CHILDREN'S ONCOLOGY GROUP

The world's childhood cancer experts

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A National Cancer Institute-supported member group of the National Clinical Trials Network

September 25, 2019

Martha Kruhm, MS, RAC
Head, Protocol and Information Office
Operations and Informatics Branch
Cancer Therapy Evaluation Program
Division of Cancer Treatment and Diagnosis
National Cancer Institute
Executive Plaza North Room 730
Bethesda, MD 20892

Re.: **AALL15P1**, *A Groupwide Pilot Study to Test the Tolerability and Biologic Activity of the Addition of Azacitidine (IND# 133688, NSC# 102816) to Chemotherapy in Infants with Acute Lymphoblastic Leukemia (ALL) and MLL Gene Rearrangement* –**Amendment #4**

Dear Ms. Kruhm,

Enclosed please find **Amendment #4** to the protocol and informed consent document for the study **AALL15P1**. This amendment is being submitted in response to a Request for Rapid Amendment (RRA) from Dr. Richard Piekarz, Dr. Jeffrey Moscow, and Dr. Meg Mooney dated September 6, 2019. In this amendment, the revised CAEPR for azacitidine (Version 2.7, July 30, 2019) has been inserted into the protocol, and the associated risk information in the informed consent document has been revised.

Sincerely,

Rachel Vasquez, Protocol Coordinator

(on behalf of)

Erin M. Guest, M.D., AALL15P1 Study Chair
Patrick Brown, M.D., AALL15P1 Study vice Chair
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Peter Adamson, M.D., COG Group Chair

AALL15P1 SUMMARY OF CHANGES
SUMMARY OF CHANGES: PROTOCOL DOCUMENT

In accordance with the above discussion, the following specific revisions have been made to the Protocol.

Section	Page	Comments
Throughout	Throughout	Updated the version date.
Table of Contents	3-7	Updated page numbers.
Study Committee	9	Rachel Vasquez has replaced Arshi Reyaz as the Protocol Coordinator.
Section 6.0	95-100	• Updated date of monograph for azacitidine • Updated date of CAEPR for azacitidine • Added New Risk: ○ Also Reported on Azacitidine Trials But With Insufficient Evidence for Attribution: Ascites; Cardiac arrest; Chronic kidney disease; Delirium; Edema face; Fracture; Gastrointestinal disorders - Other (enteritis); Gastrointestinal pain; Hallucinations; Hemolysis; Hyperphosphatemia; Immune system disorders - Other (GVHD); Investigations - Other (thrombocytosis); Laryngeal hemorrhage; Metabolism and nutrition disorders - Other (gout exacerbation); Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (metastases to central nervous system); Stroke ○ • Increase in Risk Attribution: ○ Changed to Rare but Serious from Also Reported on Azacitidine Trials But With Insufficient Evidence for Attribution: Tumor lysis syndrome • Decrease in Risk Attribution: ○ Changed to Less Likely from Likely: Anorexia; White blood cell decreased ○ Changed to Also Reported on Azacitidine Trials But With Insufficient Evidence for Attribution from Less Likely: Depression; Dyspepsia; Hyperhidrosis; Malaise; Non-cardiac chest pain; Pain; Skin and subcutaneous tissue disorders - Other (skin lesion); Vascular disorders - Other (pallor) • Provided Further Clarification: ○ Conjunctivitis, Gastrointestinal disorders - Other (diverticulitis), Respiratory, thoracic and mediastinal disorders - Other (pneumonia legionella), and Skin and subcutaneous tissue disorders - Other (ecthyma gangrenosum) are now reported as parts of Infection. ○ Postnasal drip is now reported as Postnasal drip and Rhinorrhea. ○ Acute coronary syndrome *(CTCAE 4.0 language)* is now reported as part of Chest-pain cardiac. ○ Wolff-Parkinson-White syndrome *(CTCAE 4.0 language)* is now reported as Cardiac disorders - Other (Wolff-Parkinson-White syndrome). ○ General disorders and administration site conditions - Other (Sweet's Syndrome) is now reported as Skin and subcutaneous tissue disorders - Other (Sweet's Syndrome). ○ Investigations - Other (blood LDH increased) *(CTCAE 4.0 language)* is now reported as Blood lactate dehydrogenase increased. ○ Metabolism and nutrition disorders - Other (fluid overload) is now reported as generalized edema. ○ Musculoskeletal and connective tissue disorder - Other (muscle cramps) and Musculoskeletal and connective tissue disorder - Other (muscle spasms) are now reported as Muscle cramp. ○ Sinus pain, previously listed under the NERVOUS SYSTEM DISORDERS SOC *(CTCAE 4.0 language)*, is now reported under the RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS SOC. ○ Urinary tract pain is now reported as Dysuria. ○ Respiratory, thoracic and mediastinal disorders - Other (oropharyngeal pain) is now reported as Oropharyngeal pain.

		○ Visceral arterial ischemia, previously listed under the VASCULAR DISORDERS SOC *(CTCAE 4.0 language)*, is now reported under the GASTROINTESTINAL DISORDERS SOC. ○ General disorders and administration site conditions - Other (general weakness) is now reported as part of Fatigue.

AALL15P1 SUMMARY OF CHANGES
SUMMARY OF CHANGES: INFORMED CONSENT DOCUMENT

In accordance with the above discussion, the following specific revisions have been made to the Consent.

Section	Page	Comments
Throughout	Throughout	Updated the version date.
What side effects or risks can I expect from being in the study?	7	Made the following changes to the risk profile: • Decrease in Risk Attribution: ○ Changed to Occasional from Common: Loss of appetite ○ Changed to Also Reported on Azacitidine Trials But With Insufficient Evidence for Attribution from Occasional (i.e., removed from the Risk Profile): Heartburn; Depression; Increased sweating; Sores on the skin; Pale skin • Provided Further Clarification: ○ Swelling and redness of the eye (Occasional) is now reported under Infection, especially when white blood cell count is low (Common)

Activated: 03/27/17
Closed:
Version Date: 09/25/2019
Amendment #4

CHILDREN'S ONCOLOGY GROUP

AALL15P1

A Groupwide Pilot Study to Test the Tolerability and Biologic Activity of the Addition of Azacitidine (IND# 133688, NSC# 102816) to Chemotherapy in Infants with Acute Lymphoblastic Leukemia (ALL) and *KMT2A (MLL)* Gene Rearrangement

A COG Groupwide Pilot Study

NCI Supplied Agent: Azacitidine (IND# [redacted], NSC# 102816)
IND Sponsor for Azacitidine: DCTD, NCI

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CONTACT INFORMATION		
To submit site registration documents:	**For patient enrollments:**	**Submit study data**
Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal. Regulatory Submission Portal: (Sign in at www.ctsu.org, and select the Regulatory Submission sub-tab under the Regulatory tab.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 to receive further instruction and support. Contact the CTSU Regulatory Help Desk at 1-866-651-2878 for regulatory assistance.	Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN) which can be accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions at ctsucontact@westat.com.	Data collection for this study will be done exclusively through Medidata Rave. Please see the Data Submission Schedule in the CRF packet for further instructions.
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS.		
For clinical questions (i.e. patient eligibility or treatment-related) contact the Study PI of the Lead Protocol Organization.		
For non-clinical questions (i.e. unrelated to patient eligibility, treatment, or clinical data submission) contact the CTSU Help Desk by phone or e-mail: CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.		
The CTSU Website is located at https://www.ctsu.org.		

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NCI Supplied Agent: Azacitidine, NSC#102816
Other Agents: Cytarabine NSC#63878, Commercial
Cyclophosphamide NSC#26271, Commercial
Daunorubicin NSC# 82151, Commercial
Dexamethasone NSC#34521, Commercial
Hydrocortisone NSC# 10483, Commercial
Leucovorin calcium NSC# 3590, Commercial
Mercaptopurine, NSC#755, Commercial
Methotrexate, NSC#740, Commercial
Pegaspargase, NSC#624239, Commercial
Prednisone NSC # 10023, Commercial
Thioguanine NSC#752, Commercial
Vincristine, NSC#67574, Commercial
IND#: [redacted]
IND Sponsor for azacitidine: DCTD, NCI

SEE SECTION 14.0 FOR SPECIMEN SHIPPING ADDRESSES

ABSTRACT

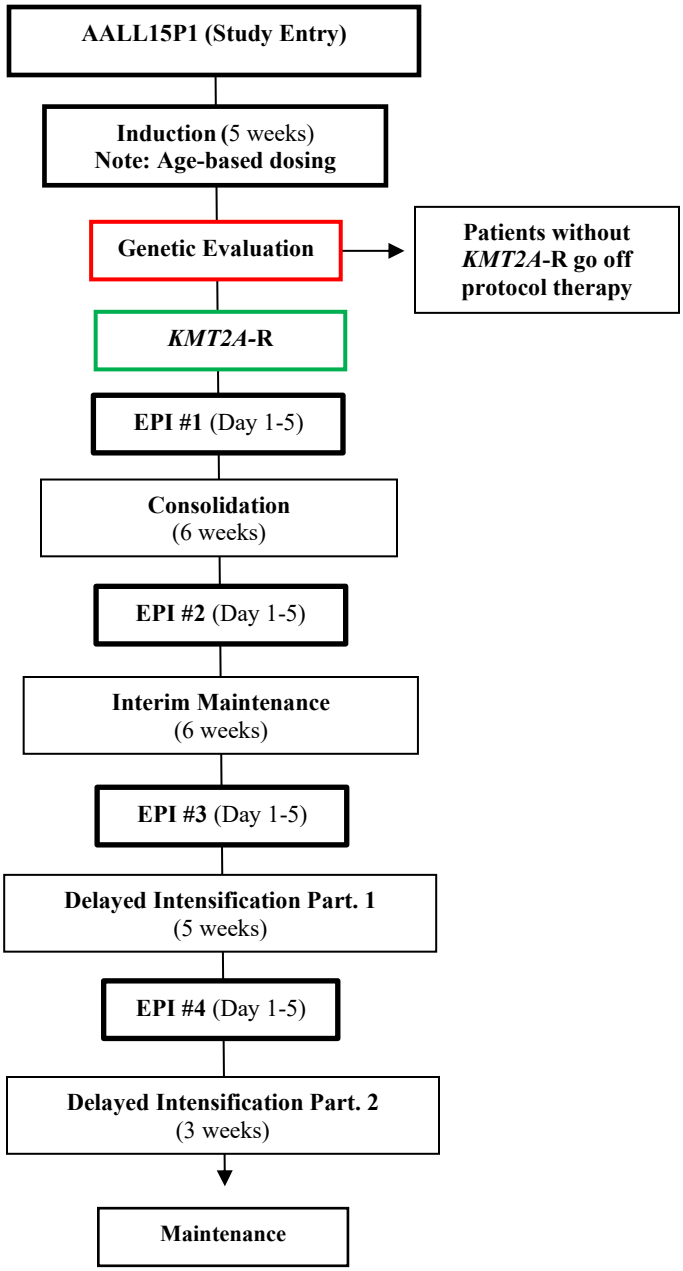
AALL15P1, a COG group-wide pilot study for infants less than 1 year of age with newly diagnosed B lymphoblastic leukemia (also termed B-precursor acute lymphoblastic leukemia) or acute leukemia of ambiguous lineage, will assess whether it is feasible to add azacitidine (VIDAZA®) to the Interfant-06 standard chemotherapy backbone. Though the rate of remission for infants with acute lymphoblastic leukemia (ALL) with *KMT2A* gene (previously referred to as the *MLL* gene) rearrangement (*KMT2A*-R) is high, the event-free survival (EFS) and overall survival (OS) for infants with *KMT2A*-R ALL remain poor. Epigenetic features of ALL cells with *KMT2A*-R, including DNA hypermethylation, may contribute to early chemotherapy resistance and relapse. DNA demethylating agents have been shown to reverse the methylation signature and improve the cytotoxicity of chemotherapy in infant ALL cells with *KMT2A*-R. Azacitidine is a DNA demethylating agent that has been used safely in combination with chemotherapy in pediatric patients 0-20 years of age with leukemia. The tolerability of azacitidine in combination with chemotherapy in infants with *KMT2A*-R ALL has not been previously tested.

