the pH. Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F). Cytarabine solutions should be protected from light.

6.4.5 Preparation and Administration:

IV Infusion: Reconstitute the lyophilized powder with Bacteriostatic Water for Injection or NS injection. Solution containing bacteriostatic agent should not be used for the preparation of doses > 200 mg/m^2. May be further diluted with dextrose or sodium chloride containing solutions. May be administered by IV push injection, by IV infusion, or by continuous infusion.

High Dose (≥ 1000 mg/m^2/dose): Administer steroid eye drops (dexamethasone or prednisolone), 2 drops each eye q.6.h. beginning immediately before the first dose and continuing 24 hours after the last dose. If patient does not tolerate steroid eye drops, administer artificial tears on a q. 2-4 hour schedule.

Stability: When reconstituted with Bacteriostatic Water for Injection, cytarabine is stable for 48 hours at room temperature. Solutions reconstituted without a preservative should be used immediately. Discard if solution appears hazy. Diluted solutions in D5W or NS are stable for 8 days at room temperature; however, the diluted cytarabine should be used within 24 hours for sterility concerns.

Intrathecal: For intrathecal administration, dilute with 5-10 mL (or volume per institutional practice) preservative free 0.9% sodium chloride injection, lactated Ringer's injection, Elliot's B solution. The volume of CSF removed should be equal to at least ½ the volume delivered.

Stability: Intrathecal cytarabine mixed in NS, lactated Ringer's injection, or Elliot's B solution is stable for 24 hours at 25 ºC but contains no preservative and should be administered as soon as possible after preparation.

6.4.6 Supplier: Cytarabine is commercially available. See package insert and prescriber information for further Information

6.5 Filgrastim (G-CSF, Neupogen)

6.5.1 Source and Pharmacology: Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. Colony-stimulating factors are glycoproteins which act on hematopoietic cells by binding to specific cell surface receptors and stimulating proliferation, differentiation commitment, and some end-cell functional activation.

Endogenous G-CSF is a lineage specific colony-stimulating factor which is produced by monocytes, fibroblasts, and endothelial cells. G-CSF regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens). G-CSF is not species-specific and has been shown to have minimal direct *in vivo* or in vitro effects on the production of hematopoietic cell types other than the neutrophil lineage.

Filgrastim is a 175 amino acid protein manufactured by recombinant DNA technology. It is produced by Escherichia coli (E coli) bacteria into which has been inserted the human granulocyte colony-stimulating factor gene. Filgrastim has a molecular weight of 18,800 daltons. The protein has an amino acid sequence that is identical to the natural sequence predicted from human DNA sequence analysis, except for the addition of an N-terminal methionine necessary for expression in E coli. Because Filgrastim is produced in E coli, the product is nonglycosylated and thus differs from G-CSF isolated from a human cell.

6.5.2 Toxicity/Adverse Events: In clinical trials involving patients receiving Filgrastim following chemotherapy, most adverse experiences were the sequelae of the underlying malignancy or cytotoxic chemotherapy. Medullary bone pain is the most common adverse reaction attributed to Filgrastim therapy. Spontaneously reversible elevation in uric acid, LDH, and alkaline phosphatase also observed in patients receiving Filgrastim.

Filgrastim (G-CSF, Neupogen)			
	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug		Local irritation at the injection site, headache	Allergic reactions (more common with IV administration than subq):skin (rash, urticaria, facial edema), respiratory (wheezing, dyspnea) and cardiovascular (hypotension, tachycardia), low grade fever
Prompt: Within 2-3 weeks, prior to the next course	Mild to moderate medullary bone pain	Increased: alkaline phosphatase, lactate dehydrogenase and uric acid, thrombocytopenia	Splenomegaly, splenic rupture, exacerbation of pre-existing skin rashes, sickle cell crises in patients with SCD, excessive leukocytosis
Delayed: Any time later during therapy, excluding the above conditions			Cutaneous vasculitis, ARDS
Late: Any time after completion of treatment			MDS or AML (confined to patients with severe chronic neutropenia and long term administration)
Unknown Frequency and Timing: Fetal toxicities and teratogenic effects of filgrastim in humans are unknown. Conflicting data exist in animal studies and filgrastim is known to pass the placental barrier. It is unknown whether the drug is excreted in breast milk.			

6.5.3 Preparation and Administration: Use only one dose per vial; do not re-enter the vial. Discard unused portions. Do not save unused drug for later administration. Use only one dose per prefilled syringe. Discard unused portions. Do not save unused drug for later administration.

Vials: Single-dose, preservative-free vials containing 300 mcg (1 mL) of Filgrastim (300 mcg/mL). Dispensing packs of 10 (NDC 55513-530-10).

Single-dose, preservative-free vials containing 480 mcg (1.6 mL) of Filgrastim (300 mcg/mL). Dispensing packs of 10 (NDC 55513-546-10).

Prefilled Syringes (SingleJect®): Single-dose, preservative-free, prefilled syringes with 27 gauge, ½ inch needles with a needle guard, containing 300 mcg (0.5 mL) of filgrastim (600 mcg/mL) Dispensing packs of 10 (NDC 55513-924-10).

Single-dose, preservative-free, prefilled syringes with 27 gauge, ½ inch needles with a needle guard, containing 480 mcg (0.8 mL) of filgrastim (600 mcg/mL). Dispensing packs of 10 (NDC 55513-209-10).

The needle cover of the prefilled syringe contains dry natural rubber (a derivative of latex).

6.5.4 Storage and Dilution: NEUPOGEN® should be stored in the refrigerator at 2 °C to 8 °C (36 °F to 46 °F). Avoid shaking. Prior to injection, NEUPOGEN® may be allowed to reach room temperature for a maximum of 24 hours. Any vial or prefilled syringe left at room temperature for greater than 24 hours should be discarded. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit; if particulates or discoloration are observed, the container should not be used.

Dilution: If required, NEUPOGEN® may be diluted in 5% dextrose. NEUPOGEN® diluted to concentrations between 5 and 15 mcg/mL should be protected from adsorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2 mg/mL. When diluted in 5% dextrose or 5% dextrose plus Albumin (Human), NEUPOGEN® is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes. Dilution of NEUPOGEN® to a final concentration of less than 5 mcg/mL is not recommended at any time. Do not dilute with saline at any time; product may precipitate.

6.5.5 Supplier: Filgrastim is commercially available. See package insert and prescriber information for further information.

7.0 REQUIRED OBSERVATIONS/MATERIAL AND DATA TO BE ASSESSIONED

All protocol-specified hematology, blood chemistries, and bone marrow aspirations and/or biopsies are to be performed in the local laboratory at each investigational site.

7.1 Clinical and Laboratory Studies

All entry/eligibility studies must be performed within 1 week prior to study enrollment. Additional assessments may be obtained as needed for good patient care.

Studies to be Obtained	Pre-Study	Course 1 & 2	Final Visit	Follow-Up
History	X	Twice Weekly	X	X
Physical Exam (Ht, Wt, BSA, VS)	X[1]	Twice Weekly[*]	X	X
CBC, differential, platelets	X[1]	Twice Weekly[*]	X	X
Chemistry Panel[2]	X[1]	Weekly[*]	X	X
Liver Panel[3]	X[1]			
Urine pregnancy or Serum β-HCG (females of childbearing potential)	X[1]			
Echocardiogram or MUGA	X[1]	Prior to Course 2	X	

Studies to be Obtained	Pre-Study	Course 1 & 2	Final Visit	Follow-Up
EKG	X[1]	Prior to Course 2	X	
Bone marrow aspirate and/or biopsy for routine morphology	X[1, ,5]	Day 35-42 or when counts recover[4]		
CSF Cell Count and Differential	X[1]	Around Day 35-42 or when counts recover		
Bone marrow sample for MRD		Around Day 35-42 or when counts recover		
Bone marrow for Required Correlative Studies	X[1]	Day 35-42 or when counts recover		
Peripheral blood for Required Correlative Studies	X[1,4]	Days 5, 14, and 35-42 or when counts recover		

* Any Grade 3 or 4 lab tests or other adverse experiences found should be reviewed and repeated within 48 hours to determine if the grade and duration meet the definition for DLT (section 6.6)
[1] Required for verification of eligibility. If a patient has labs done prior to study entry that establish eligibility, then abnormal Day 1 labs do not deem them ineligible. Height only needs to be recorded at study entry to determine BSA and not repeated with subsequent visits. Bone marrow evaluations done prior to study enrollment that establish patient's eligibility, can be waived for pre-study entry marrow if leukemia blasts are present in the peripheral blood and can be sent for biology correlative studies. Bone marrow does not have to be repeated purely for the purposes of obtaining correlative study samples. In this case, peripheral blood samples for correlative biology studies are acceptable.
[2] Chemistry panel should include: Creatinine, calcium, glucose, electrolytes (chloride, sodium, potassium, and bicarbonate) and uric acid (uric acid is required at baseline and during 1st week of therapy, otherwise only if clinically indicated),
[3] Liver panel should include: AST, ALT, total bilirubin, direct bilirubin, alkaline phosphatase, total protein, albumin
[4] Day 35-42 peripheral blood correlative studies should be done on the same day as the bone marrow aspirate and/or biopsy
[5] Relapse bone marrow sample must be sent for FLT3-ITD molecular testing pre-study.

7.2 Disease Evaluation During Therapy

All patients are required to have a bone marrow aspirate and/or biopsy and CBC to assess response around day 35-42 or when blood counts recover (ANC >500/µL AND platelet >50,000/µL), whichever occurs first.

- A bone marrow aspiration/biopsy to assess remission status should be performed approximately on day 35-42 when blood counts recover and discontinuation of G-CSF for at least 24 hours. Bone marrow aspirate may be delayed beyond day 42 if adequate peripheral count recovery has not yet occurred. See section 11 for response criteria.

- If the marrow is hypoplastic and/or there is little or no evidence of normal hematopoiesis, a repeat marrow should be performed after a further 7-21 days (based upon peripheral blood count recovery and the

clinician's judgment) and remission status assessed at this later time point. Patients who continue to have hypoplastic bone marrow aspiration beyond day 56 will be considered to have experienced a DLT. Bone marrow hypoplasia is defined as overall marrow cellularity less than 10%.

- Administration of course 2 intrathecal therapy at the time of the end of course 1 bone marrow evaluations is permitted to avoid additional sedation procedures.

- Patients who achieve CR, CRi, PR, or SD should receive a second course of chemotherapy as soon as clinically acceptable to the treating institution but no earlier than 33 days after the start of course 1, and the following criteria are met:

  – All grade 3 and 4 non-hematologic toxicities have resolved to grade 2 or less

  – Patients in CR or CRi must have an ANC > 500/ul and PLT > 50,000/ul. It is recommended but not required that ANC>750/ul and platelet count > 50,000/ul to be achieved before proceeding with a second course of therapy **EXCEPT** patients with PR, or SD. These patients, if in good enough clinical condition (based on treating physician's clinical judgment), may continue begin Course 2 irrespective of blood counts.

  – Patients who do not meet these criteria by day 56 will be considered to have a DLT

  – Administration of course 2 intrathecal therapy at the time of the end of course 1 bone marrow evaluations is permitted to avoid additional sedation procedures.

7.3 Bone Marrow Sample for MRD

(ALL MRD RESULTS MUST BE SENT TO TACL OPERATIONS AT TACL@CHLA.USC.EDU ONCE THEY HAVE BEEN RECEIVED FROM SEATTLE)

Participation in the collection of these specimens is required

Samples requested:	**Bone Marrow Sample for MRD** • At the end of course 1 and 2 around day 35-42 when counts recover • The sample should also be submitted if the bone marrow procedure is repeated after day 42 to document marrow and count recovery.
Bone marrow Collection procedure:	a. Collect minimum of 2 mL of marrow into a syringe and place marrow into a large purple EDTA tube that are commonly used in all hospitals. Mix well b. Use multiple syringes and tubes as needed. Reposition marrow aspirates needle at least once during procedure to ensure the maximum quality of marrow
Specimen Labeling:	Each tube must be labeled with the study ID number, along with the date the sample was obtained. No personal identifying patient information should be included in the specimen or transmittal form.
Specimen Packaging and	Samples are to be sent at room temperature except for international samples that

Shipping:	are expected to be delayed for more than 48 hours, place a cold pack (not ice pack) in shipment. Include the completed Specimen Shipping Form with shipment and email a copy to TACL Operations Center at tacl@chla.usc.edu. For specimen shipping, contact TACL Operations for a FED-EX label 24 hrs in advance of shipment. Please call the TACL Operations Center for any questions. Samples can be shipped to arrive Monday-Friday. For Saturday arrivals, please call the lab ahead of time: Phone: 206-288-7060 Brent Wood, MD, PhD SCCA Hematopathology Laboratory Room G7-800 825 Eastlake Ave. E. Seattle WA 98109-1028 Phone: 206-288-7060 Fax: 206-288-7127

- **All MRD results MUST be sent to TACL operations once they are received from Seattle.**

7.4 Required Observations Following Completion of Protocol Therapy

Upon completing protocol therapy or exiting the study, all patients will be followed for life or until otherwise notified by the study committee that the study is closed. Sites should submit follow-up a minimum of every 6 months. Events such as patient death, relapse or development of toxicity related to this therapy should be reported right away. The purpose is to assess safety, remission status, administration of alternative therapies, and survival. The following data will be collected:

1) Disease status information
2) Anti-cancer therapy received after exiting the protocol
3) All adverse events thought to be related to this study's treatment
4) Date and cause of death

8.0 CORRELATIVE STUDIES

8.1 Determination of Baseline

Determination of Baseline "functional epigenetic profile" on leukemic blasts isolated at study entry using the following triad of assays:

8.1.1 Reduced representation bisulfite sequencing (RRBS) will be used to analyze the genome-wide methylation profiles on a single nucleotide level; To quantitatively assess global changes in DNA methylation, a LINE-methylation assay will be utilized and specific genes monitored through advanced Infinium MethylationEPIC BeadChip from Illumina.

8.1.2 Chromatin immunoprecipitation (ChIP) with antibodies specific for histone modifications associated with transcriptional activation (H3K4me3 and H3K27ac) and repression (H3K9me3 and H3K27me3) and isotype controls, followed by DNA sequencing (ChIP-seq);

8.1.3 RNA sequencing analysis will be used to measure global transcriptome changes. The power of this approach derives from the integration of complimentary, cross-referenced genome-wide data to generate lists of candidate genes for which it is likely that over- or under-expression is truly driven by epigenetic regulation. Profiles of CD33+ umbilical cord blood cells, whole bone marrow, or Peripheral Blood Stem Cells (PBSCs) will be used as normal controls for each sample.

8.1.4 Immune assessment: Peripheral blood immune cell phenotyping and serum cytokine analysis.

8.2 Assessment of the *in Vivo* Effects

Assessment of the *in vivo* effects of combined DNMTi/HDACi on the functional epigenetic profile by comparing the following in paired pre- (Day 0) and post-exposure (Day 5, Day 14 and Day 35) leukemic blasts:

8.2.1 Reversal of DNA promoter hypermethylation of “repressed” genes of interest using RRBS, validated with Pyrosequencing-based methylation assay;

8.2.2 Increase in H3K9/14 acetylation in association with “repressed” genes of interest using H3K9/14 ChIP-seq, validated with ChIP-qPCR;

8.2.3 Reversal of transcriptional silencing of “repressed” genes of interest using RNA seq, validated by qRT-PCR. Since significant acute cell kill is unlikely during the 5-day “window” of DNMTi/HDACi, we will have a unique opportunity to assess the in vivo effects of epigenetic therapy with the Day 5 sample. The Day 14 peripheral blood and Day 35 marrow samples will also contribute in patients whose leukemic blasts persist at these time points.

8.2.4 Changes in peripheral blood immune cell subsets and serum cytokines.

8.3 Correlations of Baseline

Correlations of Baseline functional epigenetic phenotype and pharmacodynamic modulation of repressed genes of interest with clinical responses: While the ability to definitively correlate either baseline epigenetic phenotypes or successful reversal of epigenetic phenotypes with clinical response will be limited by small patient numbers, the descriptive data we generate will be invaluable in developing biomarkers to be validated in future trials.

8.4 Chemo-Sensitivity Profiling

We hypothesize that vorinostat and decitabine may influence sensitivity of leukemic cells to the standard re-induction chemotherapy. We will test blasts isolated at baseline (pre-treatment) vs. those isolated at Day 5 (post-exposure to DNMTi/HDACi) and compare cytotoxic IC50 for fludarabine and cytarabine using enzyme-based proliferation assays (WST-1, e.g.) and flow-based apoptosis assays (annexin V and 7-AAD).

8.5 Immune monitoring

It is now clear that the immune system can have an important role in controlling cancer progression. Epigenetic modifying drugs and many chemotherapeutic agents are known to have both positive and negative effects on the immune system. The combination of agents being used here could have either beneficial or detrimental effects on immune cell function and composition. We hypothesize that immune cell subsets and serum cytokines levels will be impacted by the treatment regimen. Plasma will be quantitatively analyzed for 65 different cytokines and chemokines in multi-plex assays. We will also immunophenotype peripheral blood mononuclear cells using multi-color (8 parameters) flow cytometry. Cell subsets to be analyzed will include T cells (naïve, effector and regulatory), B cells, NK cells, dendritic cells and myeloid cells.

Samples requested:	**Bone Marrow** Courses 1 and 2 • Pre-study • Day 35*	**Peripheral Blood** Courses 1 and 2 • Pre-study • Days 5, 14, 35*
Blood Sample Procedure:	Draw 5-10 mL of blood into a sodium-heparin (BD Vacutainer Green top)	
Bone Marrow Draw Procedure:	Draw 5-10 mL of bone marrow into a sodium-heparin (BD Vacutainer Green top)	
Specimen Processing:	Include the completed Correlative Sample Transmittal Form with shipment and email a copy to TACL Operations Center at tacl@chla.usc.edu. For specimen shipping, contact TACL Operations for a FED-EX label 24 hrs in advance of shipment. Please call the TACL Operations Center for any questions. No processing. Please ship immediately at room temperature using overnight FedEx shipping on Monday-Thursday (excluding holidays). If the samples cannot be shipped immediately, please store at Room Temperature until they can be shipped.	
Specimen Labeling:	Each tube must be labeled with the patient study ID number, type of specimen (blood or bone marrow) and the date and time the specimen was drawn. No identifying patient information should be included on the specimen or transmittal form.	
Specimen Packaging and Shipping:	Green Top Tubes with sample: Attn: Cary Stelloh (Rao Lab) Phone: 414-937-3878 (cell 617-504-2984) Sid Rao, MD	

	Blood Research Institute Medical College of Wisconsin 8727 Watertown Plank Rd. Milwaukee, WI 53226-3548 FedEx # 053204660

*Can be performed between day 35 and 42

9.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY, OFF STUDY CRITERIA AND STUDY TERMINATION

9.1 Criteria for Removal from Protocol Therapy

a. Progressive disease
b. Patients remains CNS positive after the first course of therapy
c. Relapse in any site following remission
d. Second malignant neoplasm
e. Completion of protocol therapy
f. Patient/parent withdrawal or refusal after beginning protocol therapy
g. Patient/parent withdrawal or refusal before beginning protocol therapy
h. Patient off treatment for other complicating disease
i. Non-compliance with protocol regimen and procedures
j. Unacceptable toxicity
k. Female patient becomes pregnant or begins breast-feeding

9.2 Off Study Criteria

1. Death
2. Patient lost to follow-up
3. Patient withdraws consent, refuses follow-up

9.3 Termination of Study by TACL

The TACL Consortium may terminate this study prematurely, either in its entirety or at an investigative site, for reasonable cause provided that written notice is submitted in advance of the intended termination. Advance notice is not required if the study is stopped due to safety concerns.

10.0 STATISTICAL CONSIDERATIONS

The primary objectives of this study, and thus of the statistical design and analysis, are to determine the maximum tolerated dose (MTD) of decitabine when used in combination with vorinostat, fludarabine, high dose cytarabine and G-CSF (FLAG), and to evaluate the ability to safely deliver this combination in children and young

adults with relapsed or refractory AML. Both of these objectives are addressed by the Phase I dose-escalation design. Secondary objectives include evaluating pharmacodynamic endpoints and their possible association with tumor response in the context of a Phase I study, and evaluating the ability to safely deliver this treatment combination in Down syndrome patients with relapse/refractory AML.

The study will enroll patients in two strata – a primary stratum of eligible patients without Down syndrome, and a secondary stratum of eligible patients with Down syndrome.

10.1 Statistical Considerations for Dose Escalation Criteria

The primary endpoint for dose escalation is the occurrence of a dose-limiting toxicity (DLT – Section 4.4) observed during the first couse of therapy. Within each of the two strata, a standard 3+3 patient cohort escalation design[33], as described below, will be used, starting at dose level 1 (Table 6):

1. Up to three evaluable patients are entered initially at the current dose level.
2. If 0/3 patients experience a DLT at the current dose level, then the dose is escalated to the next higher level, if one exists, and three patients are enrolled.
3. If 1/3 patients experience a DLT at current dose, then up to three additional patients are accrued at the same dose level.
4. If 2 or or more patients experience a DLTs in a three-patient or six-patient cohort at the current dose level, then the MTD has been exceeded, and dose escalation will be stopped. Up to three additional patients will be enrolled at the next lower dose level if one exists and fewer than six patients have already been treated at that dose level.

10.2 Study Stopping Rules

Study duration will be based on enrollment and outcome in the primary stratum. Enrollment to the study will be halted when, in the primary stratum, a provisional RP2D as defined in 10.3.1 is established and the optional expansion phase (see below) is complete, or when at least two evaluable patients treated at dose level 0 experiences DLT. In the latter case no MTD is established and we will conclude that this combination cannot be safely delivered at any of the dose levels tested.

If a provisional RP2D is established in the primary stratum, accrual can continue until a maximum of 33 DLT- and response-evaluable patients overall in the primary stratum has been enrolled, with the number of additional patients at the discretion of the study committee based on considerations of numbers of patients evaluable for secondary endpoints, accrual rate, occurrence of toxicity or severe adverse events, and existence of other competing studies. Patients who are not DLT- or response-evaluable will be replaced but will be included in statistical analysis as appropriate.

Patients with Down syndrome will be enrolled according to a 3+3 design as described, but with the additional restriction that the dose level in Down syndrome patients may never exceed the current dose level in the primary stratum. Given the rarity of Down syndrome patients, this stratum is not expected to accrue many patients. Hence the analysis of this stratum will be primarily descriptive.