Infants with newly diagnosed B lymphoblastic leukemia or acute leukemia of ambiguous lineage will be treated with standard Induction chemotherapy based on AALL0631 Induction, with pegaspargase. Following Induction, infants with *KMT2A*-R will be non-randomly assigned to receive four courses of azacitidine therapy, as epigenetic priming, prior to each major block of post-Induction chemotherapy. Infants with *KMT2A* germline (non-rearranged) leukemia will not receive azacitidine and will be removed from protocol therapy following remission assessment at the completion of Induction.

The post-Induction chemotherapy backbone for infants with *KMT2A*-R is based upon the Interfant-06 standard chemotherapy, which is considerably less intensive than prior COG protocols, but has been associated with essentially identical outcomes. This trial aims to determine whether azacitidine can be safely incorporated into the Interfant-06 chemotherapy backbone. If the combination is tolerable, then an international Phase 3 trial will test the efficacy of azacitidine in preventing relapse among infants with *KMT2A*-R ALL.

Amendment #1 extends the allowable time for Induction recovery to avoid unneccessary removal of infants from protocol therapy.

EXPERIMENTAL DESIGN SCHEMA



KMT2A-R: *KMT2A* gene- Rearrangement EPI: Azacitidine Epigenetic Therapy
Induction: Methotrexate, Predniso(lo)ne, Daunorubicin, Cytarabine, Dexamethasone, Vincristine, Pegaspargase, and Hydrocortisone
Consolidation: Cyclophosphamide, Mesna, Mercaptopurine, Cytarabine, Methotrexate, and Hydrocortisone.
Interim Maintenance: Mercaptopurine, High-dose Methotrexate, Leucovorin, Methotrexate/Hydrocortisone, High-dose Cytarabine, and Pegaspargase.
Delayed Intensification: Dexamethasone, 6-Thioguanine, Vincristine, Daunorubicin, Pegaspargase, Cyclophosphamide, Cytarabine, and Hydrocortisone
Maintenance: Mercaptopurine, Methotrexate, Hydrocortisone, and Cytarabine

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aims

1.1.1 To evaluate the tolerability of azacitidine in addition to Interfant-06 standard chemotherapy in infants with newly diagnosed ALL with *KMT2A* gene rearrangement (*KMT2A*-R).

1.2 Secondary Aims

1.2.1 To evaluate the biologic activity of azacitidine by pharmacodynamic assessment of global DNA methylation in peripheral blood mononuclear cells (PBMCs) of infants treated with azacitidine.

1.3 Exploratory Aims

1.3.1 To determine the 5 year event-free survival (EFS) of infants with *KMT2A*-R treated with azacitidine in addition to Interfant-06 standard chemotherapy.

1.3.2 To correlate minimal residual disease (MRD) with outcome in the context of the protocol therapy.

1.3.3 To perform pharmacokinetic (PK) testing of azacitidine in infants.

1.3.4 To test the expansion of infant T lymphocytes by stimulation with artificial antigen presenting cells identical to those used in CART-19 production.

1.3.5 To collect pharmacodynamic (PD) data for asparaginase activity following pegaspargase administration in infants.

2.0 BACKGROUND

2.1 Introduction/Rationale for Development

Approximately 75-80% of infant ALL cases contain *KMT2A (*previously "*MLL")*-R. These are driver mutations and there are very few, if any, other somatic mutations that accompany *KMT2A*-R in infant ALL. Survival for infants diagnosed with *KMT2A*-R ALL remains poor despite intensive chemotherapy with/without stem cell transplantation. The two largest completed infant ALL trials to date are COG P9407 and Interfant-99, and results in these two studies were quite similar for *KMT2A*-R patients: P9407 5-year event-free survival (EFS) 36% vs. Interfant 4-year EFS 37%.[1,2] The results of AALL0631, which incorporated FLT3 inhibition with lestaurtinib into therapy for infants with ALL, demonstrate similar EFS to prior trials for infants with *KMT2A*-R ALL and no significant improvement with lestaurtinib [3-year EFS 37% (n=54) with chemotherapy alone vs. 3-year EFS 37% (n=67) for chemotherapy with lestaurtinib; p=0.90] (P. Brown, SIOP Abstract, 2016). Relapse frequently occurs early, and often during therapy, for infants with *KMT2A*-R ALL, and second remission is very difficult to achieve. Thus, novel treatments are necessary to improve the rates of sustained first remission and overall survival (OS). MLL fusion proteins contribute to transformation by altering the epigenetic landscape of multiple target genes. Epigenetic modifiers, including DNA methyltransferase inhibitors (DNMTi), have shown preclinical efficacy against *KMT2A*-R ALL blasts. This study will test the tolerability and biologic activity of the addition of the DNMTi azacitidine to chemotherapy in infants with *KMT2A*-R ALL in preparation for a randomized international trial of chemotherapy +/- this agent. One dose level will be evaluated using a design that allows safe and efficient monitoring of the combination.

2.1.1 Rationale for Interfant Backbone Chemotherapy

This protocol will adopt the Interfant-06 standard as the chemotherapy backbone, based upon comparable outcomes (see above) of COG P9407 and the predecessor Interfant-99 regimen, and the lower cumulative chemotherapy exposure of the Interfant backbone compared with prior COG regimens. Complete remission (CR) rate was 94% and 4-year EFS was 47% for all patients enrolled on Interfant-99.[2] The toxicities reported were similar to previous protocols, with toxic death rate of 5.2% in remission.[2] The standard arm of Interfant-06 is based upon the standard risk arm of Interfant-99, with the addition of BFM Protocol IB therapy (cyclophosphamide, mercaptopurine, cytarabine, IT methotrexate/prednisone and IT cytarabine/prednisone) as the first post-Induction block. Interfant-06 was designed to randomize this standard treatment against additional post-Induction chemotherapy intensification. At the time of AALL15P1 development, 414 patients with *KMT2A*-R ALL have been enrolled on Interfant-06, and 215 are being treated on the standard arm. The death rate for patients on the standard arm is 3% overall. Infections are the most common serious adverse event with suspected relationship to treatment, and occur most frequently during the MARMA phase. Interfant-06 is an active study and outcomes data for the randomized treatment are not yet available for review.

Induction therapy for this pilot study will be based upon the amended Interfant-based AALL0631 induction (cohort 2), but with a change to pegaspargase. Though infectious complications and toxic mortality have been problematic in trials of infants with ALL, the modification of AALL0631 to Interfant-based induction in cohort 2 led to reduced toxicity.[3,4] Cohort 1 of AALL0631 had received P9407-

based Induction with reduced anthracycline dosing and a change to pegaspargase (2500 IU/m^2) from native L-asparaginase. Interfant-06 continues to use native L-asparaginase during Induction, with a total cumulative dose that is 2 times higher than the cumulative dose provided in the amended AALL0631 Induction, but the native L-asparaginase formulation is no longer available in the United States. Pegaspargase has the advantage of sustained asparagine depletion and it is well tolerated in infants on Interfant and COG trials during post-Induction phases. Therefore, one dose of pegaspargase will be substituted for the six doses of native L-asparaginase. During Induction, the dose of pegaspargase will be reduced by 20% from the standard dose to 2000 units per m^2 body surface area (BSA) for infants ages 6 months and older at diagnosis, with additional age-based dose reductions for younger infants. Standard dose pegaspargase was associated with excessive toxicities in AALL0631 cohort 1 and standard dose pegaspargase has not been previously administered to infants receiving Interfant-based Induction. Therefore, this trial will provide the reduced dose pegaspargase to all infants in an effort to reduce the risk of associated toxicities.

2.1.2 Rationale for Azacitidine

KMT2A-R ALL blasts demonstrate unique biological features, including characteristic gene expression profiles and epigenetic alterations. DNA promoter hypermethylation, which results in silencing of genes, including tumor suppressor genes, is a feature of infant *KMT2A*-R ALL.[5-4] Clonal methylation changes have a critical role in chemoresistance and relapse in infant ALL.[6] In vitro exposure of infant *KMT2A*-R ALL samples to the DNMTis decitabine and zebularine has been shown to reverse the methylation pattern of silenced genes and induce selective cytotoxicity for *KMT2A*-R cells.[7,8] There is growing clinical experience with DNMTis in combination with chemotherapy in acute leukemia in children, but no specific treatment experience in infants. Thus, a pilot study in infants to determine the safety of this combination with chemotherapy is warranted. For this trial in a highly vulnerable infant population, azacitidine has been chosen for study, based upon its availability and preferred profile of safety and tolerability in prior pediatric studies.

2.1.3 Interfant-06 and Azacitidine

The experimental combination of a DNMTi with chemotherapy shows preclinical efficacy in *KMT2A*-R infant blasts and this trial is designed to address the poor outcomes and high relapse rate of infants with *KMT2A*-R ALL. Many infants with *KMT2A*-R ALL present with features of mixed lineage or biphenotypic leukemia, and infants with mixed lineage leukemia will also be eligible, as long as the predominant features are lymphoblastic. Azacitidine will be given to infants with *KMT2A*-R ALL as 4 courses of epigenetic priming therapy prior to blocks of post-Induction chemotherapy. Infants who enroll and are later found to have *KMT2A* germline ALL will continue on protocol therapy for Induction and remission assessment, but will be removed from protocol prior to the first course of azacitidine.

2.2 Preclinical Studies

Preclinical studies of methylation patterns in infant ALL cell lines and patient samples have identified DNA hypermethylation as an important pathway driving leukemogenesis and is a potential target for testing in infant ALL models.[7,9,10] Kostadinov *et al*., reported the

critical role of subclonal methylation changes in chemoresistance and relapse of infant ALL.[6] Azacitidine has established efficacy as a demethylating agent in myelodysplastic syndrome and demonstrated a direct cytotoxic effect *in vitro* against pre-B ALL cells.[11] Decitabine and zebularine are similar DNA demethylation inhibitors that have demonstrated preclinical efficacy in the treatment of *KMT2A*-R ALL cells. Bhatla *et al*., described reversal of the gene methylation signature and cytotoxicity of the combination of decitabine with vorinostat in B-ALL patient samples and *KMT2A*-R cell lines.[12] Stumpel *et al*., further demonstrated that infant ALL cells with *KMT2A*-R are susceptible to demethylating agents.[5] In a study of the methylation patterns of infant ALL, Shaefer *et al.* identified significantly more promoter hypermethylation in *KMT2A*-R patient-derived cells, compared with *KMT2A*-wild type infant ALL cells.[7] Genes with promoter hypermethylation correlated with down-regulated or silenced expression. Treatment with decitabine preferentially led to cytotoxicity of *KMT2A*-R cell lines, compared with *KMT2A*-wild type cell lines, supporting the role for demethylating agents in inducing cytotoxicity and chemosensitivity in the treatment of infant *KMT2A*-R ALL.

2.3 Adult Studies

Azacitidine is indicated for the treatment of all types of myelodysplastic syndrome (MDS). Typical dosing of azacitidine as monotherapy for MDS is 75 mg/m^2/day IV or subcutaneous daily for 7 days, repeated in cycles every 28 days. Common adverse reactions include neutropenia, thrombocytopenia, and anemia, gastrointestinal toxicity, and infusion site reactions with subcutaneous administration [VIDAZA ® package insert]. Azacitidine has moderate emetogenic potential. In adult patients, azacitidine has been administered in combination with chemotherapy to treat acute myeloid leukemia (AML) and prostate cancer. The results have been mixed with regards to efficacy, and the dose limiting toxicities of azacitidine in combination with chemotherapy have been related to myelosuppression. In a pilot study of azacitidine as epigenetic treatment prior to standard 7 + 3 cytarabine and daunorubicin chemotherapy for adult patients with AML, the combination was feasible.[13] The doses given were 37.5 mg/m^2/day and 75 mg/m^2/day IV for 5 days. There were no dose limiting toxicities, but there were fatal adverse events in this older population during Induction treatment. In a randomized study of chemotherapy with or without azacitidine for older patients with AML by Muller-Tidow *et al.*, azacitidine was dosed at 75 mg/m^2/day IV for 5 days prior to each course of chemotherapy and every 4 weeks during Maintenance therapy.[14] The median age of patients in the study was 70 years and the study authors concluded that azacitidine added toxicity, but did not provide benefit for unselected older patients. Singal *et al*. conducted a Phase 1/2 study of azacitidine prior to docetaxel and prednisone in men with metastatic castration-resistant prostate cancer, and the treatment combination demonstrated biologic activity without dose limiting toxicity.[15] The recommended Phase II dose was 75 mg/m^2/day IV for 5 days, followed by docetaxel with growth factor support. Objective response was noted in 3 of 10 evaluable patients.