10.3 Definitions

10.3.1 Maximum Tolerated Dose (MTD)/Highest Tested Dose (HTD)/Recommended Phase 2 Dose (RP2D): The MTD is the highest dose level tested at which 0/6 or 1/6 patients experience DLT

with at least 2/3 or 2/6 patients encountering DLT at the next higher dose. If the highest specified dose level in this study is reached with 0/6 or 1/6 patients experiencing DLT, i.e., the MTD has not been reached, this dose level will be referred to as the Highest Tested Dose (HTD). The MTD or HTD will be the provisional recommended phase 2 dose (RP2D). If 2 or more DLTs are observed at the lowest specified dose, an MTD, and therefore an RP2D, does not exist. The decision about the final RP2D, or whether an RP2D exists, will be made after the completion of the expansion phase based on the additional toxicity, SAE, and other data that is generated during the expansion phase.

10.3.2 Definition of a Patient Evaluable for DLT: Any patient not experiencing a DLT who receives less than 80% of the prescribed total dose of any of the systemic anticancer agents (i.e., fewer than 4 of the 5 prescribed doses of Decitabine, Vorinostat, Fludarabine, or IV Cytarabine) for reasons unequivocally unrelated to Decitabine toxicity, or who starts subsequent anti-cancer therapy before the required observation times specified in the DLT definition, will be considered NOT EVALUABLE for DLT and will be replaced. All other patients who receive any portion of treatment will be considered EVALUABLE for DLT.

10.3.3 Definition of a Patient Evaluable for Response: A patient will be considered evaluable for response if the patient receives all or part of protocol therapy and the patient is under follow-up for a sufficient period to evaluate the disease or to meet the definition of progressive disease. A patient who is removed from therapy for problems possibly related to disease progression will be considered a non responder.

10.4 Patient Accrual and Study Duration

10.4.1 Patient Accrual: This study will enroll a minimum of 4 and a maximum of 33 non-Down syndrome patients who are evaluable for dose-limiting toxicity (DLT) to complete the dose escalation and expansion at the RP2D. A small number of Down syndrome patients will also be enrolled.

10.4.2 Study Duration: The study will require approximately 18 to 24 months of accrual.

10.5 Interim Monitoring of Toxic Death

The occurrence of toxic death (TD) at any time during protocol therapy will be a primary endpoint for safety monitoring. A population toxic death rate that exceeds p0=0.10 will be considered unacceptable. A Bayesian monitoring rule based on a pessimistic Beta (1,3) prior distribution on the parameter p0 will be used to judge the evidence that the population toxic death rate exceeds this quantity. This prior has mean 0.25, median 0.21, and 90% of support under 0.54. A posterior probability of greater than 80% that p0>0.10 will be considered statistical evidence that the population toxic death rates may exceed 0.10. Operationally, this criterion will be satisfied if the following fractions of TDs out of total patients treated are exceeded: ≥1/5, ≥2/12, ≥3/20, ≥4/28, and so on as dictated by this rule. If this criterion is satisfied at any time 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. The monitoring rule will apply across both study strata combined.

10.6 Analysis of Correlative Studies

The analysis of genome wide and specific gene methylation, histone modification, RNA expression, peripheral blood immune cell parameters, and chemosensitivity pre- and post-exposure and, where applicable, their possible assocation with tumor response and achievement of MRD- status, will be performed using standard statistical methods (e.g. linear regression, logistic regression). These analyses will be primarily descriptive, as the sample size will not provide sufficient power to detect any but extremely large effects, but they nevertheless will provide useful prelminary information that will inform planning of successor studies or investigations.

10.7 Inclusion of Women and Minorities

The study is open to all participants regardless of gender or ethnicity. Review of accrual to past studies of new agents demonstrates the accrual of both genders and all NIH-identified ethnicities to such studies. The small number of patients entered into this trial will obviate any analysis of variation in response rate with gender or ethnicity.

11.0 RESPONSE CRITERIA

11.1 Bone Marrow Response Criteria

11.1.1 Complete Remission, MRD negative (CR MRD-)

- A bone marrow with <5% blasts by morphology; and
- MRD <0.1% by flow or molecular testing (e.g. PCR); and
- No evidence of circulating blasts or extramedullary disease; and
- Recovery of peripheral counts (ANC > 500/µL and PLT count > 50,000 µL).
- Qualifying marrow and peripheral counts should be performed within 1 week of each other

11.1.2 Complete Remission (CR)

- A bone marrow with <5% blasts by morphology; and
- No evidence of circulating blasts or extramedullary disease; and
- Recovery of peripheral counts (ANC > 500/µL and PLT count > 50,000 µL).
- Qualifying marrow and peripheral counts should be performed within 1 week of each other.

11.1.3 Complete Response with Incomplete Count Recovery (CRi)

- A bone marrow with <5% blasts by morphology; and
- No evidence of circulating blasts or extramedullary disease; and
- Insufficient recovery of absolute neutrophil counts (ANC <500/µL), and or insufficient recovery of platelets (PLT counts <50,000/ul),

11.1.4 Partial Remission (PR)

- Complete disappearance of circulating blasts and one of the following:
- A decrease of at least 50% of blasts in the bone marrow with >5% and ≤ 20% blasts by morphology with recovery of peripheral counts (ANC > 500/µL and PLT count > 50,000 µL)

Note: only patients who entered the study with ≥ 20% blasts in the marrow may be assessed as PR

11.1.5 Stable Disease (SD)

Patient does not satisfy the criterion for either CR, CRi, PR or disease progression.

11.1.6 Progressive Disease (PD)

An increase of at least 25% in the absolute number of bone marrow or circulating leukemic cells, development of new sites of extramedullary disease, or other laboratory or clinical evidence of progression of disease.

11.1.7 Not evaluable (NE)

Aplastic or severely hypoplastic marrow with any blast percentage. Bone marrow aplasia/hypoplasia is defined as overall marrow cellularity less than 10-20%. In this instance, marrow evaluation should be repeated every 1-2 weeks until response determination can be made or patient meets criteria for a DLT of prolonged myelosuppression (as defined in section 4.3.b).

11.1.8 Relapse

After documentation of remission, one bone marrow aspirate and/or biopsy showing ⩾5% leukemic blasts or pathological evidence of extramedullary disease.

11.1.9 MRD Response Criteria

Patient who achieves CR or CRi will additionally be classified according to their minimum residual disease (MRD) status as follows, based on the results of an MRD assay of contemporaneously obtained BM sample:

- MRD-: MRD is undetectable – i.e. below the level of quantifiability of the assay
- MRD+: MRD is detectable (the quantified level of MRD will also be recorded)
- MRD unknown: MRD assay result is not available"

11.2 CNS Response Criteria

11.2.1 CNS Disease Definition for patients with AML

AML patients with CNS disease is defined as:

- Any number of blasts on a cytospin prep in an atraumatic (< 100 RBCs) lumbar puncture, OR
- Blasts in a traumatic tap in which the WBC/RBC ratio in the CSF is twice that in the peripheral blood OR
- Clinical signs of CNS leukemia (such as facial nerve palsy, brain/eye involvement or hypothalamic syndrome). Extra-ocular orbital masses are not considered CNS leukemia, OR
- Radiographic evidence of an intracranial, intradural mass consistent with a chloroma.

11.2.2 Method of Evaluating Initial Traumatic Lumbar Punctures:

If the patient has leukemic cells in the peripheral blood and the lumbar puncture is traumatic and contains ≥ 5 WBC/μL and blasts, the following Steinherz/Bleyer algorithm should be used

$$\frac{\text{CSF WBC}}{\text{CSF RBC}} > 2X \frac{\text{Blood WBC}}{\text{Blood RBC}}$$

A patient with CSF blasts, whose CSF WBC/RBC is 2X greater than the blood WBC/RBC ratio, has CNS disease at diagnosis.

Example: CSF WBC = 60/μL; CSF RBC = 1500/μL; blood WBC = 46000/μL; blood RBC = 3 X 10^6/μL:

60/1500 **= 0.04 > 2X** 4600/3x10^6 **= 0.015**

Therefore this patient has CNS disease because 0.04 > 2x 0.015

12.0 ADVERSE EVENT REPORTING REQUIREMENTS

12.1 Definitions

Adverse Event: An adverse event means 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. Reasonable possibility means there is evidence to suggest a causal relationship between the drug and the adverse event.

Unexpected Adverse Event or Unexpected Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not available, is not consistent with the risk information described in the general investigational plan.

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

Death of Patient	An event that results in the death of a patient.
Life-Threatening	An event that, in the opinion of the investigator, would have resulted in immediate fatality if medical intervention had not been taken. This does not include an event that would have been fatal if it had occurred in a more severe form.
Hospitalization	An event that results in an admission to the hospital for any length of time. This does not include an emergency room visit or admission to an outpatient facility.
Prolongation of Hospitalization	An event that occurs while the study patient is hospitalized and prolongs the patient's hospital stay.
Congenital Anomaly	An anomaly detected at or after birth or any anomaly that results in fetal loss.
Persistent or Significant Disability/ Incapacity	An event that results in a condition that substantially interferes with the activities of daily living of a study patient. Disability is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (*e.g.,* sprained ankle).
Important Medical Event Requiring Medical or Surgical Intervention to Prevent Serious Outcome	An important medical event that may not be immediately life-threatening or result in death or hospitalization, but based on medical judgment may jeopardize the patient and may require medical or surgical intervention to prevent any of the outcomes listed above (*i.e.,* death of patient, life-threatening, hospitalization, prolongation of hospitalization, congenital anomaly, or persistent or significant disability/incapacity). Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

12.2 Data Collection

Adverse events and suspected adverse reactions will be collected and reported on the electronic CRFs beginning with the first dose of study therapy until 30 days following the last dose of study therapy. All toxicities both hematologic and non-hematologic should be reported in the eCRF. This includes toxicities of all grades (i.e. 1 through 5) and all grade fluctuations. The investigator will evaluate all adverse events and suspected adverse reactions as to their severity and relationship to decitabine and vorinostat as well as the regimen as a whole. Serious adverse events and suspected adverse reactions will require expedited reporting to the TACL Operations Center as described below.

12.3 Reporting Serious Adverse Events or Serious Suspected Adverse Reactions

12.3.1 The following serious adverse events or serious suspected adverse reactions requires expedited reporting:

- All Grade 5 events regardless of causality.
- All Grade 4 non-hematologic events that are possibly, probably or definitely related to decitabine and vorinostat or the regimen as a whole.
- All unexpected Grade 3 events that are possibly, probably or definitely related to decitabine and vorinostat or the regimen as a whole.

12.3.2 Steps for Reporting

Step 1: Identify the adverse event or suspected adverse reaction using the NCI Common Toxicity Criteria (CTC), version 4.0.

The CTC provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTC can be downloaded at
http://evs.nci.nih.gov/ftp1/CTCAE/About.html.

Step 2: Grade the event using the NCI CTCAE version 4.0.

Step 3: Determine if the adverse event or suspected adverse reaction meets the criteria of being "serious".

Step 4: Determine the relationship of DRUG and the regimen as a whole to the event
The investigator will assess the causal relationship between the investigational product and the regimen as a whole and the adverse event. The investigator will use his/her clinical expertise and judgment to select the attribution category below that best fits the circumstances of the AE.

Attribution categories are as follows: Unrelated, Unlikely, Possible, Probable, and Definite.

Unrelated:
- The adverse event is *clearly unrelated* to the investigational agent(s).
- Does not follow a known response pattern to the suspect investigational product (if response pattern is previously known).
- Can be explained by the known characteristics of the patient's clinical state or therapy administered to the patient.

Unlikely:
- The adverse event is *doubtfully* related to the investigational agent(s).
- May or may not follow a reasonable temporal sequence from administration of the investigational product.
- Likely explained by the known characteristics of the patient's clinical state or other therapy administered to the patient.

Possibly Related:
- The adverse event *may be* related to the investigational agent(s).
- Follows a reasonable temporal sequence from administration of the investigational product.
- May also be reasonably explained by the patient's clinical state or therapy administered to the patient.
- May follow a known response pattern to the investigational product (if response pattern is previously known)

Probably Related:
- The adverse event *is likely* related to the investigational agent(s).
- Follows a reasonable temporal sequence from administration of the investigational product.
- May follow a known response pattern to the investigational product (if response pattern is previously known)
- Could not be reasonably explained by the known characteristics of the patient's clinical state or other modes of therapy administered to the patient, if applicable;

Definitely Related:
- The adverse event is *clearly related* to the investigational agent(s).
- Follows the temporal sequence from administration of the investigational product.
- Follows a known response pattern to the investigational product.
- Cannot be explained by the known characteristics of the patient's clinical state or other therapy administered to the patient.
- Is confirmed by improvement of symptoms on stopping or slowing administration of the investigational product and re-emergence of symptoms on restarting administration of the investigational product, (if applicable).

Step 5: Determine if the adverse event or suspected adverse reaction is "unexpected".

Step 6: Notify the TACL Operations Center by telephone or email

The following information should be submitted within 24 hours of event notification by either phone (323)361-3022, or Email tacl@chla.usc.edu.

1. Patient TACL study ID and initials
2. Event description
3. Severity (NCI Grade)
4. Onset date
5. Reason event is considered serious
6. Dose of study drug and dates of administration
7. Investigator opinion of relationship to DRUG and the regimen as a whole
8. Name and phone number of physician in charge of the case
9. Name and phone number of CRA or Research nurse working with the case

Step 7: Submit a written report to the TACL Operations Center

Complete the TACL SAE Notification Form within 72 hours of learning of the event. The completed form should be emailed to tacl@chla.usc.edu. The form may also be faxed to (877) 904-2166. A follow-up SAE Notification Form must be submitted upon resolution of the event or at the request of the study chair or study vice-chair. Please confirm via email or phone that the TACL Operations Center has received this notification.

12.4 Institutional Reporting to the IRB

All SAE's should be reported to the treating institutions IRB or Ethics board. The TACL Operations Center will also report all SAE's to the TACL IRB. The TACL Operations Center will distribute SAE's to all TACL sites as appropriate for submission to their own IRB's.

13.0 DATA AND SAFETY MONITORING

13.1 Data Submission

All study data will be submitted via electronic data capture forms using the Rave-Medidata/TACL Website. Please refer to the TACL web site (https://tacl.chla.usc.edu) for the Rave user manual or contact the TACL Operations Center at (323) 361-3022 if you need assistance.

The following are required to be submitted to the Operations Center for all patients entered:
Scanned copy of following**:** Roadmaps and Bone Marrow Reports (include both aspirate and biopsy reports). These forms are to be emailed to the TACL Operations Office at tacl@chla.usc.edu at the end of each course during which the bone marrows are done.

13.2 Weekly Safety Review

The TACL Operations Center (TOC) conducts weekly (as needed) patient safety and review meetings with the protocol chair, protocol vice-chair, research coordinators and other administrative TACL team members to review all data submitted, non-serious adverse events and other correspondence pertaining to patients. Serious adverse events will be immediately evaluated by the study team and determination regarding notification of participating sites will be made. All serious adverse events will be sent to the CHLA CCI and DSMC if required. Any interim results that would affect patient safety would be immediately communicated to all participating TACL sites. All correspondence with sites will be done via email, with all information also being posted on a member's only section of the TACL website.

13.3 Data Safety and Monitory Committee

DSMC meetings will occur every 6 months. Every 6 months, a DSMC report for each protocol will be prepared by the study statistician and study PI detailing patient accrual, toxicities, deaths on study, current study status, responses (responses will be blinded until study completes accrual), summary of amendments to protocol/consent, lists of any publications from study, and plans for study in coming year. Any publications from the study (abstract or manuscript) will be attached to the DSMC report. After DSMC review, the DSMC will issue a confidential report for each study to the study PI and TACL Operations Center.

Not more than 8 weeks after the DSMC meeting, a DSMC public review report will also be created for each protocol after approval of the confidential report and resolution of any issues by the PI The public report will then be emailed to the participating sites for each study. These can be filed at the IRB at each site if required per local IRB guidelines.

Local IRB changes to this document are allowed. Changes within the document should not substantially alter the meaning or intent of the consent document. If the institution or IRB insists on making deletions or more substantive modifications to the consent, especially in the risks sections, they should be reviewed and approved by the TACL Operations Center.

14.0 SAMPLE INFORMED CONSENT

SAMPLE INFORMED ASSENT*/CONSENT DOCUMENT / PARENTAL PERMISSION FOR PARTICIPATION IN RESEARCH

***Assent for patients >13 years of age**

***Assent for patients <13 years of age should be written per institutional guidelines**

TACL Protocol

Subject Name:	
Medical Record #	
Physician:	

When we say "you" in this consent form, we mean you or your child; "we" means the doctors and other staff.

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family. You can also discuss it with your health care team. If you have any questions, you can ask your study doctor for more explanation.

This study is being carried out by the Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) Consortium. TACL is a group of Universities and Children's Hospitals that are working together to find treatments for children with leukemia and lymphoma.

You are being asked to take part in this research study because your acute myelogenous leukemia (AML) has relapsed. Relapse means your leukemia has come back after treatment. DECITABINE (DEC) and VORINOSTAT (VOR) are drugs approved by the FDA (Food and Drug Administration) for the treatment of myelodysplastic syndrome, and T-Cell Lymphoma, respectively, which are cancers similar to leukemia. These drugs are thought to work by turning on genes that limit the growth of cancer cells. DEC and VOR have been given to children and adults with AML, and has been given together with other chemotherapy. This study is being done to find out if DEC and VOR can be given safely together before treatment with standard chemotherapy drugs in patients with AML.

It is important for you to know why this study is being done before you decide to take part in this research study. This consent will tell you about the study. This consent will also tell you about risks and side effects that might happen to you if you take part in this study. You also need to know you do not have to take part in this study. You can talk to your doctor about other cancer treatments. Because all of the drugs used in this study are each available to doctors, it is possible for you to receive these drugs without taking part in this study. Taking part in this study is voluntary.

Why is this study being done?

The goals of this study are to improve survival rates in children and young adults with relapsed AML through the combination of DEC and VOR. We believe the addition of DEC and VOR in combination with standard AML chemotherapy will provide greater treatment response rates than without these drugs but the effectiveness and safety of this approach is not known.

How many people will take part in this study?

It is expected that approximately 12 to 33 children and young adults will take part in this study.

What will happen if I take part in this research study?

Before you begin the study…

You will need to have the following exams, tests or procedures to find out if you can be in the study. These exams, tests or procedures are part of your regular cancer care and may be done even if you do not join the study. If you have had some of them recently, they may not need to be repeated. The results of these tests will be reviewed. It is possible that after these tests are reviewed, you will not be able to take part in the study. If you are not able to take part in the study, your doctor will discuss with you the reasons why.

- A medical history
- Physical exam with vital signs
- Bone marrow test to check your cancer (see Tests on the Bone Marrow, below)
- Lumbar puncture to test the fluid in your spinal cord (see Lumbar Punctures, below)
- Blood tests to check your organ function
- Tests to make sure you are not pregnant (if you are a female and old enough to become pregnant)
- Tests of liver function
- Echocardiogram (ECHO) which uses sound waves to test heart function
- EKG to check your heart rhythm

<u>Tests on the Bone Marrow</u>
Examinations of the bone marrow will be performed routinely and may be done at the discretion of your study doctor. You have already had many tests of your bone marrow for your previous treatment of AML. Many children receive some form of sedation or anesthesia during this procedure. A small area over your hip bone on the back will be cleaned and numbed with lidocaine and/or with an anesthetic cream. Approximately 2 teaspoons of bone marrow will be withdrawn through a needle inserted into the bone. The test is painful, especially when the bone marrow is withdrawn. There is also a small risk of bleeding or infection from this procedure.

<u>Lumbar Punctures ("L.P.s", "spinal taps")</u>
You are familiar with spinal taps since they were done during your initial therapy for AML. Whether you decide to participate or not in this study, additional spinal taps will need to be done to give medicines, which are necessary

to prevent the leukemia from spreading to the spinal fluid. The drugs cytarabine, hydrocortisone and methotrexate will be injected into your spine while you are having a lumbar puncture. If you are found to have leukemia in your spinal fluid you may receive cytarabine, hydrocortisone and methotrexate otherwise all patients will receive only cytarabine. This way of giving medicine is called "intrathecal" or IT. Many children receive some form of sedation or anesthesia during this procedure. Spinal taps are painful and may cause headaches. The skin at the site of needle insertion is usually numbed with an anesthetic cream or lidocaine before the procedure is performed. Approximately 1 teaspoon of spinal fluid will be withdrawn prior to injection of the medicine.

During the study…

Dose Escalation Schedule

Decitabine Dose Level	Dose	Days Given
0	5 mg/m^2	1-5
1	7.5 mg/m^2	1-5
2	10 mg/m^2	1-5
3	15 mg/m^2	1-5

Treatment
You will receive a total of 2 planned courses

Days (Courses 1 and 2)		1	2	3	4	5	6	7	8	9	10	11	12	…	35
Decitabine		•	•	•	•	•									
Vorinostat		•	•	•	•	•									
Fludarabine							•	•	•	•	•				
Cytarabine							•	•	•	•	•				
Filgrastim (G-CSF)						•	•	•	•	•	•	•	•	•#	
IT Cytarabine	•														
ITT Therapy	•														
Bone Marrow Asp/Bx	**X**														**X**

Treatment will last about 5 to 7 weeks. At the end of each treatment course, you will have an evaluation to see how your leukemia is responding to therapy. Additional therapy is recommended after completion of this treatment and your doctor will discuss those options with you.

Medical Tests During Treatment

Whether you are on this study or not the following medical tests will be done to monitor for response to treatment as well as side effects related to treatment. You will have regular medical appointments throughout treatment. The timing of the appointments will be the same regardless if you participate in the research study. These include:

- Physical exams with vital signs
- Blood tests to check your organ function including
  - CBC to look at your blood cells
  - Chemistries to look at elements and minerals in your blood
  - Blood tests to look at your kidney function
- Bone Marrow Tests
- Lumbar Punctures
- ECHO and EKG before course 2

Tests for Research Purposes:

In addition to the routine tests listed above, we would like to do other tests while you are enrolled on the study.

Bone marrow for Required Correlative Studies
Peripheral blood for Required Correlative Studies

Minimal Residual Disease (MRD)

Doctors have developed tests to detect whether very small amounts of cancer cells can be found in blood or bone marrow. During treatment 1/2 teaspoon (2mL) of bone marrow will be taken. Samples will be taken at the end of course 1 and 2. This testing is done at the time of scheduled bone marrow procedures to determine response and disease status so it does not require any additional procedures. Any leftover bone marrow that is not used for this test will be destroyed.

Final Study Visit

Within 30 days after you finish the last dose of study drugs, your doctor will need to check to see how you are doing. The doctor will ask you how you feel, if you have trouble doing your daily routine, and what drugs you are taking. You will also have the following tests done:

- Physical exam, weight and vital signs
- CBC and chemistry blood tests
- Echocardiogram if required
- EKG

Follow-up Tests

After completing the treatment on this study we would like to continue to collect some medical information about how you are doing for as long as you are willing to let us or until the study is completely closed. We will collect information on how your AML is doing, what kind of therapy you may be getting and if you have any long term side effects.

What are my responsibilities?