2.4 Pediatric Studies

2.4.1 Azacitidine: Cytotoxic Drug

Azacitidine has been studied in high doses as a cytotoxic drug for acute leukemia since the 1970s. Karon *et al*., published the use of azacitidine in 37 children with acute leukemia in 1973.[16] The maximum tolerated dose (MTD) was estimated to be 150 mg/m^2 to 200 mg/m^2 IV when given for 5 days every 14 days. The major dose limiting toxicities were nausea, vomiting, and diarrhea. In 1981 Look *et al*., treated relapsed AML in pediatric patients with 200 mg/m^2/day of azacitidine for

2 days following etoposide on Days 1-3.[17] The doses were repeated after 1-2 days and until the marrow became hypoplastic. Of 22 patients treated with this schedule, 18 developed marrow hypoplasia and 10 entered CR. The major toxicity of the combination was prolonged pancytopenia. Azacitidine (300 mg/m^2/day) IV for 2 days, in combination with etoposide, was incorporated into a 5-drug regimen for newly diagnosed AML and reported by Kalwinsky *et al*., in 1988.[18] Sixty-eight children were treated on the study, the CR rate was 85% after Induction, but the failure free survival rate showed no improvement over prior trials. The toxicities reported included fungal infections, febrile neutropenia, myelosuppression, and one patient with cardiomyopathy. These toxicities were not specific to the addition of azacitidine. In 1996, Steuber *et al*., reported the results of a randomized study of a 3-drug combination of azacitidine (250 mg/m^2/day), etoposide, and amsacrine compared with the control group, etoposide and amsacrine alone.[19] The CR rate for refractory AML was significantly higher (18% vs 53%, p=0.03) with the addition of azacitidine.[19] There was increased infectious toxicity in the 3-drug combination, though reported as comparable to standard AML Induction therapies.

2.4.2 Azacitidine: DNA Methyltransferase Inhibitor

More recently, azacitidine and decitabine have been administered in lower doses as DNA methyltransferase inhibitors rather than as cytotoxic agents. Demonstrated efficacy in adults with myelodysplastic syndrome has led to FDA approval of both drugs as single agents. A number of studies have further explored the use of azacitidine and decitabine as "epigenetic priming" agents in combination with chemotherapy for resistant cancers.[15,20-22] The major adverse effect of this approach is dose-limiting myelosuppression and the combination has increased toxicity without demonstrable benefit for elderly patients with acute myeloid leukemia.[13,23] Dose-limiting myelosuppression has also been noted in children with refractory solid tumors who received decitabine with intensive chemotherapy.[24] However, Benton *et al*., trialed decitabine in combination with the first 5 days of Hyper-CVAD therapy for children and adults (median age 33 years, range 4-67 years) with relapsed or refractory ALL, and the results were encouraging.[25] The most common Grade 3 or 4 adverse events were hepatic dysfunction and hyperglycemia, an MTD was not reached, and the response rate to the combination was 56%. Thus, younger patients with ALL may tolerate the combination reasonably well at doses required for demethylation.

Epigenetic priming therapy with a demethylating agent has been introduced into two pediatric trials of relapsed or refractory leukemia. The Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) consortium tested azacitidine as a priming agent prior to fludarabine and cytarabine in relapsed or refractory AML and ALL pediatric patients, ages 1-21 years.[26] Azacitidine 75 mg/m^2/day was given subcutaneously on Days 1-5, prior to the chemotherapy on Days 6-10. The rationale for this protocol was that low dose azacitidine as an epigenetic priming agent could reverse aberrant DNA methylation, overcome drug resistance, and improve the cytotoxic effect of the chemotherapy medications. Fifteen patients were enrolled and none experienced DLT. Non-hematologic toxicities ≥ Grade 3 attributed to azacitidine included febrile neutropenia, infections, AST elevation, oral hemorrhage, and hypokalemia. Evidence of demethylation was seen in every patient by G-LINE analysis. The treatment was well tolerated in this population.

The TACL consortium is also testing a combination of epigenetic therapy (decitabine and the histone deacetylase inhibitor vorinostat) and intensive re-Induction chemotherapy in children, ages 1-25 years, with relapsed ALL. Enrollment was temporarily suspended when non-albicans Candidemia developed in 4 of the first 5 enrolled patients, despite anti-fungal prophylaxis.[27] After an amendment to reduce the decitabine dose and duration and to require empiric non-azole fungal treatment, the infectious toxicity improved, but remains a significant concern. Thus, AALL15P1 incorporates extensive supportive care recommendations for infants receiving azacitidine and chemotherapy (see Appendix IV).

2.5 Dosing Rationale

Azacitidine will be given as pre-treatment prior to chemotherapy in 4 courses post-Induction to infants with *KMT2A*-R. The rationale for pre-treatment is based upon the hypothesis that DNMT inhibition will reverse the epigenetic signature of the blasts and enhance the cytotoxicity of the chemotherapy. Following peripheral blood cell count recovery from the prior block, azacitidine will be given IV daily for 5 days. Chemotherapy will begin on the first day immediately following each azacitidine course.

The dosing of azacitidine will be weight-based, with a BSA:weight conversion factor of 30:1. The effect of the 30:1 conversion of BSA:weight dosing is to reduce doses to approximately 50% of BSA-based doses for newborns, up to approximately 75% of BSA-based doses for 1-year-olds. Considering that the safe and biologically active BSA-based dose of azacitidine is 75 mg/m^2 in older children, the starting dose (DL1) for this study will be 2.5 mg/kg (Table 1). If DL1 is deemed too toxic, then the azacitidine dose will be reduced by 30% to DL0, 1.8 mg/kg, for all infants with remaining azacitidine doses and for all subsequently enrolled infants.

This pilot study does not seek to define maximum tolerated dose or to escalate doses to evaluate for clinical efficacy. Rather, DL1 (and DL0 if necessary) will be evaluated for tolerability and evidence of epigenetic activity in combination with chemotherapy. If both DL1 and DL0 are too toxic, but show evidence of biologic activity, then consideration will be given to amending the study to test lower doses. If not, the strategy will not be pursued further.

Table 1: Experimental doses for infants with *KMT2A*-R

Dose Level	Azacitidine IV daily on Days 1-5
1	2.5 mg/kg
0	1.8 mg/kg

2.6 Recovery Weeks

Interfant-06 standard therapy includes a minimum of 2 weeks of recovery time between MARMA and OCTADAD and 1 week of recovery time between Parts I & II of OCTADAD. Patients on Interfant-06 therapy must have an ANC > 500/µL and platelets > 50,000/µL with resolution of mucositis to begin Protocol IB, MARMA, and OCTADAD. They must also meet specified ANC and platelet parameters for dosing of cyclophosphamide in Protocol IB, high dose cytarabine on Day 15 of MARMA and for all doses of vincristine and daunorubicin in OCTADAD. AALL15P1 includes 1 to 2 weeks of minimum recovery time prior to the start of each azacitidine course. Course 1 of azacitidine begins on Day 36 of Induction, 1 week following the end of Induction chemotherapy on

Day 29. Recovery of ANC ≥ 500/µL and platelets ≥ 50,000/µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2, is required prior to the start of each azacitidine course.

2.6.1 Extension in the length of Induction therapy recovery time.

Prior to Amendment #1 patients were required to meet criteria to begin EPI Block #1 by Day 50 of Induction. Induction chemotherapy on AALL15P1 begins with a week of prednisone on Days 1-7 and lasts for 5 weeks. The end-Induction bone marrow is requested on Day 35 and patients may begin EPI Block #1 as early as Day 36. A review of the first 5 patients enrolled on AALL15P1 demonstrated delayed recovery periods without excessive or unexpected adverse events. Similarly, Induction recovery times on AALL0631 post-Amendment #1A, which utilized the same chemotherapy with the exception that AALL0631 included Erwinia asparaginase instead of pegaspargase, resulted in mean and median lengths of 47 and 46 days, respectively. Induction length was > 50 days for 33 infants (28%) and was ≥ 55 days for 17 infants (15%). The longest recovery time was 59 days, for 2 infants (Unpublished results). Therefore, Amendment #1 extends the allowable time for Induction recovery to Day 64, to avoid unnecessary removal of infants from protocol therapy. Subjects who do not meet recovery criteria by Day 64 will be removed from protocol and will not receive azacitidine.

2.7 Pharmacodynamic Assessment of DNA Methylation

A secondary endpoint of this trial is to determine if there is biologic activity of azacitidine at a tolerable dose level in infants with *KMT2A*-R ALL. The starting dose level is reduced from the typical adult BSA-based dosing of azacitidine and the metabolism of azacitidine may be altered in infants, so this trial will assess for evidence of demethylation at each dose level tested. Global methylation assessment of peripheral blood mononuclear cells (PBMCs) will be performed using a commercial ELISA LINE-1 kit which measures 5-methylcytosine (5mC) content. Samples of peripheral blood will be collected on Day 1 prior to azacitidine and on Day 5 of the first two courses of azacitidine. The mean 5mC content will be calculated for all patients before and after azacitidine, and a paired t-test will be performed to determine if there is significant demethylation in the study population for each tested dose level. See Sections 14.1 and Appendix VI for additional details.

2.8 Molecular Profiling of Diagnostic, Remission and Refractory/Relapse Leukemic Cells (Banking for Future Research)

Submission of bone marrow aspirate material, peripheral blood, and leukapheresis material for molecular profiling is optional. Samples from consenting patients will be collected from all time points where a bone marrow evaluation is clinically indicated (at diagnosis, at the end of Induction, Consolidation and Interim Maintenance, and at the time of relapse). Submitted samples will be banked for the purpose of performing retrospective studies to refine risk stratification, identify new targets for therapy, identify biomarkers to predict response, and to link host polymorphisms with various disease characteristics and toxicities. Refer to Section 14.3 for details related to sample processing and shipping.

2.9 Minimal Residual Disease (MRD)

As a part of standard care, MRD by flow cytometry in a COG-approved laboratory is required with bone marrow evaluations at the end of Induction, Consolidation, and Interim

Maintenance. Results will be collected from local institutions and correlated with disease outcome. MRD will not be used to determine treatment allocation.

2.10 Pharmacokinetics (PK) of azacitidine

Participation in the PK of azacitidine study is optional for sites and patients. Please review the details outlined in Appendix VIII. Peripheral blood samples (1 mL) will be collected from an in-dwelling catheter or by venipuncture at 7 time points during the first course of azacitidine. Azacitidine is unstable in blood, and therefore all blood samples will be processed and plasma harvested immediately per the instructions provided in a study laboratory manual. A validated high-performance liquid chromatography/tandem mass spectrometric method (LC-MS/MS) will be used for azacitidine plasma concentration analysis by the bioanalytical laboratory. Sample collection kits and supplies will be provided by the sponsor or sponsor-designated vendor. Sample collection, processing, storage, and shipment instructions will be provided to study sites in a separate laboratory manual.

2.11 Feasibility of CAR T-cell production for infants with ALL

This trial includes a correlative study testing the feasibility of T-cell collection for the purposes of chimeric antigen receptor (CAR) T-cell production from the peripheral blood in infants with ALL. For those patients who consent, peripheral blood samples will be collected prior to therapy initiation, at the end of Induction, and at the end of Consolidation therapy. Lymphocyte subsets will be quantified, and functional studies will be performed to assess the ability of the lymphocytes to expand in response to artificial antigen presenting cell stimulation ex vivo. The ability to expand in response to this stimulus is a critical determinant of the feasibility of adoptive immunotherapy, specifically for CART-19. See Section 14.5 and Appendix VII for details.

2.12 Pharmacodynamics of Pegaspargase

Asparaginase activity levels are recommended on the 7th day following the administration of pegaspargase during Induction, Interim Maintenance, and Delayed Intensification part 1. For patients who consent, results will be collected from local institutions and correlated with outcome. Asparaginase activity levels are optional, are not reimbursed by the study, and will not be used to determine treatment allocation. Local investigators may choose to substitute *Erwinia* asparaginase for pegaspargase based on the activity level results, per each institution's standard practice. Patients who develop clinical hypersensitivity to pegaspargase should be given *Erwinia* asparaginase (see Section 5.8)

3.0 STUDY ENROLLMENT PROCEDURES AND PATIENT ELIGIBILITY

3.1 Study Enrollment

3.1.1 Patient Registration

Prior to enrollment on this study, patients must be assigned a COG patient ID number. This number is obtained via the Patient Registry module in OPEN once authorization for the release of protected health information (PHI) has been obtained. The COG patient ID number is used to identify the patient in all future interactions with COG. If you have problems with the registration, please refer to the online help. For additional help or information, please contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

A Biopathology Center (BPC) number will be assigned as part of the registration process. Each patient will be assigned only one BPC number per COG Patient ID. For additional information about the labeling of specimens please refer to the Pathology and/or Biology Guidelines in this protocol.