- During the study you will be asked to take all your chemotherapy drugs as prescribed. It is very important that you follow your doctor's instructions regarding when and how to take your study medications. Be sure to ask your study doctor or nurse if you have any questions about taking your study medications.

- If you experience any unusual side effects as explained by your study doctor, you should contact the study center immediately. You should also contact your study doctor if you are hospitalized for any reason during the study or within 30 days after completion of therapy.

How long will I be in the study?

You will be in the study for two courses of therapy which will last approximately 2 months. After completing the study therapy, your doctor will discuss with you the options for additional treatment.These options will vary depending on whether or not your leukemia responded to the therapy.

Can I stop being in the study?

Yes. You can decide to stop at any time. Your clinical care will not be affected by your decision to withdraw. Tell your doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop safely. You will be asked to visit the hospital or clinic for some follow-up tests to make sure all the side effects you may have experienced have gone away.

It is important to tell your doctor if you are thinking about stopping so any risks of the study treatment and chemotherapy drugs can be evaluated by your doctor. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow-up care and testing could be most helpful for you.

Your doctor may stop you from taking part in this study at any time without your permission if he/she believes it is in your best interest; if you do not follow the study rules; if you become pregnant or begin to breast feed; or if the study is stopped.

What side effects or risks can I expect from being in the study?

All people who receive cancer treatment are at risk of having side effects. In addition to killing leukemia cells, chemotherapy drugs can damage normal tissues and produce side effects. Side effects are usually reversible when the medication is stopped, but occasionally can persist and cause serious complications or death. The therapy used in this clinical trial is intensified so that it can kill cancer cells quickly before they can become resistant to treatment. Protocols used to treat relapsed leukemia are more intense than are those to treat newly diagnosed disease. It is not possible to predict whether the side effects listed below, or other rare side effects may occur. Side effects can be increased when chemotherapy drugs are combined.

Common side effects include nausea, vomiting, hair loss and fatigue. Chemotherapy causes temporary bone marrow depression. Bone marrow depression results in a decreased production of red cells causing anemia; decreased platelet production causing bruising and an increased chance of bleeding; and decreased white cell production, causing a risk of serious and potentially life threatening infections. Red blood cell and platelet transfusions may be required

Risks of having the bone marrow procedure include bleeding, chance of an infection and long lasting pain at the place where the needle is inserted into the bone.

Each drug will have a unique set of side effects. Side effects related to drugs occur in people at different rates or frequencies.

DRUG

.

Decitabine		
System Organ Class	**Adverse Events Reported more than 10 of 100 adult patients who received decitabine in a Phase 3 MDS trial**	**Adverse Events Reported in 5-10 of 100 adult patients who received decitabine in a Phase 3 MDS trial**
Blood and Lymphatic System	▪ Neutropenia (condition in which the number of white blood cells called neutrophils is abnormally low) ▪ Thrombocytopenia (low number of cells called platelets which may cause bleeding and brusing) ▪ Anemia (Low number of red blood cells) ▪ Febrile neutropenia (condition in which the number of white blood cells called neutrophils is abnormally low along with fever ▪ Leukopenia (low number of white blood cells) ▪ Lymphadenopathy (disease where lymph nodes are abnormal)	▪ Thrombocythemia (disease where a large amount of cells in clotting called platelets are produced)

Decitabine		
System Organ Class	**Adverse Events Reported more than 10 of 100 adult patients who received decitabine in a Phase 3 MDS trial**	**Adverse Events Reported in 5-10 of 100 adult patients who received decitabine in a Phase 3 MDS trial**
Gastrointestinal	▪ Nausea (feeling sick to the stomach) ▪ Constipation (difficulty passing stools) ▪ Diarrhea ▪ Vomiting ▪ Abdominal pain (pain in the belly) ▪ Decreased appetite (less hungry than normal) ▪ Anorexia (illness where person does not want to eat) ▪ Oral Mucosal petechia (Small red dots in the mouth caused by problems with clotting) ▪ Stomatitis (sores in the mouth) ▪ Dyspepsia (indigestion) ▪ Ascites (build up of fluid in the belly which causes bloating and discomfort)	▪ Gum bleeding ▪ Hemorrhoids (swollen veins in the lower part of the rectum and anus) ▪ Tongue ulceration (small parts of the tongue are eroded away) ▪ Difficulty swallowing ▪ Oral soft tissue disorders (disorders of the skin in the mouth) ▪ Lip ulceration (small parts of the lip are eroded away) ▪ Abdominal distention (bloating) ▪ Upper abdominal pain (pain in the upper part of the belly) ▪ Gastro-esophogeal reflux disease (acid reflux) ▪ Pain in tongue
General Disorders	▪ Pyrexia (fever) ▪ Peripheral edema (swelling of the limbs) ▪ Rigors (chills and shivering) ▪ Edema NOS (swelling) ▪ Pain NOS ▪ Lethargy (tiredness)	▪ Falls ▪ Chest discomfort ▪ Intermittent pyrexia (fevers that come and go) ▪ Malaise (general feeling of unwellness) ▪ Crepitations (a crackling or rattling sound made in breathing) ▪ Catheter site redness (reddness around the site where a tube is entered into the body) ▪ Catheter site pain (pain around the site where a tube is entered into the tbody) ▪ Injection site swelling

Decitabine		
System Organ Class	**Adverse Events Reported more than 10 of 100 adult patients who received decitabine in a Phase 3 MDS trial**	**Adverse Events Reported in 5-10 of 100 adult patients who received decitabine in a Phase 3 MDS trial**
Infection	▪ Pneumonia NOS (condition with severe coughing, fever and chest pain that has no known cause) ▪ Cellulitis (infection of th skin) ▪ Candidal infection NOS (infection caused by a yeast called candida)	▪ Catheter related infection ▪ Urinary tract infection (infection of the part of the body that urine comes out of) ▪ Staphylococcal infection (infection caused by a bacteria called staphylococci) ▪ Oral candidiasis (infection of the mouth by a yeast called candida) ▪ Sinusitis (infection of the sinuses) ▪ Bacteremia (infection in the blood)

Decitabine		
System Organ Class	**Adverse Events Reported more than 10 of 100 adult patients who received decitabine in a Phase 3 MDS trial**	**Adverse Events Reported in 5-10 of 100 adult patients who received decitabine in a Phase 3 MDS trial**
Metabolic/Laboratory	▪ Hyperbilirubinemia (a build up of a substance called bilirubin in the blood) ▪ Elevated alkaline phophatase (abnormally high levels in a liver test of a protein called alkaline phosphatase) ▪ Elevated AST (abnormally high levels in a liver test of a protein called aspartate aminotransaminase) ▪ Elevated BUN (High levels in a kidney test of a molecule ▪ Hyperglycemia (high levels of sugar in the blood) ▪ Hypoalbuminemia (low levels of a protein called albumin in the blood) ▪ Hypomagnesemia (low levels of an ion called magnesium in the blood) ▪ Hypokalemia (low levels of an ion called potassium in the blood) ▪ Hyponatremia (low levels of an ion called sodium in the blood) ▪ Hyperkalemia (high levels of an ion called potassium in the blood) ▪	▪ Dehydration (sickness resulting from not have enough fluids) ▪ Lactate dehydrogenase increased (high levels of a molecule called lactate dehydrogenase in the blood) ▪ Increased blood bicarbonate ▪ Decreased blood bicarobonate ▪ Decreased blood chloride ▪ Blood protein decreased ▪ Blood bilirubin decreased
Musculoskeletal	▪ Arthralgia (pain in the joins) ▪ Limb pain (pain in the arms or legs) ▪ Back pain	▪ Chest wall pain ▪ Musculoskeletal discomfort (pain in the muscles or bones) ▪ Muscle pain
Neurological	▪ Headache ▪ Dizziness ▪ Hypoesthesia (reduced sense of touch) ▪ Insomnia (difficulty sleeping) ▪ Confusional state ▪ Anxiety	

Decitabine		
System Organ Class	**Adverse Events Reported more than 10 of 100 adult patients who received decitabine in a Phase 3 MDS trial**	**Adverse Events Reported in 5-10 of 100 adult patients who received decitabine in a Phase 3 MDS trial**
Pulmonary	▪ Cough ▪ Pharyngitis (infection in the throat) ▪ Crackles lung (lungs with a build up of fluid in them) ▪ Breath sounds decreased ▪ Hypoxia (low levels of oxygen reaching the body)	▪ Rales (abnormal rattling sound when listening to unhealthy lungs with a stethoscope) ▪ Postnasal drip (when fluid from the sinuses drain into the throat)
Renal		▪ Dysuria (pain when urinating) ▪ Urinary frequency (urinating frequently
Skin	▪ Ecchymosis (discoloration of the skin caused by bleeding underneath, for example by bruising) ▪ Rash ▪ Erythema (reddness of the skin) ▪ Skin lesions NOS (general wounds of the skin) ▪ Pruritus (itching) ▪ Petechiae (small points of discoloration of the skin caused by bleeding underneath) ▪ Pallor (paleness)	▪ Alopecia (Baldness) ▪ Urticaria (a type of skin rash) ▪ Facial swelling (enlargening of the face caused by fluid)
Cardiac	▪ Cardiac murmur NOS (sound the heart makes when the valves inside of it generally don't work properly)	▪ Hypotension (low blood pressure) ▪ Hematoma (bruise)

Vorinostat	
Adverse Events Reported in more than 10 of 100 adult patients With Cutaneous T-cell Lymphoma Who Were Receiving Vorinostat (Regardless of Causality)	
▪ Fatigue (tiredness) ▪ Diarrhea (loose stools) ▪ Nausea (feeling of wanthing to throw up) ▪ Dysguesia (change in sense of taste) ▪ Thrombocytopenia (low number of cells caused platelets resulting in blood clotting problems) ▪ Anorexia (disease of an abnormally low appetite that often causes extreme weight loss) ▪ Weight decrease ▪ Muscle spasms (when muscles contract uncontrollably) ▪ Alopecia (baldness) ▪ Dry mouth ▪ Blood creatinine increased (test of the kidneys where there is a high level of a protein called creatinine in the blood) ▪ Chills (shivering)	▪ Vomiting ▪ Constipation (difficulty passing stools) ▪ Dizziness ▪ Anemia (Low number of red blood cells that causes tiredness) ▪ Decreased appetite (less desire to eat) ▪ Peripheral edema (swelling of the arms or legs) ▪ Headache ▪ Pruritus (itchiness) ▪ Cough ▪ Upper respiratory infection (infection of the nose, throat and upper parts of the lungs) ▪ Pyrexia (fever)
Adverse Events Reported in 5-10 of 100 adult patients With Cutaneous T-cell Lymphoma Who Were Receiving Vorinostat (Regardless of Causality)	
▪ Pulmonary embolism (clot in the lungs)	▪ Hyperglycemia (high levels of sugar in the blood)
Rare Adverse Events Reported in Other Patients Who Were Receiving Vorinostat (Regardless of Causality)	
▪ Deep vein thrombosis (clot in the deep blood vessels of the leg) ▪ Abnormal electrolytes (including blood creatinine, magnesium, potassium and calcium) (abnormal levels of ions in the blood) ▪ Ischemic stroke (blocking of blood vessels in the brain causing a lower level of oxygen reaching the brain) ▪ Pelviureteric obstruction (when part of the kidney is blocked) ▪ Spinal cord injury (damage to part of the spine) ▪ Syncope (temporary loss of consciousness caused by a fall in blood pressure) ▪ Ureteric obstruction (blockage of tube that carries urine from the kidney to the bladder)	▪ Squamous cell carcinoma (cancer of the skin) ▪ Cholecystitis (inflammation of the gallbladder) ▪ Death ▪ Blood stream infections (including specifically, for example: enterococcal, streptococcal) ▪ Exfoliative dermatitis (redness and peeling of the skin in large parts of the body) ▪ Gastrointestinal hemorrhage (bleeding within the belly) ▪ Lobar pneumonia (severe infection of a section of the lung often causing fever and cough) ▪ Myocardial infarction (heart attack) ▪ T-cell lymphoma (a type of cancer of the immune system) ▪ Prolonged QT interval (heart rhythm disorder)

Fludarabine	
Frequency of Adverse Event:	**Common: Happens to 21-100 children out of every 100**
Timing of AE Onset in Relation to Drug Administration:	**Adverse Events**
Immediate: Within 1-2 days of receiving drug	▪ Fever ▪ Fatigue (tiredness) ▪ weakness ▪ pain ▪ nausea (Feeling of wanting to vomit) ▪ vomiting ▪ anorexia (disease of severely decreased appetite often causing a large amount of weight loss) ▪ cough ▪ dyspnea (difficulty breathing)
Prompt: Within 2-3 weeks, prior to the next course	▪ Myelosuppression including: o anemia (decrease in red blood cells) o Neutropenia (decrease in cells of the immune system called neutrophils) o thrombocytopenia (decrease in cells called platelets in the blood which causes problems in clotting) ▪ Infection including: o urinary tract infection (infection causing pain when peeing) o herpes simplex infection (infection caused by a virus called herpes simplex virus) o pneumonia (severe infection of the chest causing fever and coughing) o upper respiratory infection (infection of the nose, airways and upper parts of the lungs)
Delayed: Any time later during therapy, excluding the above conditions	
Late: Any time after completion of treatment	
Frequency of Adverse Event:	**Occasional: Happens to 5-20 children out of every 100**
Timing of AE Onset in Relation to Drug Administration:	**Adverse Events**
Immediate: Within 1-2 days of receiving drug	▪ Edema including peripheral edema (swelling including swelling in the limbs)

	▪ Chills (shivering) ▪ rash ▪ diarrhea (loose stools) ▪ rhinitis (stuffy and runny nose) ▪ diaphoresis (heavy sweating) ▪ malaise (general feeling of being unwell) ▪ abdominal pain (pain in the belly) ▪ headache ▪ back pain ▪ myalgia (muscle pains) ▪ stomatitis (inflamed and sore mouth) ▪ flu-like syndrome
Prompt: Within 2-3 weeks, prior to the next course	▪ Weight loss, ▪ gastrointestinal bleeding (bleeding in the belly) ▪ hemoptysis (coughing with blood) ▪ paresthesia (burning or prickling sensation in the skin) ▪ allergic pneumonitis (inflammation within the lungs due to irritation from a substance breathed in) ▪ bronchitis (inflammation of air passages) ▪ pharyngitis (inflammation of the throat) ▪ visual disturbance (problems in seeing) ▪ hearing loss ▪ hyperglycemia (high levels of sugar in the blood)
Delayed: Any time later during therapy, excluding the above conditions	
Late: Any time after completion of treatment	
Frequency of Adverse Event:	**Rare: Happens to < 5 children out of every 100**
Timing of AE Onset in Relation to Drug Administration:	**Adverse Events**
Immediate: Within 1-2 days of receiving drug	▪ Anaphylaxis (severe allergic reaction) ▪ tumor lysis syndrome (disease caused by a large amount of tumor cells being killed off at the same time) ▪ dehydration (lack of fluid in the body)
Prompt: Within 2-3 weeks, prior to the next course	▪ Sinusitis (inflammation of the sinus) ▪ dysuria (painful or difficulty urinating) ▪ opportunistic infections and reactivation of latent viral infections like (infections caused by weakened immune system) o Epstein-Barr virus (EBV) o herpes zoster o John Cunningham (JC) virus (progressive multifocal leukoencephalopathy [PML]) ▪ EBV associated lymphoproliferative disorder (large production of a cell in the immune system called lymphocytes due to an infection

	with a virus called Epstein Barr virus) ▪ panycytopenia (can be prolonged) (low levels of red blood cells, white blood cells and platelets) ▪ pulmonary hypersensitivity o dyspnea (difficulty breathing) o cough o hypoxia (low levels of oxygen in the body) o interstitial pulmonary infiltrate (build up of fluid in the walls around the air sacs in the lungs) ▪ pulmonary toxicity (acute respiratory distress syndrome [ARDS] ▪ pulmonary fibrosis (scarring within the lungs) ▪ pulmonary hemorrhage (bleeding within the lungs) ▪ respiratory distress ▪ respiratory failure (failure of respiratory system) ▪ pericardial effusion (fluid build up in the space around the heart) ▪ skin toxicity including: o erythema multiforme (a severe allergic reaction associated with certain infections and medications) o Stevens-Johnson syndrome (severe skin reaction associated with fever and flu like symptoms) o toxic epidermal necrolysis (widespread redness and peeling off of the skin) o pemphigus (skin disease with watery blisters) ▪ liver failure ▪ renal failure ▪ hemorrhage (bleeding) ▪ transfusion-associated graft-versus-host disease following transfusion of non-irradiated blood products (Immune system attack of the cells that were transfused) ▪ phlebitis (inflammation of a vein) ▪ sleep disorder ▪ cerebellar syndrome (dysfunction of a part of the brain called the cerebellum) ▪ depression ▪ mentation impaired (memory lapses) ▪ alopecia (hair loss) ▪ pruritus (itchiness) ▪ seborrhea (chronic dandruff) ▪ esophagitis (inflammation of the tube between the mouth and stomach that may cause difficulty swallowing and chest pain) ▪ constipation ▪ mucositis ▪ dysphagia ▪ hesitancy ▪ cholelithiasis ▪ abnormal liver function tests ▪ osteoporosis ▪ arthralgia

	▪ abnormal renal function test ▪ proteinuria ▪ epistaxis ▪ hemorrhagic cystitis ▪ eosinophilia

Cytarabine Intravenous			
	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, anorexia (weight loss)	Flu-like symptoms with fever, rash	Ara-C syndrome (fever, myalgia, bone pain, occasionally chest pain, maculopapular rash, malaise, conjunctivitis), anaphylaxis (severe allergic reaction)
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (anemia, thrombocytopenia, leukopenia, megaloblastosis, reticulocytopenia)(low levels of red blood cells, platelets, white blodd cells, several large immature red blood cells, marrow failure) Stomatitis (inflammation of the mouth and lips) alopecia (hairloss)	Diarrhea (loose stools), hypokalemia (low levels of potassium in the blood), hypocalcemia (low levels of calcium in the blood) hyperuricemia (high levels of uric acid in the blood)	Hepatotoxicity (damage to the liver), sinusoidal obstruction syndrome (SOS, formerly VOD), urinary retention (inability to completely empty the bladder), renal dysfunction (kidney problems), pain and erythema (redness) of the palms and soles
Delayed: Any time later during therapy, excluding the above conditions			Asymptomatic nonoliguric rhabdomyolysis (kidney failure as a consequence of muscle breakdown)
Unknown Frequency and Timing:	Fetal toxicities (toxicities of a baby while mother is still pregnant) and teratogenic effects (birth defects) of cytarabine have been noted in humans. It is unknown whether the drug is excreted in breast milk.		

Cytarabine Intrathecal			
	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	▪ Nausea (feeling of wanting to vomit) ▪ vomiting ▪ fever ▪ headache	▪ Arachnoiditis (inflammation of tissues that surround the brain called the arachnoid)	▪ Rash ▪ Somnolence (sleepiness) ▪ meningismus (irritation of the tissue around the brain called meninges) ▪ convulsions (a sudden, violent irregular movement of a limb) ▪ paresis (muscular damage caused by weakness)
Prompt: Within 2-3 weeks, prior to the next course			▪ Myelosuppression (bone marrow activitiy is decreased which result in cells called red blood cells, white blood cells and platelets being decreased) ▪ Ataxia (the loss of full control of body movements)
Delayed: Any time later during therapy, excluding the above conditions			▪ Necrotizing leukoencephalopathy (changes in the brain that result from chemotherapy) ▪ Paraplegia (paralysis of the legs) ▪ blindness (in combination with XRT & systemic therapy)

Cytarabine, Methotrexate and Hydrocortisone Intrathecal			
	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
	▪ Nausea ▪ Vomiting ▪ Fever ▪ Headache	▪ Swelling of the brain which may cause stiff neck, sensitivity to light,	▪ Rash ▪ Bleeding in the brain ▪ Paralysis, weakness ▪ Dizziness ▪ Damage to the brain which may cause changes in thinking, blindness

Cytarabine, Methotrexate and Hydrocortisone Intrathecal			
	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
		headache, vomiting ▪ Major change in thinking patterns ▪ Difficulty learning ▪ Confusion ▪ Tiredness ▪ Seizure	▪ Infection ▪ 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 ▪ Allow number of platelets causes you to bruise and bleed more easily

Filgrastim (G-CSF, Neupogen)			
	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug		▪ Local irritation at the injection site, ▪ headache	▪ Allergic reactions (more common with IV administration than subq): ▪ skin o rash o urticaria (hives) o facial edema (facial swelling) ▪ respiratory o wheezing o dyspnea (difficulty breathing) ▪ cardiovascular o hypotension (low blood pressure) o Tachycardia (rapid heart rate) ▪ low grade fever

Filgrastim (G-CSF, Neupogen)			
	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Prompt: Within 2-3 weeks, prior to the next course	Mild to moderate medullary bone pain (pain inside the bone)	▪ Increased: alkaline phosphatase, lactate dehydrogenase and uric acid, (increased levels of molecules called alkaline phosphatase, lactate dedhyrogenase and uric acid in the blood) ▪ Thrombocytopenia (low levels of cells called platelets that causes problems with clotting)	▪ Splenomegaly (enlargement of the spleen) ▪ splenic rupture (major injury to the spleen) ▪ exacerbation of pre-existing skin rashes (Worsening of skin rashes that were already present) ▪ sickle cell crises in patients with SCD (severe pain in patients with a disease called sickle cell disease) ▪ excessive leukocytosis (high levels of white blood cells in the blood)
Delayed: Any time later during therapy, excluding the above conditions			▪ Cutaneous vasculitis (inflamed blood vessels of the skin) ▪ ARDS (fluid build up of the tiny, elastic air cells of the lungs)
Late: Any time after completion of treatment			▪ MDS or AML (confined to patients with severe chronic neutropenia and long term administration) (types of cancer that develop when patients have either a low amount of cells in the blood called neutrophils or are given anti-cancer medications for a long priod of time)

Filgrastim (G-CSF, Neupogen)			
	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Unknown Frequency and Timing: Fetal toxicities (toxicities in a baby while the mother is still pregnant) and teratogenic effects (effects that cause birth defects)of filgrastim in humans are unknown. Conflicting data exist in animal studies and filgrastim is known to pass the placental barrier. It is unknown whether the drug is excreted in breast milk.			

Fungal Risks

With high doses of chemotherapy and decitabine there has been some evidence of greater risks of fungal infections. You will be required to take medication to prevent these infections.

Reproductive risks

Because the drugs in this study can affect an unborn baby, you should not become pregnant or father a baby while on this study. You should not breast-feed a baby while on this study. It is a condition of this study that adequate birth control methods be used by all participants while enrolled in the study. Examples of these include total abstinence (no sex), oral contraceptives ("the pill"), an intrauterine device (IUD), Levonorgestrol implants (Norplant), or medroxyprogesterone acetate injections (Depo-provera shots). If one of these methods of birth control cannot be used, contraceptive foam with a condom is recommended. Ask your doctor about counseling and more information about preventing pregnancy.