Please see Appendix I for detailed CTEP Registration Procedures for Investigators and Associates, and Cancer Trials Support Unit (CTSU) Registration Procedures including: how to download site registration documents; requirements for site registration, submission of regulatory documents and how to check your site's registration status.

NOTE: In order for an institution to maintain COG membership requirements, every patient with a known or suspected neoplasm needs to be offered participation in APEC14B1, *Project:EveryChild A Registry, Eligibility Screening, Biology and Outcome Study*.

3.1.2 IRB Approval

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federal Wide Assurance (FWA) number
- An active roster affiliation with the Lead Network or a participating organization
- A valid IRB approval
- Compliance with all protocol specific requirements.

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site.

For information about the submission of IRB/REB approval documents and other regulatory documents as well as checking the status of study center registration packets, please see Appendix I.

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support. For general (non-regulatory) questions call the CTSU General Helpdesk at: 1-888-823-5923.

Note: Sites participating on the NCI CIRB initiative and accepting CIRB approval for the study are not required to submit separate IRB approval documentation to the CTSU Regulatory Office for initial, continuing or amendment review. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory

Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.. Other site registration requirements (e.g., laboratory certifications, protocol-specific training certifications, or modality credentialing) must be submitted to the CTSU Regulatory Office or compliance communicated per protocol instructions.

3.1.3 Reservation Requirements

Prior to obtaining informed consent and enrolling a patient, a reservation must be made following the steps below. Reservations may be obtained 24 hours a day through the Oncology Patient Enrollment Network (OPEN) system.

Patient enrollment for this study will be facilitated using the Slot-Reservation System in conjunction with the Registration system in OPEN. Prior to discussing protocol entry with the patient, site staff must use the CTSU OPEN Slot Reservation System to ensure that a slot on the protocol is available for the patient. Once a slot-reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

If the study is active, a reservation can be made by following the steps below:

1) Log in to https://open.ctsu.org/open/ using your CTEP IAM user name and password.
2) In order to make a reservation, the patient must have an OPEN patient number. Click on the 'Slot Reservation' tab to create an OPEN patient number, under 'Patients'.
3) Using the OPEN patient number '**RESERVE'** a slot for that patient.
4) On the 'Create Slot Reservation' page, select the Protocol Number, enter the COG Patient ID, and choose the required stratum (if applicable) in order to obtain a reservation.

   Refer to the 'SITE – Slot Reservation Quick Reference' guide posted under the 'Help' tab in OPEN for detailed instructions:

   https://www.ctsu.org/readfile.aspx?fname=OPEN/OPEN_SlotReservation_QuickReference_SiteUserGuide_102612.pdf&ftype=PDF

3.1.4 Study Enrollment

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. To access OPEN, the site user must have an active CTEP-IAM account (check at https://ctepcore.nci.nih.gov/iam) and a 'Registrar' role on either the lead protocol organization (LPO) or participating organization roster. Registrars must hold a minimum of an AP registration type. If a DTL is required for the study, the registrar(s) must also be assigned the OPEN Registrar task on the DTL.

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient position in the Rave database. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org. To assign an IVR or NPIVR as the treating, crediting, consenting, drug shipment (IVR only), or investigator receiving a transfer in OPEN, the IVR or NPIVR must list on their Form FDA 1572 in RCR the IRB number used on the site's IRB approval. If a DTL is required for the study, the IVR or NPIVR must also be assigned the appropriate OPEN-related tasks on the DTL.

Patient enrollment for this study will be facilitated using the Slot-Reservation System in conjunction with the Registration system in the Oncology Patient Enrollment Network (OPEN). Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot- reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

Prior to accessing OPEN, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Further instructional information is provided on the CTSU members' web site OPEN tab or within the OPEN URL (https://open.ctsu.org). For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

3.1.5 Timing

Patients must be enrolled before treatment begins. The date protocol therapy is projected to start must be no later than **five (5)** calendar days after the date of study enrollment. **Patients who are started on protocol therapy prior to study enrollment will be considered ineligible**. **The only exceptions to this are steroid pretreatment or the administration of intrathecal methotrexate or intrathecal cytarabine as described in Section 3.2.3.4.**

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated in the eligibility section below.

3.2 Patient Eligibility Criteria

Important note: The eligibility criteria listed below are interpreted literally and cannot be waived. All clinical and laboratory data required for determining eligibility

of a patient enrolled on this trial must be available in the patient's medical/research record which will serve as the source document for verification at the time of audit.

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated. Laboratory values used to assess eligibility must be no older than seven (7) days at the start of therapy. Laboratory tests need not be repeated if therapy starts within seven (7) days of obtaining labs to assess eligibility. If a post-enrollment lab value is outside the limits of eligibility, or laboratory values are > 7 days old, then the following laboratory evaluations must be re-checked within 48 hours prior to initiating therapy: CBC with differential, bilirubin, ALT (SGPT) and serum creatinine. If the recheck is outside the limits of eligibility, the patient may not receive protocol therapy and will be considered off protocol therapy. Imaging studies, if applicable, must be obtained within 2 weeks prior to start of protocol therapy (repeat the tumor imaging if necessary).

See Section 4.2.3 for required studies to be obtained prior to starting protocol therapy.

3.2.1 Age

Infants less than 1 year of age on the date of diagnosis are eligible; infants must be > 36 weeks gestational age at the time of enrollment.

3.2.2 Diagnosis

3.2.2.1 Patients must have newly diagnosed B lymphoblastic leukemia (2008 WHO classification) also termed B-precursor acute lymphoblastic leukemia) or acute leukemia of ambiguous lineage (ALUL), which includes mixed phenotype acute leukemia (MPAL). For patients with ALUL, the morphology and immunophenotype must be at least 50% B lymphoblastic.

CNS status must be determined based on a sample obtained prior to the administration of any systemic or intrathecal chemotherapy, with the exception of steroid pretreatment.

3.2.3 Exclusion Criteria

3.2.3.1 Patients with known absence of *KMT2A*-Rearrangement leukemia prior to enrollment.

3.2.3.2 Patients with Down syndrome.

3.2.3.3 Patients with secondary B-ALL that developed after treatment of a prior malignancy with cytotoxic chemotherapy.

3.2.3.4 With the exception of steroid pretreatment (defined in Section 3.4) or the administration of intrathecal methotrexate or intrathecal cytarabine, receipt of any other prior cytotoxic chemotherapy for either the current diagnosis of B-ALL or any cancer diagnosed prior to the initiation of

protocol therapy on AALL15P1.

3.2.4 Regulatory Requirements

3.2.4.1 All patients and/or their parents or legal guardians must sign a written informed consent.

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

3.3 *KMT2A* Rearrangement (*KMT2A*-R*)* Status

All patients must undergo cytogenetic FISH testing at a COG-approved laboratory for *KMT2A*-R determination. Results must be submitted for central review and confirmation of *KMT2A*-R status. Patients will be eligible to remain on protocol therapy post-Induction if *KMT2A*-R is determined and confirmed by central review. Please refer to Appendix X and Section 14.7 for sample requirements and details regarding central review.

3.4 Definitions

INITIAL WBC: The first WBC at the treating COG institution, or the WBC prior to intravenous fluids, whichever occurred first. If prior therapy (i.e. steroids) has been administered and a CBC is available that was obtained within 72 hrs prior to steroid therapy, then this pre-steroid WBC should be used.

INITIAL PLATELET COUNT: The first platelet count at the treating COG institution, or the count before transfusion of platelets if transfused prior to arrival.

INITIAL HEMOGLOBIN: The first hemoglobin at the treating COG institution, or the hemoglobin prior to intravenous fluid or red cell transfusions, whichever occurred first.

STEROID PRETREATMENT: Patients who have received any amount of oral or IV steroids prior to study entry will be eligible for enrollment, provided patients meet all other eligibility requirements. Inhalational steroids and topical steroids are not considered to be pretreatment.

CNS LEUKEMIA AT DIAGNOSIS:

CNS 1: In cerebrospinal fluid (CSF), absence of blasts on cytospin preparation, regardless of the number of white blood cells (WBCs).

CNS 2: In CSF, presence < 5/μL WBCs and cytospin positive for blasts, or traumatic LP, ≥ 5/μL WBCs, cytospin positive for blasts, but negative by Steinherz/Bleyer algorithm:

CNS 2a: < 10/μL RBCs; < 5/μL WBCs and cytospin positive for blasts;
CNS 2b: ≥ 10/μL RBCs; < 5/μL WBCs and cytospin positive for blasts; and
CNS 2c: ≥ 10/μL RBCs; ≥ 5/μL WBCs and cytospin positive for blasts but negative by Steinherz/Bleyer algorithm (see below).

CNS3: In CSF, after traumatic LP presence of ≥ 5/μL WBCs and cytospin positive for blasts and/or clinical signs of CNS leukemia:

CNS 3a: < 10/μL RBCs; ≥ 5/μL WBCs and cytospin positive for blasts;
CNS 3b: ≥ 10/μL RBCs, ≥ 5/μL WBCs and positive by Steinherz/Bleyer algorithm (see below);
CNS 3c: Clinical signs of CNS leukemia (such as facial nerve palsy, brain/eye involvement or hypothalamic syndrome).

METHOD OF EVALUATING INITIAL TRAUMATIC LUMBAR PUNCTURES:
If the patient has leukemic cells in the peripheral blood and the lumbar puncture is traumatic and the CSF contains ≥ 5 WBC/μL and blasts, the following Steinherz/Bleyer algorithm should be used to distinguished between CNS2 and CNS3 disease:

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

A patient with CSF WBC ≥ 5/μL blasts, whose CSF WBC/RBC ≥ 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 = 3.0 X 10^6/μL:

$$\frac{60}{1500} = 0.04 \text{ and is } > 2X \frac{46000}{3.0 \times 10^6} = 0.015$$

TESTICULAR LEUKEMIA AT DIAGNOSIS:
Unilateral or bilateral testiculomegaly. Biopsy is required if clinical findings are equivocal or suggestive of hydrocele or a non-leukemic mass.

BONE MARROW STATUS:
M1: < 5% lymphoblasts
M2: 5%- 25% lymphoblasts
M3: > 25% lymphoblasts.

REMISSION
M1 marrow with complete resolution of extramedullary leukemia. Must have absence of blasts on cytospin preparation and no clinical signs of CNS leukemia.

BONE MARROW MRD STATUS (Day 35):
Positive: ≥ 0.01% detectable leukemia cells
Negative: < 0.01% detectable leukemia cells

REFRACTORY DISEASE:
Failure to achieve remission by the end of Consolidation therapy.

RELAPSE:
Any recurrence of disease, whether in marrow or extramedullary site(s), at any point after achieving remission. Relapse should be histopathologically confirmed.

1) ISOLATED BONE MARROW RELAPSE:
Patients with an M3 marrow at any point after achieving remission without involvement

of the CNS and/or testicles.

2) COMBINED RELAPSE:
M2 or M3 marrow at any point after achieving remission with concomitant CNS and/or testicular and/or other extramedullary site relapse.

3) CNS RELAPSE:
Positive cytomorphology and WBC ≥ 5/μL OR clinical signs of CNS leukemia such as facial nerve palsy, brain/eye involvement, or hypothalamic syndrome. If any CSF evaluation shows positive cytomorphology and WBC < 5/μL, a second CSF evaluation is required within 2-4 weeks. While identification of a leukemic clone in CSF by flow cytometry (TdT, CD19, CD10, etc) or FISH for diagnostic karyotypic abnormality may be useful, definitive evidence of CNS involvement (i.e. WBC ≥ 5/μL OR clinical signs of CNS leukemia) is required for the diagnosis of a CNS relapse.

4) TESTICULAR RELAPSE:
Must be documented by testicular biopsy, if not associated with a marrow relapse.

5) ISOLATED EXTRAMEDULLARY RELAPSE
Must be documented by histopathology, if not associated with a marrow relapse.

DISEASE EVALUATION DURING FOLLOW-UP:
A disease evaluation is a procedure ordered with the intent to measure or assess the disease status of a patient. The most common evaluations are a bone marrow aspirate and/or biopsy and a lumbar puncture (LP). If a CBC has findings that raise suspicion for relapse, a bone marrow aspirate must be performed to confirm the relapse.