If you become pregnant or father a child during this study, contact your doctor immediately to discuss the requirements for pregnancy outcome follow-up. Female subjects will be given instructions for discontinuation of study medication.

Confidential Information

There is the potential risk of accidental disclosure (release) of confidential information when you participate in research. Please see the "Will my medical information be kept private?" section of this document for more details.

Developing a Second Cancer

It is possible that you may develop a second form of cancer as a result of this treatment. Experience so far suggests that the chance of this happening is very small. Not enough information has been gathered in children to be able to give an accurate prediction, although it may be in the range of one in every 50 to 800 children treated.

Are there benefits to subjects taking part in the study?

Participation in this study may or may not benefit you. Participating in this study will not cure your relapsed leukemia. Based on experience with the drugs used in the treatment plan, researchers believe this therapy may cause your leukemia to stop growing or go into remission for a period of time. Your cancer may not have any response to the therapy received while participating in this study.

It is hoped that the information learned from this study may help future children or young adults with relapsed AML.

What other choices do I have if I do not take part in this study?

You do not have to participate in this study to receive treatment for recurrent leukemia. There is no "standard" therapy for recurrent leukemia. Most treatment plans have used drugs similar to those used in this protocol, although these drugs may be given in different combinations, and at different times. You can receive other combinations of chemotherapy without participating in this study.

As an alternative to this study, you may decide you don't want additional treatment for your relapsed leukemia. You will always receive medicines to help you feel more comfortable and deal with problems caused by your cancer or treatment whether you participate in this study or not.

Talk to your doctor about your choices before you decide if you will take part in this study.

Will my medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law.

Members of the research team and, if appropriate, your primary care physicians and nurses will know that you are a research subject. All results will be kept confidential, but may be made available to you, and/or your physician if you wish. Because this study involves the treatment of a medical condition, a copy of this consent form will be placed in your medical record. This will allow the doctors that are caring for you to obtain information about what medications or procedures you are receiving in the study and treat you appropriately. You may read your medical record. The records are available to those caring for you at this hospital.

Organizations that may inspect/or copy your research records for quality assurance and data analysis include:

- Therapeutic Advances in Childhood Leukemia Consortium (TACL)
- The United States Food and Drug Administration (FDA)
- Health Canada
- Therapeutic Goods Administration, Australia
- The Department of Health and Human Services (DHHS)
- The Institutional Review Board (IRB) of CHLA - Committee on Clinical Investigations

As a result, these organizations may see your name; but they are bound by rules of confidentiality not to reveal your identity to others. These organizations will also have access to study data in a form that does not mention your name in order to complete the research including processing, analyzing (studying), using, and storing data. Reasonable steps will be taken to protect your right to privacy. No information about you, or provided by you during the research, will be shared with others without your written permission, except as explained below:

- If necessary to protect your rights or welfare (for example, if you are injured and need emergency care); or

- If required by law (i.e., child abuse, reports of certain infectious diseases)

The information collected will be used to meet the purpose of this clinical study. In addition, this information may be used to support applications to market the studied drug in the United States and in other countries. It may also be used in reports of the study or for scientific publications and presentations that will not identify study participants by name.

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

What are the costs of taking part in this study?

The health care costs during your participation in this study that are considered part of the standard treatment of your disease will be billed to your insurance or other third-party payer. This includes blood tests, hospitalizations, procedures that will be done and medications.

You will not have to pay for the following tests that will be done for research purposes only.

- Tests for MRD
- Tests for how the DEC and VOR are affecting your leukemia cells

Your family is responsible for other costs which may result from your participation in the study, such as, but not limited to, time off of work, car fare, baby sitter fees, food purchased while at the hospital, etc. You will not receive any type of payment for participating in this study. Taking part in this study may lead to added costs to your insurance company. Please ask about any expected added costs or insurance problems.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at *http://cancer.gov/clinicaltrials/understanding/insurance-coverage* . You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

What happens if I am injured because I took part in this study?

It is important that you tell your study doctor, _____ *[investigator's name(s)],* if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ *[telephone number].*

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

What are my rights if I take part in this study?

Taking part in this study is voluntary. You may choose not to participate in this study. If you decide not to participate, you will not be penalized and you will still receive the standard treatment.

If you choose to participate, you may discontinue your participation in the study at any time. If you discontinue participation in the study, physicians and hospital personnel will still take care of you.

You also have the right to know about new information that may affect your health, welfare, or your willingness to participate in the study. You will be provided with this information as soon as it becomes available.

Whether you participate or not, you will continue to get the best medical care this hospital can provide.

Who can answer my questions about the study?

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor _____ *[name(s)]* at _____ *[telephone number].*

For questions about your rights while taking part in this study, call the _____ *[*name of center*] Institutional Review Board (a group of people who review the research to protect your rights) at* _____ *[*telephone number*].* *[*Note to Local Investigator: Contact information for patient representatives or other individuals in a local institution who are not on the IRB or research team but take calls regarding clinical trial questions can be listed here.*]*

Where can I get more information?

Call the National Cancer Institute's Cancer Information Service:
1-800-4-CANCER (1-800-422-6237) OR 1-800-332-8615 (for the hearing impaired)

- You will be given a copy of this consent form.
- You will be given a copy of this treatment plan upon request.
- CancerNet™: http:/cancernet.nci.nih.gov This site provides accurate cancer information including the Physicians Data Query (PDQ). The PDQ is the National Cancer Institute's comprehensive cancer database. It contains peer-reviewed summaries on cancer treatment, screening, prevention, and supportive care; a registry of about 1,700 open and 10,300 closed cancer clinical trials from around the world; and directories of physicians, genetic counselors, and organizations that provide cancer care.
- Visit the TACL Consortium Website at https://tacl.chla.usc.edu

SIGNATURE OF RESEARCH SUBJECT

Your signature below indicates:

- You have read this document and understand its meaning;
- You have had a chance to ask questions and have had these questions answered to your satisfaction;
- You agree to participate in this research study; and
- You will be given a copy of the signed permission form.

_____
Name of Subject

_____ _____
Signature of Subject Date

SIGNATURE OF PARENT(S)/GUARDIAN (if the subject is a minor)

Your signature below indicates that you have read this document; understand its meaning; have had a chance to ask questions; have had these questions answered to your satisfaction; and agree to your child's participation in this research study. You have been given a signed copy of this assent/permission form.

_____ _____
Name(s) of Parent(s)/Guardian Name(s) of Parent(s)/Guardian

_____ _____
Signature of Parent (Guardian) Date

_____ _____
Signature of Parent (Guardian) Date

SIGNATURE OF INVESTIGATOR

I have explained the research to the subject and/or the subject's parent(s)/guardian(s) and have answered all of their questions. I believe that they understand the information described in this document and freely give consent/permission/assent to participate.

_____
Name of Investigator/Person obtaining consent

_____ _____
Signature of Investigator/Person obtaining consent Date

SIGNATURE OF WITNESS (if applicable)

My signature as witness certified that the subject and/or the subject's parent(s)/guardian(s) signed this permission form in my presence as their voluntary act and deed.

_____
Name of Witness

_____ _____
Signature of Witness Date

APPENDIX I: DISEASE CLASSIFICATION

WHO CLASSIFICATION OF ACUTE MYELOID LEUKEMIA [Blood, October 1, 2002 vol 100(7)]

Acute myeloid leukemia with recurrent genetic abnormalities

Acute myeloid leukemia with t(8;21)(q22;q22), AML1/ETO)
Acute myeloid leukemia with abnormal bone marrow eosinophils and inv(16)(p13q22) t(16;16)(p13;q22), (CBFβ/MYH11)
Acute promyelocytic leukemia with t(15;17)(q22;q12), (PML/RARα) and variants
Acute myeloid leukemia with 11q23 (MLL) abnormalities

Acute myeloid leukemia with multilineage dysplasia

Following MDS or MDS/MPD
Without antecedent MDS or MDS/MPD, but with dysplasia in at least 50% of cells in 2 or more myeloid lineages

Acute myeloid leukemia and myelodysplastic syndromes, therapy related

Alkylating agent/radiation-related type
Topoisomerase II inhibitor-related type (some may be lymphoid)
Others

Acute myeloid leukemia, not otherwise categorized

Acute myeloid leukemia, minimally differentiated
Acute myeloid leukemia without maturation
Acute myeloid leukemia with maturation
Acute myelomonocytic leukemia
Acute monoblastic/acute monocytic leukemia
Acute erythroid leukemia (erythroid/myeloid and pure erythroleukemia)
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma

APPENDIX II: PERFORMANCE STATUS SCALES/SCORES

Performance Status Criteria Karnofsky and Lansky performance scores are intended to be multiples of 10					
ECOG (Zubrod)		**Karnofsky**		**Lansky**	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
		90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

APPENDIX III: ORAL SUSPENSION RECIPE

*****Use compounding equipment designated for hazardous drugs & wear gloves and mask while preparing*****

Product Description	Recipe		References
Name: Vorinostat **Strength:** 50 mg/ml **Dosage Form:** Suspension **Route:** PO **Stability:** expires 14 days after preparation **Packaging:** Package in tight light-resistant ***GLASS*** bottles **Storage:** Room Temperature	Items Needed #20 Vorinostat 100mg capsules 20ml Ora-Plus 20ml Ora-Sweet	Preparation Instructions 1.Place 20ml of Ora-Plus (Paddock Labs brand) in glass bottle 2.Place contents of 20 vorinostat 100mg capsules in same bottle and shake to disperse. Note shaking could take upwards of 3 minutes. 3.Add 20ml of Ora-Sweet (Paddock Labs brand) and shake to disperse.	**REF:** unpublished information from CTEP pediatric clinical trials provided by Merck. **Ref: Reviewed Date:** 04/2011 **Date Updated:** 04/2009

Please use the Chemo Hood

Date	CMH		Total Qty Prepared	Manufacturer						Initals		
	Control Number	Exp Date		Name	Manufacturer	NDC	Lot Number	Exp Date	Qty	Prep	Check	Final Check
				Vorinostat 100mg capsules								
				Ora-Plus								
				Ora-Sweet								
				Vorinostat 100mg capsules								
				Ora-Plus								
				Ora-Sweet								
				Vorinostat 100mg capsules								
				Ora-Plus								
				Ora-Sweet								
				Vorinostat 100mg capsules								
				Ora-Plus								

DO NOT PHOTOCOPY THIS FORM

					Ora-Sweet								

APPENDIX IV: DOSING SCHEDULE

Vorinostat is only available in 100 mg capsules. Vorinostat is given for 5 days. The dose of vorinostat is 180 mg/m^2/day on days1 through 5. For patients whose body surface area falls between those noted on the table, round up or down to the closest BSA value on the table and treat patient at that level. If your patient's BSA is less than 0.35 m^2 or above 2 m^2, call the TACL Operations Center (323) 361-5429 for the correct dose.