4.0 TREATMENT PROGRAM

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable (except where explicitly prohibited within the protocol).

4.1 Overview of Treatment Plan

Any infant < 1 year of age on the day of diagnosis with ALL or acute ambiguous lineage leukemia (ALUL) that is predominantly B lymphoblastic, which includes mixed phenotype acute leukemia (MPAL), is potentially eligible for enrollment on AALL15P1. Patients will be treated with a common 5-week Induction course, as detailed in Section 4.2. After Induction, only subjects with *KMT2A*-rearrangement (*KMT2A*-R) are eligible to continue on protocol therapy at which point they will receive azacitidine designated "EPI" prior to chemotherapy during 4 cycles of post-Induction therapy.

Phases of Therapy:
Post-Induction therapy includes 4 cycles of Azacitidine epigenetic therapy, Consolidation, 1 Interim Maintenance phase with high dose methotrexate and high dose cytarabine, 2 Delayed Intensification phases (as based on 1 OCTADAD phase from the Interfant backbone) and Maintenance.

Chemotherapy Dosing:
Chemotherapy dosing for infants on AALL15P1 is based on age and BSA. Induction chemotherapy is similar to the amended AALL0631 Induction (cohort 2), with the exception of a change to pegaspargase dosing. Post-Induction chemotherapy dosing is based upon the Interfant-06 standard backbone. Post-Induction, infants ≥ 12 months of age receive the full doses of chemotherapy, infants ≥ 6 months and < 12 months of age receive 3/4 of the full doses, and infants <6 months of age receive 2/3 of the full doses. The AALL15P1 age-based dose-reductions are the same as the age-based dose reductions of Interfant-06. The appropriate BSA-based doses for each age group are listed on the therapy delivery maps.

4.1.1 Concomitant Therapy Restrictions

4.1.1.1 Patients cannot receive any non-protocol chemotherapy or investigational therapy while on this study.

4.1.1.2 Patients should avoid the use of Echinacea.

4.1.1.3 Cytochrome P450 Interactions with Antileukemic Drugs

Since concurrent use of enzyme inducing anticonvulsants (e.g., phenytoin, phenobarbital, and carbamazepine) with antileukemic therapy has recently been associated with inferior EFS, every effort should be made to avoid these agents, as well as rifampin, which also induces many drug metabolizing enzymes.[28] Neither gabapentin nor levetiracetam induce hepatic drug metabolizing enzymes and if clinically indicated may be suitable alternative anticonvulsants. Azole

antifungals (listed in the table below) and the macrolide group of antibiotics (listed in the table below) may have potent inhibitory effects on drug-metabolizing enzymes. Patients receiving some antileukemic drugs (e.g., vincristine, anthracyclines, etoposide) may experience excess toxicity when these agents are given concomitantly; alternate antifungal and antibacterial therapy should be used whenever possible (see table below).

DRUGS	POTENTIAL INTERACTION	ACTION TO BE TAKEN
Anticonvulsants	Induction of drug metabolizing enzymes Lowered EFS	AVOID fosphenytoin, phenytoin, phenobarbital, carbamazepine Consider gabapentin or levetiracetam as alternative
Rifampin Rifabutin St. Johns Wort	Induction of drug metabolizing enzymes	DO NOT USE
Azole Antifungals (fluconazole, itraconazole*, posaconazole voriconazole, ketoconazole)	Inhibition of drug metabolizing enzymes	CONSIDER ALTERNATIVE MEDICATIONS May need dose reductions of vincristine*, anthracyclines, etoposide, steroids
Macrolide Antibiotics (erythromycin, clarithromycin, roxithromycin, telithromycin)	Inhibition of drug metabolizing enzymes	CONSIDER ALTERNATIVE MEDICATIONS May need dose reductions of vincristine, anthracyclines, etoposide, steroids

* Itraconazole should NOT be used in patients who are receiving vincristine due to a serious drug-drug interaction leading to severe neurotoxicity.[29,30]
For a more complete list of CYP3A 4/5 Inhibitors and Inducers, see Appendix II.

4.1.1.4 Possible Drug Interactions with High or Intermediate Dose Methotrexate

Avoid non-steroidal anti-inflammatory drugs (NSAIDs), trimethoprim/sulfamethoxazole (TMP/SMX), penicillins, probenecid, IV contrast media, proton pump inhibitors, phenytoin and fosphenytoin. Urinary acidifiers can cause methotrexate to precipitate in the urinary tract.

4.1.1.5 Supportive Care Guidelines

Study-specific supportive care guidelines are provided in Appendix IV. In addition, for COG Supportive Care Guidelines see: https://childrensoncologygroup.org/index.php/cog-supportive-care-guidelines under Standard Sections for Protocols.

4.2 Induction

4.2.1 Therapy Delivery Map - INDUCTION

All patients will receive the same Induction therapy with regards to agents and schedule. **Non-intrathecal doses during Induction are based on the age on the day of diagnosis. Intrathecal doses are based on the age on the day of administration**. Induction therapy is 5 weeks (35 days) duration.

_____ Patient COG ID number

_____ DOB

Treatment details and criteria to start are in Section 4.2.4. This Therapy Delivery Map is **three (3)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Intrathecal Methotrexate (IT MTX)	IT	Age (yr) / Dose < 1 / 6 mg ≥ 1 / 8 mg	1 & 29	See Section 4.2.4 for additional guidelines.
PrediniSO(LO)NE (PRED)	PO or NG	Age / Dose < 7d / 10 mg/m^2/dose TID ≥7d and <6mo / 13 mg/m^2/dose TID ≥ 6mo / 15 mg/m^2/dose TID	1-7	Administer 3 doses per day. See Section 4.2.4 for additional guidelines May substitute with methylprednisone IV at 80% of predniSO(LO)NE dose.
DAUNOrubicin (DAUN)	IV infusion over 1-15 min	Age / Dose < 7d / 15 mg/m^2/dose ≥7d and <6mo / 20 mg/m^2/dose ≥ 6mo / 23 mg/m^2/dose	8 & 9	
Cytarabine (ARAC)	IV infusion over 30 min	Age / Dose < 7d / 35 mg/m^2/dose ≥7d and <6mo / 50 mg/m^2/dose ≥ 6mo / 60 mg/m^2/dose	8-21	
Dexamethasone (DEX)	PO or NG or IV	Age / Dose < 7d / 1 mg/m^2/dose TID ≥7d and <6mo / 1.3 mg/m^2/dose TID ≥ 6mo / 1.5 mg/m^2/dose TID	8-28	Administer 3 doses per day.
VinCRIStine (VCR)	IV push over 1 min$^+$	Age / Dose < 7d / 0.8 mg/m^2/dose ≥7d and <6mo / 1 mg/m^2/dose ≥ 6mo / 1.2 mg/m^2/dose	8, 15, 22 & 29	Round final dose to the nearest 0.01 mg $^+$Or infusion via minibag as per institutional policy Max dose 2mg
Pegaspargase (PEG-ASP)	IV over 1-2 hours or IM	Age / Dose < 7d / 1250 IU/m^2/dose ≥7d and <6mo / 1750 IU/m^2/dose ≥ 6mo / 2000 IU/m^2/dose	12	
Intrathecal Cytarabine (IT ARAC)	IT	Age (yr) / Dose < 1 / 15 mg ≥ 1 / 20 mg	15	
Intrathecal Hydrocortisone (IT HC)	IT	Age (yr) / Dose < 1 / 12 mg ≥ 1 / 16 mg	15 & 29	

Continue to the next page for the therapy log.

4.2.2 Therapy Delivery Map – INDUCTION Continued

All patients will receive the same Induction therapy with regards to agents and schedule. **Non-intrathecal doses during Induction will be based on the age on the day of diagnosis. Intrathecal doses are based on the age on the day of administration**. Induction therapy is 5 weeks (35 days) duration.

_____ Patient COG ID number

_____ DOB

Ht_____cm Wt_____kg BSA_____m²

Date Due	Date Given	Day	IT MTX _____mg	PRED ___mg ____mg ____mg	DAUN ____mg	ARAC ____mg	DEX ____mg ____mg ____mg	VCR _____mg	PEG-ASP _____IU	IT ARAC ____mg	IT HC _____mg	Studies
				Enter calculated dose above and actual dose administered below								
		1	_____mg	____mg ____mg ____mg								a-h, j, l, m
		7										
		8			____mg	____mg		____mg				b
		9			____mg	____mg	____mg ____mg					
		10				____mg	____mg					
		11				____mg						
		12				____mg			_____IU			
		13				____mg						
		14				____mg						
		15				____mg		____mg		____mg	____mg	b, c, f
		16				____mg						
		17				____mg						
		18				____mg						
		19				____mg						k
		20				____mg						
		21				____mg						
		22						____mg				b
		28										
		29	____mg					____mg			____mg	f
		35										b, c, h, i, j,
		56										h*
		36	Patients who **do not** have *KMT2A*-R ALL go off protocol therapy. For *KMT2A* -R ALL patients who have **M1** or **M2** marrow, following the completion of Induction therapy, begin EPI#1 (Section 4.3), when ANC ≥ 500/µL and platelets ≥ 50,000/ µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later). Patients who do not meet criteria to start by Day 64 go off-protocol therapy. For *KMT2A* -R ALL patients **with M3** marrow at the end of Induction, proceed to EPI #1 (Section 4.3), as soon as marrow results are known, irrespective of hematologic values, mucositis, and/or diaper dermatitis, and provided there is no active infection or life threatening organ malfunction.								

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

4.2.3 Required Observations in Induction

All baseline studies must be performed prior to starting protocol therapy unless otherwise indicated below.
*If count requirements are not met by Day 56 to begin EPI course #1, complete observation h.

a) History & physical exam (including weight, height, BSA)
b) CBC/diff/platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili
d) Echo or MUGA
e) CrCl or GFR (may be estimated using Schwartz formula)
f) CSF cell count/diff/cytospin
g) TPMT and NUDT15 genotype (TPMT highly recommended for all subjects; NUDT15 is highly recommended for subjects of Hispanic/Native American or East Asian ancestry, and optional for all other subjects (See Section 5.7))
h) Local bone marrow evaluation (pre-treatment must include *KMT2A* FISH and standard cytogenetic studies performed at COG-approved cytogenetics laboratory). Peripheral blood may be substituted pre-treatment if the bone marrow cannot be performed for medical reasons or inadequate marrow material is obtained, refer to Appendix X for sample requirements. SUBMIT RESULTS BY INDUCTION DAY 10 FOR CENTRAL REVIEW, see Section 14.7 and Appendix X
i) MRD by flow cytometry in a COG-approved laboratory, please refer to the Section 7.3 for laboratory details. **Note**: For patients who consent, enter MRD results into RAVE (Section 14.2).
j) Peripheral blood for the **optional** (CAR) T-cell study (Section 14.5).
k) Report Asparaginase activity levels for **optional** pegaspargase pharmacodynamic study (Section 14.6).
l) Bone marrow aspirate for the **optional** molecular profiling study (peripheral blood may be substituted in some cases, see Section 14.3).
m) If apheresis is performed for clinical purposes, submit an apheresis sample for the **optional** molecular profiling study (Section 14.3).

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.2.4 Induction Treatment Details

Patients must be enrolled on study prior to the start of treatment with the exception of steroid pretreatment, IT methotrexate or IT cytarabine as defined in Section 3.2.3.4.

All patients will receive the same Induction chemotherapy with regards to agents and schedule. Non-intrathecal drug doses during Induction are based on age on the day of diagnosis. Intrathecal drug doses are based on age on the day of administration.

See the Parenteral Chemotherapy Administration Guidelines (CAGs) on the COG website at: https://cogmembers.org/_files/disc/Pharmacy/ChemoAdminGuidelines.pdf for special precautions and suggestions for patient monitoring during the infusions. As applicable, also see the CAGs for suggestions on hydration, or hydrate according to institutional guidelines.

Methotrexate: Intrathecal (IT)
Days: 1 and 29
Dose: Age-based dosing.
Note: If a dose was received within 7 days prior to the start of protocol therapy and is per protocol dosing, Day 1 administration does not need to be repeated. Count this dose of IT therapy as the initial intrathecal dose.

Age:	**Dose:**
< 1 year	6 mg
≥ 1 year	8 mg

PrediSO(LO)NE: Oral (PO) or Nasogastric (NG)
Days: 1-7
Dose: Age-based dosing. Administer 3 doses per day.
Notes: If equivalent or higher doses are received within 7 days prior to the start of protocol therapy and are per protocol dosing, doses do not need to be repeated. Count protocol therapy beginning with the first dose of prednisone and adjust Day 8 therapy accordingly.

Age:	**Dose:**
< 7 days	10 mg/m^2/dose TID
≥7 d and <6 mo	13 mg/m^2/dose TID
≥6 months	15 mg/m^2/dose TID

Note: If a patient is unable to take prediSO(LO)NE by mouth or nasogastrically, IV methylprednisolone may be given at 80% of prediSO(LO)NE dose.

DAUNOrubicin: Intravenous (IV) over 1-15 minutes
Days: 8 & 9
Dose: Age-based dosing.

Age:	Dose:
< 7 days	15 mg/m^2/dose
≥ 7 d and <6 mo	20 mg/m^2/dose
≥6 months	23 mg/m^2/dose

Special precautions: Medication errors have occurred due to confusion between DAUNOrubicin and DOXOrubicin. DAUNOrubicin is available in a liposomal formulation (DAUNOrubicin citrate, DaunXome®). The conventional and liposomal formulations are NOT interchangeable; use of the liposomal formulation is not permitted in this trial.

Cytarabine: Intravenous (IV) over 30 minutes
Days: 8-21
Dose: Age-based dosing.

Age:	Dose:
< 7 days	35 mg/m^2/dose
≥ 7 d and <6 mo	50 mg/m^2/dose
≥6 months	60 mg/m^2/dose

Dexamethasone: Oral (PO), Nasogastric (NG) or Intravenous (IV)
Days: 8-28
Dose: Age-based dosing. Administer 3 doses per day.

Age:	Dose:
< 7 days	1 mg/m^2/dose TID
≥7 d and <6 mo	1.3 mg/m^2/dose TID
≥ 6 months	1.5 mg/m^2/dose TID

VinCRIStine: Intravenous (IV) over 1 minute or infusion via minibag as per institutional policy
Days: 8, 15, 22, 29
Dose: Age-based dosing. Max dose 2 mg.

Age:	Dose:
< 7 days	0.8 mg/m^2/dose
≥7 d and <6 mo	1 mg/m^2/dose
≥ 6 months	1.2 mg/m^2/dose

Note: round vinCRIStine dose to the nearest 0.01 mg

Special precautions: FOR INTRAVENOUS USE ONLY.
The container or the syringe containing vinCRIStine should be enclosed in an overwrap bearing the statement "Do not remove covering until moment of injection. For intravenous use only- Fatal if given by other routes."

Medication errors have occurred due to confusion between vinCRIStine and vinBLAStine. VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). The conventional and liposomal formulations are NOT interchangeable; use of the liposomal formulation is not permitted in this trial.

Pegaspargase: Intravenous (IV) over 1-2 hours or Intramuscular (IM)
Day: 12
Dose: Age-based dosing.

Age:	Dose:
< 7 days	1250 International Units (IU)/m^2/dose
≥ 7 d and <6 mo	1750 International Units/m^2/dose
≥ 6 months	2000 International Units/m^2/dose

Cytarabine: Intrathecal (IT)
Days: 15
Dose: Age-based dosing.

Age:	Dose:
< 1 year	15 mg
≥ 1 year	20 mg

Hydrocortisone: Intrathecal (IT)
Days: 15 & 29
Dose: Age-based dosing.

Age:	Dose:
< 1 year	12 mg
≥ 1 year	16 mg

See Section 5.0 for Dose Modifications based on Toxicities.

Patients who **do not** have *KMT2A*-R ALL go off protocol therapy.

Patients with *KMT2A*-R ALL who have **M1** or **M2** marrow following the completion of Induction therapy should begin Azacitidine Epigenetic Therapy 1 (EPI#1, Section 4.3), when ANC ≥ 500/μL and platelets ≥ 50,000/ μL, and resolution of mucositis and/or diaper area dermatitis to ≤ Grade 2 (whichever occurs later). Patients with *KMT2A*-R ALL who do not meet criteria to start by Day 64 go off protocol therapy (See Section 8.1).

For *KMT2A*-R ALL patients with **M3** marrow at the end of Induction, proceed to EPI #1 (Section 4.3), as soon as marrow results are known, irrespective of hematologic values mucositis, and/or diaper dermatitis, and provided there is no active infection or life threatening organ malfunction.

4.3 Azacitidine EPI BLOCK #1

4.3.1 Therapy Delivery Map – Azacitidine EPI BLOCK #1	
Following Induction therapy and the recovery of peripheral blood cell counts, azacitidine will be administered as a pre-treatment prior to Consolidation therapy, for 5 days. Azacitidine therapy is 5 days.	_____ Patient COG ID number _____ DOB

Patients with *KMT2A*-R **M3** marrow, start EPI Block #1 immediately following the completion of Induction therapy. Patients with *KMT2A*-R **M1** or **M2** marrow, start EPI Block # 1 when ANC ≥ 500/µL and platelets ≥ 50,000/µL, and resolution of mucositis and/or diaper area dermatitis to ≤ Grade 2 (whichever occurs later).Treatment details and criteria to start are in Section 4.3.3. This Therapy Delivery Map is **two (2)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Azacitidine (AZA) IND# [redacted] Do not use commercial supply	IV over 10-40 mins	2.5 mg/**kg**/dose	1-5	Note: Infusion must be completed within 45 minutes of vial reconstitution.

Ht _____cm Wt _____kg BSA _____m^2

Date Due	Date Given	Day	Azacitidine _____mg	Studies
			Enter actual dose administered below	
		1	_____mg	a-d
		2	_____mg	
		3	_____mg	e
		4	_____mg	e
		5	_____mg	d-e
		6	Continue to Consolidation (Section 4.4) on Day 6 irrespective of ANC and platelet counts.	

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

4.3.2 Required Observations in Azacitidine EPI BLOCK #1

a) History & physical exam (including length, weight, BSA, and performance status (if ≥ 12 months of age))
b) CBC/diff/ platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili
d) Peripheral blood for the **required** pharmacodynamic assessment of DNA methylation (Section 14.1).
e) Peripheral blood for the **optional** azacitidine pharmacokinetic study (Section 14.4).

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.3.3 Treatment Details for EPI BLOCK #1

Azacitidine will be given as pre-treatment prior to Consolidation therapy.

Criteria to Start Azacitidine EPI Block #1

Begin Azacitidine EPI Block #1 on Day 36 or when peripheral counts recover with ANC ≥ 500/µL and platelets ≥ 50,000/µL, and resolution of mucositis and/or diaper area dermatitis to ≤ Grade 2 (whichever occurs later).

Azacitidine: Intravenous (IV) over 10-40 mins
Days: 1-5
Dose: 2.5 mg/**kg**/dose
Note: infusion must be completed within 45 minutes of vial reconstitution
Do not use commercial supply.

See Section 5.0 for Dose Modifications on Toxicities.

Following completion of Azacitidine Epigenetic Therapy Block 1 (EPI#1), begin Consolidation therapy (Section 4.4) on Day 6 irrespective of ANC and platelet counts.

4.4 Consolidation

4.4.1 Therapy Delivery Map - CONSOLIDATION

All patients will receive the same Consolidation therapy with regards to agents and schedule. **Non-IT drugs are dosed based on age on Day 1 of Consolidation and BSA, as outlined below. IT doses are based on age on the day of administration**. Consolidation therapy is 6 weeks (42 days) duration.

_____ _____
Patient COG ID Number DOB

Following completion of Azacitidine Block 1 (EPI#1), begin Consolidation therapy on Day 6 irrespective of peripheral blood cell counts. Details and criteria to start are in Section 4.4.3. This Therapy Delivery Map is **two (2)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Cyclophosphamide (CPM)	IV over 30-60 min	Age / Dose < 6 mo: 670 mg/m²/dose ≥ 6 mo & < 12 mo: 750 mg/m²/dose ≥ 12 mo: 1000 mg/m²/dose	1 & 29	Patients should have ANC ≥ 500/µL and platelets ≥ **30,000**/µL to begin Day 29 therapy. Refer to Section 4.4.3 for admin guidelines.
Mesna	IV over 15min	Age / Dose < 6 mo: 134 mg/m²/dose ≥ 6 mo & < 12 mo: 150 mg/m²/dose ≥ 12 mo: 200 mg/m²/dose	1 & 29	Administer at 0, 4 and 8 hours from the start of CPM infusion. Refer to Section 4.4.3 for admin guidelines.
Mercaptopurine (MP)	PO or NG	Age / Dose < 6 mo: 40 mg/m²/dose ≥ 6 mo & < 12 mo: 45 mg/m²/dose ≥ 12 mo: 60 mg/m²/dose	1-28	Refer to Section 4.4.3 and Section 5.7 for admin guidelines.
Cytarabine (ARAC)	IV push or SubQ	Age / Dose < 6 mo: 50 mg/m²/dose ≥ 6 mo & < 12 mo: 56 mg/m²/dose ≥ 12 mo: 75 mg/m²/dose	3-6, 10-13, 17-20, & 24-27	Patients should have ANC ≥ 300/µL and platelets ≥ **30,000**/µL to start each 4-day cytarabine block beginning on Days 10, 17, and 24. Refer to Section 4.4.3 for admin guidelines.
Cytarabine (IT ARAC)	IT	Age (yr) / Dose < 1: 15 mg ≥ 1: 20 mg	10	
Intrathecal Hydrocortisone (IT HC)	IT	Age (yr) / Dose < 1: 12 mg ≥ 1: 16 mg	10 & 24	
Intrathecal Methotrexate (IT MTX)	IT	Age (yr) / Dose < 1: 6 mg ≥ 1: 8 mg	24	

Ht _____cm Wt _____kg BSA _____m²

Date Due	Date Given	Day	CPM ___mg	MESNA ___mg ___mg ___mg	MP ___mg	ARAC ___mg	IT ARAC ___mg	IT HC ___mg	IT MTX ___mg	Studies
			Enter calculated dose above and actual dose administered below							
		1	___mg	___mg ___mg ___mg	___mg					b-c
		3				___mg				
		4				___mg				
		5				___mg				
		6				___mg				
		10				___mg	___mg	___mg		b, d
		11				___mg				
		12				___mg				
		13				___mg				
		17				___mg				b
		18				___mg				
		19				___mg				
		20				___mg				
		24				___mg		___mg	___mg	b, d
		25				___mg				
		26				___mg				
		27				___mg				
		28								
		29	___mg	___mg ___mg ___mg						a-c
		42								b, e-g
		43	If M1 marrow is achieved, continue to azacitidine EPI Block #2 (Section 4.5) on Day 43 or when ANC ≥ 500/µL and platelets ≥ 50,000/µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).							

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

4.4.2 Required Observations in CONSOLIDATION

a) History & physical exam (including length, weight, BSA, and performance status (if ≥ 12 months of age))
b) CBC/diff/ platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili
d) CSF cell count/diff/cytospin
e) Local bone marrow evaluation. Obtain on Day 42 or when ANC is rising for 2 consecutive days post-nadir (whichever occurs later). MRD by flow cytometry in a COG-approved laboratory please refer to the Section 7.3 for laboratory details. **Note**: For patients who consent, enter MRD results into RAVE (Section 14.2).
f) Peripheral blood for the **optional** (CAR) T-cell study (Section 14.5).
g) For patients that consent, collect additional bone marrow sample for the **optional** molecular profiling study (Section 14.3).

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.4.3 Treatment Details for CONSOLIDATION

Administer Non-Intrathecal drug dosages based on the age on Day 1 of Consolidation. Administer Intrathecal drug dosages based on the age on the day of administration.

Criteria to start Consolidation

Begin Consolidation on Day 6 following Azacitidine Epigenetic Block #1 (EPI #1 Section 4.3) irrespective of peripheral blood cell counts

See the Parenteral Chemotherapy Administration Guidelines (CAGs) on the COG website at: https://cogmembers.org/_files/disc/Pharmacy/ChemoAdminGuidelines.pdf for special precautions and suggestions for patient monitoring during the infusions. As applicable, also see the CAGs for suggestions on hydration, or hydrate according to institutional guidelines.