Vorinostat Dose 180 mg/m^2/day Given days 1 to 5				
BSA (m^2)	**Calculated daily dose**	**Calculated dose Over 5 days**	**Actual dose Over 5 days**	**Daily Dosing Schedule**
0.35	63 mg	315 mg	300 mg	1 capsule given on days 1, 2, 3, no vorinostat on days 4 and 5
0.4	72 mg	360 mg	400 mg	1 capsule given on days 1, 2, 3, 4, no vorinostat on day 5
0.45	81 mg	405 mg	400 mg	1 capsule given on days 1, 2, 3, 4, no vorinostat on day 5
0.5	90 mg	450 mg	500 mg	1 capsule given on days 1 through 5
0.55	99 mg	495 mg	500 mg	1 capsule given on days 1 through 5
0.6	108 mg	540 mg	500 mg	1 capsule given on days 1 through 5
0.65	117 mg	585 mg	600 mg	1 capsule given on days 1 through 4, (2) capsules given on day 5
0.7	126 mg	630 mg	600 mg	1 capsule given on days 1 through 4, (2) capsules given on day 5
0.75	135 mg	675 mg	700 mg	1 capsule given on days 1 through 3, (2) capsules given on days 4, 5
0.8	144 mg	720 mg	700 mg	1 capsule given on days 1 through 3, (2) capsules given on days 4, 5

Vorinostat Dose 180 mg/m^2/day Given days 1 to 5				
BSA (m^2)	**Calculated daily dose**	**Calculated dose Over 5 days**	**Actual dose Over 5 days**	**Daily Dosing Schedule**
0.85	153 mg	765 mg	800 mg	(2) capsules given on days 1 through 4
0.9	162 mg	810 mg	800 mg	(2) capsules given on days 1 through 4
0.95	171 mg	855 mg	900 mg	1 capsule given on days 1, (2) capsules given on days 2 through 5 **
1	180 mg	900 mg	900 mg	1 capsule given on days 1, (2) capsules given on days 2 through 4 **
1.05	189 mg	945 mg	900 mg	1 capsule given on days 1, (2) capsules given on days 2 through 4 **
1.1	198 mg	990 mg	1000 mg	(2) capsules given on days 1 through 5
1.15	207 mg	1035 mg	1000 mg	(2) capsules given on days 1 through 5
1.2	216 mg	1080 mg	1100 mg	(2) capsules given on days 1 through 4, (3) capsules given on day 5
1.25	225 mg	1125 mg	1100 mg	(2) capsules given on days 1 through 4, (3) capsules given on day 5
1.3	234 mg	1170 mg	1200 mg	(2) capsules given on days 1 through 3, (3) capsules given on days 4 and 5
1.35	243 mg	1215 mg	1200 mg	(2) capsules given on days 1 through 3, (3) capsules given on days 4 and 5
1.4	252 mg	1260 mg	1300 mg	(2) capsules given on days 1 and 2, (3) capsules given on days 3 through 5
1.45	261 mg	1305 mg	1300 mg	(2) capsules given on days 1 and 2, (3) capsules given on days 3 through 5
1.5	270 mg	1350 mg	1400 mg	(2) capsules given on day 1 (3) capsules given on days 2 through 5
1.55	279 mg	1395 mg	1400 mg	(2) capsules given on day 1 (3) capsules given on days 2 through 5
1.6	288 mg	1440 mg	1400 mg	(2) capsules given on day 1 (3) capsules given on days 2 through 5
1.65	297 mg	1485 mg	1500 mg	(3) capsules given on days 1 through 5
1.7	306 mg	1530 mg	1500 mg	(3) capsules given on days 1 through 5
1.75	315 mg	1575 mg	1600 mg	(3) capsules given on days 1 through 4, (4) capsules given on day 5
1.8	324 mg	1620 mg	1600 mg	(3) capsules given on days 1 through 4, (4) capsules given on day 5

Vorinostat Dose 180 mg/m^2/day Given days 1 to 5				
BSA (m^2)	**Calculated daily dose**	**Calculated dose Over 5 days**	**Actual dose Over 5 days**	**Daily Dosing Schedule**
1.85	333 mg	1665 mg	1700 mg	(3) capsules given on days 1 through 3, (4) capsules given on days 4 and 5
1.9	342 mg	1710 mg	1700 mg	(3) capsules given on days 1 through 3, (4) capsules given on days 4 and 5
2	360 mg	1800 mg	1800 mg	(2) capsules given on day 1, (4) capsules given on days 2 through 5

15.0 REFERENCES

1. Abrahamsson J, Clausen N, Gustafsson G, et al. Improved outcome after relapse in children with acute myeloid leukaemia. *Br J Haematol.* 2007;136(2):229-236.
2. Aladjidi N, Auvrignon A, Leblanc T, et al. Outcome in children with relapsed acute myeloid leukemia after initial treatment with the French Leucemie Aique Myeloide Enfant (LAME) 89/91 protocol of the French Society of Pediatric Hematology and Immunology. *J Clin Oncol.* 2003;21(23):4377-4385.
3. Gorman MF, Ji L, Ko RH, et al. Outcome for children treated for relapsed or refractory acute myelogenous leukemia (rAML): a Therapeutic Advances in Childhood Leukemia (TACL) Consortium study. *Pediatr Blood Cancer.* 2010;55(3):421-429.
4. Rubnitz JE, Razzouk BI, Lensing S, Pounds S, Pui CH, Ribeiro RC. Prognostic factors and outcome of recurrence in childhood acute myeloid leukemia. *Cancer.* 2007;109(1):157-163.
5. Webb DK, Wheatley K, Harrison G, Stevens RF, Hann IM. Outcome for children with relapsed acute myeloid leukaemia following initial therapy in the Medical Research Council (MRC) AML 10 trial. MRC Childhood Leukaemia Working Party. *Leukemia.* 1999;13(1):25-31.
6. Wells RJ, Adams MT, Alonzo TA, et al. Mitoxantrone and cytarabine induction, high-dose cytarabine, and etoposide intensification for pediatric patients with relapsed or refractory acute myeloid leukemia: Children's Cancer Group Study 2951. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2003;21(15):2940-2947.
7. Breems DA, Van Putten WL, Huijgens PC, et al. Prognostic index for adult patients with acute myeloid leukemia in first relapse. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2005;23(9):1969-1978.
8. Esteller M. Profiling aberrant DNA methylation in hematologic neoplasms: a view from the tip of the iceberg. *Clinical immunology.* 2003;109(1):80-88.

9. Issa JP, Gharibyan V, Cortes J, et al. Phase II study of low-dose decitabine in patients with chronic myelogenous leukemia resistant to imatinib mesylate. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2005;23(17):3948-3956.
10. Qin T, Youssef EM, Jelinek J, et al. Effect of cytarabine and decitabine in combination in human leukemic cell lines. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2007;13(14):4225-4232.
11. Yang H, Hoshino K, Sanchez-Gonzalez B, Kantarjian H, Garcia-Manero G. Antileukemia activity of the combination of 5-aza-2'-deoxycytidine with valproic acid. *Leukemia research.* 2005;29(7):739-748.
12. Issa JP, Garcia-Manero G, Giles FJ, et al. Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies. *Blood.* 2004;103(5):1635-1640.
13. Blum W, Garzon R, Klisovic RB, et al. Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine. *Proceedings of the National Academy of Sciences of the United States of America.* 2010;107(16):7473-7478.
14. Ritchie EK, Feldman EJ, Christos PJ, et al. Decitabine in patients with newly diagnosed and relapsed acute myeloid leukemia. *Leukemia & lymphoma.* 2013;54(9):2003-2007.
15. Nieto M, Demolis P, Behanzin E, et al. The European Medicines Agency Review of Decitabine (Dacogen) for the Treatment of Adult Patients With Acute Myeloid Leukemia: Summary of the Scientific Assessment of the Committee for Medicinal Products for Human Use. *The oncologist.* 2016.
16. Blum W, Klisovic RB, Hackanson B, et al. Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2007;25(25):3884-3891.
17. Scandura JM, Roboz GJ, Moh M, et al. Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML. *Blood.* 2011;118(6):1472-1480.
18. Sun Y, Xu Y, Wu D, et al. [Outcomes of refractory or relapsed DNMT3A + cytogenetically normal acute myeloid leukemia patients followed the therapy including decitabine combined with CAG or CAG-like regimen]. *Zhonghua Xue Ye Xue Za Zhi.* 2015;36(12):1025-1030.
19. Ye XN, Zhou XP, Wei JY, et al. Epigenetic priming with decitabine followed by low-dose idarubicin/cytarabine has an increased anti-leukemic effect compared to traditional chemotherapy in high-risk myeloid neoplasms. *Leukemia & lymphoma.* 2016;57(6):1311-1318.
20. Gillenwater AM, Zhong M, Lotan R. Histone deacetylase inhibitor suberoylanilide hydroxamic acid induces apoptosis through both mitochondrial and Fas (Cd95) signaling in head and neck squamous carcinoma cells. *Molecular cancer therapeutics.* 2007;6(11):2967-2975.
21. Garcia-Manero G, Yang H, Bueso-Ramos C, et al. Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [SAHA]) in patients with advanced leukemias and myelodysplastic syndromes. *Blood.* 2008;111(3):1060-1066.

22. Kadia TM, Yang H, Ferrajoli A, et al. A phase I study of vorinostat in combination with idarubicin in relapsed or refractory leukaemia. *British journal of haematology.* 2010;150(1):72-82.
23. Garcia-Manero G, Tambaro FP, Bekele NB, et al. Phase II trial of vorinostat with idarubicin and cytarabine for patients with newly diagnosed acute myelogenous leukemia or myelodysplastic syndrome. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2012;30(18):2204-2210.
24. Desmond JC, Raynaud S, Tung E, Hofmann WK, Haferlach T, Koeffler HP. Discovery of epigenetically silenced genes in acute myeloid leukemias. *Leukemia.* 2007;21(5):1026-1034.
25. Venturelli S, Armeanu S, Pathil A, et al. Epigenetic combination therapy as a tumor-selective treatment approach for hepatocellular carcinoma. *Cancer.* 2007;109(10):2132-2141.
26. Yee KWL, Minden MD, Brandwein J, et al. A Phase I Trial of Two Sequence-Specific Schedules of Decitabine and Vorinostat in Patients with Acute Myeloid Leukemia (AML). *ASH Annual Meeting Abstracts.* 2007;110(11):908-.
27. Ravandi F, Faderl S, Thomas D, et al. Phase I Study of Suberoylanilide Hydroxamic Acid (SAHA) and Decitabine in Patients with Relapsed, Refractory or Poor Prognosis Leukemia. *ASH Annual Meeting Abstracts.* 2007;110(11):897-.
28. How J, Minden MD, Brian L, et al. A phase I trial of two sequence-specific schedules of decitabine and vorinostat in patients with acute myeloid leukemia. *Leukemia & lymphoma.* 2015;56(10):2793-2802.
29. Burke MJ, Brown P, Gore L, et al. *Invasive Candida Infections in Pediatric Patients Treated on the Pilot Study of Decitabine and Vorinostat with Chemotherapy for Relapsed ALL: A Report from the Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) Consortium.* Vol 1242014.
30. Ko RH, Ji L, Barnette P, et al. Outcome of patients treated for relapsed or refractory acute lymphoblastic leukemia: a Therapeutic Advances in Childhood Leukemia Consortium study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2010;28(4):648-654.
31. Melillo L, Valente D, D'Arena G, et al. Combination treatment of flag with non-pegylated liposomal doxorubicin (MYOCET(TM)) in elderly patients with acute myeloid leukemia: a single center experience. *International journal of immunopathology and pharmacology.* 2011;24(3):703-709.
32. Fouladi M, Park JR, Stewart CF, et al. Pediatric phase I trial and pharmacokinetic study of vorinostat: a Children's Oncology Group phase I consortium report. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2010;28(22):3623-3629.
33. Le Tourneau C, Lee JJ, Siu LL. Dose Escalation Methods in Phase I Cancer Clinical Trials. *J Natl Cancer Inst.* 2009;101(10):708-720.