Cyclophosphamide: Intravenous (IV) over 30-60 minutes
Days: 1 and 29
Dose: Age based dosing

Age	Dose
< 6 months (mo)	670 mg/m^2/dose
≥ 6 mo & < 12 mo	750 mg/m^2/dose
≥ 12 mo	1000 mg/m^2/dose

Note: Infants should have an ANC ≥ 500/µL and platelets ≥ **30,000/µL** to begin Day 29 therapy.

Mesna: Intravenous (IV) over 15 min at hours 0, 4 and 8 hours from the start of cyclophosphamide infusion
Days: 1 and 29
Dose: Age based dosing

Age	Dose
< 6 mo	134 mg/m^2/dose
≥ 6 mo & < 12 mo	150 mg/m^2/dose
≥ 12 mo	200 mg/m^2/dose

Note: Total of 3 doses per day.

Mercaptopurine: Oral (PO) or Nasogastric (NG) daily
Days: 1-28
Dose: Aged based dosing

Age	Dose
< 6 mo	40 mg/m^2/dose
≥ 6 mo & < 12 mo	45 mg/m^2/dose
≥ 12 mo	60 mg/m^2/dose

See Section 5.7 for suggested starting dose based on TPMT and NUDT15 status (if

status is known)
It is strongly recommended that mercaptopurine be taken at the same time each day. The liquid or tablet formulation may be used.

Note: Mercaptopurine should be interrupted if cytarabine blocks are delayed or interrupted. Omitted mercaptopurine doses should be made up until the planned cumulative dose 1680 mg/m^2 (60 mg/m^2 x28 doses) for infants ≥ 12 months, 1260 mg/m^2 (45 mg/m^2 x28 doses) for infants 6 to < 12 months, 1120 mg/m^2 (40 mg/m^2 x28 doses) for infants < 6 months has been administered.

Cytarabine: Intravenous (IV) push or Subcutaneous (SubQ) daily
Days: 3-6, 10-13, 17-20, and 24-27
Dose: Age based dosing

Age	Dose
< 6 mo	50 mg/m^2/dose
≥ 6 mo & < 12 mo	56 mg/m^2/dose
≥ 12 mo	75 mg/m^2/dose

Note: Patients should have ANC ≥ 300/µL and platelets ≥ **30,000**/µL to start each 4-day cytarabine block beginning on Days 10, 17, and 24. Once a 4-day block has started, do not interrupt for uncomplicated myelosuppression.

Cytarabine: Intrathecal (IT)
Day: 10
Dose: Age based dosing

Age:	**Dose:**
< 1 year	15 mg
≥ 1 year	20 mg

Hydrocortisone: Intrathecal (IT)
Days: 10 & 24
Dose: Age based dosing

Age:	**Dose:**
< 1 year	12 mg
≥ 1 year	16 mg

Methotrexate: Intrathecal (IT)
Day: 24
Dose: Age based dosing

Age:	**Dose:**
< 1 year	6 mg
≥ 1 year	8 mg

Note: If a 4-day cytarabine block is delayed due to myelosuppression, then intrathecal therapy should be delayed until the 4-day cytarabine block and mercaptopurine resume.

See Section 5.0 for Dose Modifications on Toxicities.

Following the completion of Consolidation, infants must have an **M1** marrow to remain on protocol therapy. Infants with **M2** or **M3** marrow are removed from protocol (see Section 8.1). Begin Azacitidine Epigenetic Block #2 (EPI #2, Section 4.5) on Day 43 after determination of **M1** marrow status or when ANC ≥ 500/μL and platelets ≥ 50,000/ μL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).

4.5 Azacitidine EPI BLOCK #2

4.5.1 Therapy Delivery Map – EPI BLOCK #2	
Following Consolidation and peripheral blood count recovery, azacitidine will be administered as a pre-treatment prior to Interim Maintenance for 5 days. Azacitidine therapy is 5 days.	_____ Patient COG ID number _____ DOB

Following the completion of Consolidation, begin Azacitidine Block #2 on Day 43 or when ANC ≥ 500/µL and platelets ≥ 50,000/ µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later). Extensive details and criteria to start are in Section 4.5.3 (treatment overview). This Therapy Delivery Map is **two (2)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Azacitidine (AZA) IND# [redacted] Do not use commercial supply	IV over 10-40 mins	2.5 mg/**kg**/dose	1-5	Note: infusion must be completed within 45 minutes of vial reconstitution.

Ht _____cm Wt _____kg BSA _____m^2

Date Due	Date Given	Day	Azacitidine _____mg	Studies
			Enter actual dose administered below	
		1	_____mg	a-d
		2	_____mg	
		3	_____mg	
		4	_____mg	
		5	_____mg	d
		6	Continue to Interim Maintenance (Section 4.6) on Day 6 irrespective of ANC and platelet counts.	

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

4.5.2 Required Observations in Azacitidine EPI BLOCK #2

a) History & physical exam (including length, weight, BSA, and performance status (if ≥ 12 months of age))
b) CBC/diff/ platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili
d) Peripheral blood for the **required** pharmacodynamic assessment of DNA methylation (Section 14.1).

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.5.3 Treatment Details for Azacitidine EPI BLOCK #2

Azacitidine will be given as pre-treatment prior to Interim Maintenance (IM) therapy.

Criteria to Start Azacitidine EPI Block #2

Begin Azacitidine EPI Block #2 on Day 43 following Consolidation and determination of **M1** marrow or when peripheral counts recover with ANC ≥ 500/μL and platelets ≥ 50,000/μL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).

Azacitidine: Intravenous (IV) over 10-40 mins
Days: 1-5
Dose: 2.5 mg/**kg**/dose
Note: Infusion must be completed within 45 minutes of vial reconstitution
Do not use commercial supply.

See Section 5.0 for Dose Modifications on Toxicities.

Following completion of Azacitidine Epigenetic Therapy Block #2 (EPI#2), begin Interim Maintenance therapy (Section 4.6) on Day 6 irrespective of ANC and platelet counts.

4.6 Interim Maintenance

4.6.1 Therapy Delivery Map – INTERIM MAINTENANCE (IM) Phase 1

All patients will receive the same Interim Maintenance Phase 1 therapy with regards to agents and schedule. **Non-IT drugs are dosed based on age on Day 1 of IM and BSA, as outlined below. IT doses are based on age on the day of administration**. Interim Maintenance therapy is 6 weeks (42 days) duration.

_____
Patient COG ID number

_____
DOB

Following completion of Azacitidine Block #2, begin Interim Maintenance therapy on Day 6 irrespective of ANC and platelet counts. Treatment details and criteria to start are in Section 4.6.4. This Therapy Delivery Map is **three (3)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Mercaptopurine (MP)	PO/NG	Age: < 6 mo; ≥ 6 mo & < 12 mo; ≥ 12 mo Dose: 17 mg/m²/dose; 19 mg/m²/dose; 25 mg/m²/dose	1-14	Refer to Section 4.6.4 for admin guidelines. If Day 8 HD MTX is delayed due to toxicity, then interrupt MP. Resume MP when Day 8 HD MTX is given and complete the planned 14 days of MP (Section 5.7)
High Dose Methotrexate (HD MTX)	IV over 24 hours	Age: < 6 mo; ≥ 6 mo & < 12 mo; ≥ 12 mo Dose: 3300 mg/m²/dose; 3750 mg/m²/dose; 5000 mg/m²/dose	1 & 8	Refer to Section 4.6.4 and Section 5.6.1 for admin guidelines. The Day 8 dose of MTX may be given regardless of blood counts, but should be held until mucositis is ≤ Grade 2. Administer 10% of the dose over 30 minutes, followed by the remainder of the dose (90%) over 23.5 hours.
Leucovorin (LCV)	PO/IV	Age: All ages Dose: 15 mg/m²/dose	3-4 & 10-11	Refer to Section 4.6.4 for admin guidelines. Leucovorin should be given at 42, 48, and 54 hours after the start of methotrexate infusion and continued every 6 hours until plasma methotrexate level is < 0.1 µM.
Intrathecal Methotrexate (IT MTX)	IT	Age (yr): < 1; ≥ 1 Dose: 6 mg; 8 mg	1 & 8	Deliver the IT therapy within 6 hours of the beginning of the IV MTX infusion (hour -6 to +6, with 0 being the start of the MTX bolus).
Intrathecal Hydrocortisone (IT HC)	IT	Age (yr): < 1; ≥ 1 Dose: 12 mg; 16 mg	1 & 8	Deliver the IT therapy within 6 hours of the beginning of the IV MTX infusion (hour -6 to +6, with 0 being the start of the MTX bolus).

Ht _____cm **Wt _____kg** **BSA _____m²**

Date Due	Date Given	Day	MP ____mg	HD MTX ____mg	LCV ____mg ____mg ____mg	IT MTX ____mg	IT HC ____mg	Studies
			Enter calculated dose above and actual dose administered below					
		1	____mg ↓	____mg		____mg	____mg	b-d
		2						
		3			____mg____mg____mg			
		4						
		8		____mg		____mg	____mg	b-d
		9						
		10			____mg____mg____mg			
		11						
		14		Continue to Day 15 of Interim Maintenance Phase 2 when there is no mucositis and ANC ≥ 500 µL and platelets ≥ 50,000/µL				

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

Interim Maintenance

4.6.2 Therapy Delivery Map – INTERIM MAINTENANCE (IM) Phase 2 Continued

All patients will receive the same Interim Maintenance Phase 2 therapy with regards to agents and schedule. Non-IT drugs are dosed based on age on day 1 of IM and BSA, as outlined below. Begin Day 15 of IM when there is no mucositis and when the absolute neutrophil count (ANC) is ≥ 500/µL and platelets ≥ 50,000/µL. Interim Maintenance therapy is 6 weeks (42 days) duration.

_____
Patient COG ID number

_____
DOB

Treatment details and criteria to start are in Section 4.6.4. This Therapy Delivery Map is **three (3)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
High Dose Cytarabine (HD ARAC)	IV over 3hrs	Age: < 6 mo; ≥ 6 mo & < 12 mo; ≥ 12 mo Dose: 2000 mg/m²/dose; 2250 mg/m²/dose; 3000 mg/m²/dose	15-16 & 22-23	Refer to Section 4.6.4 for admin guidelines. Administer every 12 hours (for a total of 8 doses over the 2 courses)
Pegaspargase (PEG-ASP)	IV over 1-2 hours or IM	Age: < 6 mo; ≥ 6 mo & < 12 mo; ≥ 12 mo Dose: 1650 IU/m²/dose; 1875 IU/m²/dose; 2500 IU/m²/dose	23	Administer 3 hours after the completion of the last cytarabine infusion on Day 23 Refer to Section 4.6.4 for admin guidelines.

Ht _____cm **Wt _____kg** **BSA _____m²**

Date Due	Date Given	Day	HD ARAC mg mg	PEG-ASP IU	Studies
			Enter calculated dose above and actual dose administered below		
		15	____mg ____mg		a-c
		16	____mg ____mg		
		22	mg mg		
		23	____mg ____mg	____IU	
		30			f
		36			
		42			e, g
		43	Begin Azacitidine EPI Block #3 (Section 4.7) on Day 43 or when ANC ≥ 500/µL and platelets ≥ 50,000/µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).		

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

4.6.3 Required Observations in INTERIM MAINTENANCE (IM)

a) History & physical exam (including length, weight, BSA, and performance status (if ≥ 12 months of age))
b) CBC/diff/ platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili
d) CSF cell count/diff/cytospin
e) Local bone marrow evaluation. Obtain on Day 42 or when ANC is rising for 2 consecutive days post-nadir (whichever occurs later). MRD by flow cytometry in a COG-approved laboratory, please refer to the Section 7.3 for laboratory details. **Note**: For patients that consent, enter MRD results into RAVE (Section 14.2).
f) Report asparaginase activity levels for **optional** pegaspargase pharmacodynamic study (Section 14.6).
g) For patients that consent, collect additional bone marrow aspirate for the **optional** molecular profiling study (Section 14.3).

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.6.4 Treatment Details for INTERIM MAINTENANCE (IM)
Interim Maintenance is 2 phases.

Administer IM Phase 1 and 2 non-Intrathecal drug dosages based on the age on Day 1 of IM.

Intrathecal drug doses are based on age on the day of administration.

Criteria to start Interim Maintenance
Begin Interim Maintenance on Day 6 following Azacitidine Epigenetic Block #2 irrespective of peripheral blood cell counts.

See the Parenteral Chemotherapy Administration Guidelines (CAGs) on the COG website at:
https://cogmembers.org/_files/disc/Pharmacy/ChemoAdminGuidelines.pdf for special precautions and suggestions for patient monitoring during the infusions. As applicable, also see the CAGs for suggestions on hydration, or hydrate according to institutional guidelines.

Interim Maintenance Phase 1:

Mercaptopurine: Oral (PO) or Nasogastric (NG) daily
Days: 1-14
Dose: Age based dosing

Age	Dose
< 6 mo	17 mg/m^2/dose
≥ 6 mo & < 12 mo	19 mg/m^2/dose
≥ 12 mo	25 mg/m^2/dose

See Section 5.8 for suggested starting dose based on TPMT and NUDT15 status (if status is known)

It is strongly recommended that mercaptopurine be taken at the same time each day. The liquid or tablet formulation may be used.

High Dose Methotrexate: Intravenous (IV) over 24 hours
Days: 1 and 8
Dose: Age based dosing

Age	Dose
< 6 mo	3300 mg/m^2/dose
≥ 6 mo & < 12 mo	3750 mg/m^2/dose
≥ 12 mo	5000 mg/m^2/dose

Note: The Day 8 dose of MTX should be given regardless of blood counts, but should be held until mucositis is ≤ Grade 2.

Administer over 24 hours on Days 1 and 8 (10% of the dose in 30 minutes followed by 90% of the dose in 23.5 hours).

See Section 5.6.1 for hydration, leucovorin rescue and high dose methotrexate infusion guidelines.

Leucovorin: Oral (PO) or Intravenous (IV)
Days 3-4 and 10-11
Dose: 15 mg/m^2/dose. Administer at 42, 48, and 54 hrs after the start of the MTX infusion and every 6 hours until the plasma MTX level is < 0.1 µM.

See Section 5.6.1 for hydration, leucovorin rescue and high dose methotrexate infusion guidelines.

Methotrexate: Intrathecal (IT)
Days: 1 & 8

Age:	**Dose:**
< 1 year	6 mg
≥ 1 year	8 mg

Deliver the IT therapy within 6 hours of the beginning of the IV MTX infusion (hour -6 to +6, with 0 being the start of the MTX bolus).

Hydrocortisone: Intrathecal (IT)
Days: 1 & 8

Age:	**Dose:**
< 1 year	12 mg
≥ 1 year	16 mg

Deliver the IT therapy within 6 hours of the beginning of the IV MTX infusion (hour -6 to +6, with 0 being the start of the MTX bolus).

Interim Maintenance Phase 2:
Day 15 may start only when there is no mucositis and when the absolute neutrophil count (ANC) is ≥ 500/µL and platelets ≥ 50,000/µL. The HD Cytarabine and Pegaspargase on Days 22 and 23 should be given irrespective of blood counts.

High Dose Cytarabine: Intravenous (IV) over 3 hours
Days: 15-16 and 22-23. Administer every 12 hours x 4 doses on Days 15-16 and on Days 22-23 (for a total of 8 doses).
Dose: Age based dosing.

Age	Dose
< 6 months (mo)	2000 mg/m^2/dose
≥ 6 mo & < 12 mo	2250 mg/m^2/dose
≥ 12 mo	3000 mg/m^2/dose

Note: Days 22-23 should be given irrespective of blood counts.

Suggested premedications and supportive care: Administer steroid eye drops (0.1% dexamethasone or 1% predniso(lo)ne ophthalmic solution), 2 drops to each eye every 6 hours, beginning immediately before the first dose and continuing for 24 hours after the last dose. If patient does not tolerate steroid eye drops, may administer artificial tears on an every 2-4 hour schedule.

Pegaspargase: Intravenous (IV) over 1-2 hours or Intramuscular (IM)
Day: 23
Dose: Age based dosing. Administer 3 hours after the completion of the last cytarabine infusion on Day 23.

Age	Dose
< 6 mo	1650 International Units/m^2/dose
≥ 6 mo & < 12 mo	1875 International Units/m^2/dose
≥ 12 mo	2500 International Units/m^2/dose

See Section 5.0 for Dose Modifications on Toxicities.

Following completion of Interim Maintenance, begin Azacitidine Epigenetic Block #3 (EPI #3, Section 4.7) on Day 43 or when ANC ≥ 500/μL and platelets ≥ 50,000/μL, and resolution of mucositis and/or diaper area dermatitis to ≤ Grade 2 (whichever occurs later)

.

4.7 Azacitidine EPI BLOCK #3

4.7.1 Therapy Delivery Map – Azacitidine EPI BLOCK #3	
Following IM and peripheral blood cell count recovery, azacitidine will be administered as a pre-treatment prior to Delayed Intensification Pt. 1 for 5 days. Azacitidine therapy is 5 days.	_____ Patient COG ID number _____ DOB

Begin Azacitidine Block #3 on Day 43 or when ANC ≥ 500/µl and platelets ≥ 50,000/µl and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later). Treatment details and criteria to start are in Section 4.7.3. This Therapy Delivery Map is **two (2)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Azacitidine (AZA) IND# [redacted] Do not use commercial supply	IV over 10-40 mins	2.5 mg/**kg**/dose	1-5	Note: infusion must be completed within 45 minutes of vial reconstitution

Ht _____cm Wt _____kg BSA _____m²

Date Due	Date Given	Day	Azacitidine _____mg	Studies
			Enter actual dose administered below	
		1	_____mg	a-c
		2	_____mg	
		3	_____mg	
		4	_____mg	
		5	_____mg	
		6	Continue to Delayed Intensification Pt.1 (Section 4.8) on Day 6 irrespective of ANC and platelet counts.	

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

a) History & physical exam (including length, weight, BSA, and performance status (if ≥ 12 months of age))
b) CBC/diff/ platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.7.3 Treatment Details for Azacitidine EPI BLOCK #3

Azacitidine will be given as pre-treatment prior to Delayed Intensification Part. 1 therapy.

Criteria to Start Azacitidine EPI Block #3

Begin Azacitidine EPI Block #3 on Day 43 of Interim Maintenance or when peripheral counts recover with ANC ≥ 500/µL and platelets ≥ 50,000/µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).

Azacitidine: Intravenous (IV) over 10-40 mins
Days: 1-5
Dose: 2.5 mg/**kg**/dose
Note: infusion must be completed within 45 minutes of vial reconstitution
Do not use commercial supply.

See Section 5.0 for Dose Modifications on Toxicities.

Following completion of Azacitidine Epigenetic Therapy Block #3 (EPI#3), begin Delayed Intensification Part 1 therapy (Section 4.8) on Day 6 irrespective of ANC and platelet counts.

4.8 Delayed Intensification Part 1

4.8.1 Therapy Delivery Map – DELAYED INTENSIFICATION PART 1

All patients will receive the same Delayed Intensification therapy with regards to agents and schedule. **Non-IT drugs are dosed based on age on Day 1 of DI Pt. 1 and BSA, as outlined below. IT drugs are dosed based on age on the day of administration**. Delayed Intensification Pt. 1 therapy is 5 weeks (35 days) duration.

_____ Patient COG ID number

_____ DOB

Following completion of Azacitidine Block #3, begin DI Pt. 1 therapy on Day 6 irrespective of peripheral blood cell counts. Treatment details and criteria to start are in Section 4.8.4 This Therapy Delivery Map is **three (3)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Pegaspargase (PEG-ASP)	IV over 1-2 hours or IM	Age / Dose < 6 mo: 1650 IU/m²/dose ≥ 6 mo & < 12 mo: 1875 IU/m²/dose ≥ 12 mo: 2500 IU/m²/dose	1	Refer to Section 4.8.4 for admin guidelines.
Dexamethasone (DEX)	PO or NG or IV	Age / Dose < 6 mo: 1.3 mg/m²/dose TID ≥ 6 mo & < 12 mo: 1.5 mg/m²/dose TID ≥ 12 mo: 2 mg/m²/dose TID	1-14 & 15-21	Administer 3 doses per day. **Taper to 0mg over Days 15-21**
6-Thioguanine (TG)	PO or NG	Age / Dose < 6 mo: 40 mg/m²/dose ≥ 6 mo & < 12 mo: 45 mg/m²/dose ≥ 12 mo: 60 mg/m²/dose	1-28	Refer to Section 4.8.4 for admin guidelines.
VinCRIStine (VCR)	IV over 1 min+	Age / Dose < 6 mo: 1 mg/m²/dose ≥ 6 mo & < 12 mo: 1.1 mg/m²/dose ≥ 12 mo: 1.5 mg/m²/dose	1, 8, 15 & 22	Round dose to the nearest 0.01 mg. +Or infusion via minibag as per institutional policy. Max dose 2 mg Refer to Section 4.8.4 for admin guidelines.
DAUNOrubicin (DAUN)	IV over 1-15 min	Age / Dose < 6 mo: 20 mg/m²/dose ≥ 6 mo & < 12 mo: 23 mg/m²/dose ≥ 12 mo: 30 mg/m²/dose	1, 8, 15 & 22	Refer to Section 4.8.4 for admin guidelines.
Cytarabine (ARAC)	IV push or SubQ	Age / Dose < 6 mo: 50 mg/m²/dose ≥ 6 mo & < 12 mo: 56 mg/m²/dose ≥ 12 mo: 75 mg/m²/dose	2-5, 9-12, 16-19 & 23-26	Patients should have ANC ≥ 300/µL and platelets ≥ **30,000**/µL to start each 4-day cytarabine block beginning on Days 9, 16, and 23. Refer to Section 4.8.4 for admin guidelines.
Intrathecal Hydrocortisone (IT HC)	IT	Age (yr) / Dose < 1: 12 mg ≥ 1: 16 mg	1 & 15	Refer to Section 4.8.4 for admin guidelines.
Intrathecal Cytarabine (IT ARAC)	IT	Age (yr) / Dose < 1: 15 mg ≥ 1: 20 mg	1 & 15	Refer to Section 4.8.4 for admin guidelines.

Ht _____ **cm** **Wt** _____ **kg** **BSA** _____ **m²**

Date Due	Date Given	Day	PEG-ASP ___IU	DEX ___mg ___mg ___mg	TG ___mg	VCR ___mg	DAUN ___mg	ARAC ___mg	IT HC ___mg	IT ARAC ___mg	Studies
			Enter calculated dose above and actual dose administered below								
		1	___IU	___mg ___mg ___mg	___mg	___mg	___mg		___mg	___mg	b-d
		2						___mg			
		3						___mg			
		4						___mg			
		5						___mg			
		8				___mg	___mg				b, f
		9						___mg			
		10						___mg			
		11						___mg			
		12						___mg			
		14				Continue DI Pt. 1 (Days 15-36) on the next page.					

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

Delayed Intensification Pt. 1

4.8.2 Therapy Delivery Map – DELAYED INTENSIFICATION PART 1 Continued

All patients will receive the same Delayed Intensification therapy with regards to agents and schedule. **Non-IT drugs are dosed based on age on Day 1 of DI Pt. 1 and BSA, as outlined below. IT drugs are based on age on the day of administration.** Delayed Intensification Pt. 1 therapy is 5 weeks (35 days).

Patient COG ID number

DOB

Treatment details and criteria to start are in Section 4.8.4 This Therapy Delivery Map is **three (3)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Dexamethasone (DEX)	PO or NG or IV	Taper dexamethasone stepwise to zero mg over 7 days	15-21	**Taper to 0 mg over 7 days (Days 15-21).** Suggested taper: reduce dexamethasone dose by 20-30% of the administered dose every 1-2 days, then discontinue. Consider tapering to BID or once daily schedule as clinically appropriate.
6-Thioguanine (TG)	PO or NG	Age / Dose < 6 mo: 40 mg/m²/dose ≥ 6 mo & < 12 mo: 45 mg/m²/dose ≥ 12 mo: 60 mg/m²/dose	15-28	Refer to Section 4.8.4 for admin guidelines.
VinCRIStine (VCR)	IV over 1 min+	Age / Dose < 6 mo: 1 mg/m²/dose ≥ 6 mo & < 12 mo: 1.1 mg/m²/dose ≥ 12 mo: 1.5 mg/m²/dose	15 & 22	Round dose to the nearest 0.01 mg. +Or infusion via minibag as per institutional protocol Max dose 2 mg. Refer to Section 4.8.4 for admin guidelines.
DAUNOrubicin (DAUN)	IV over 1-15 min	Age / Dose < 6 mo: 20 mg/m²/dose ≥ 6 mo & < 12 mo: 23 mg/m²/dose ≥ 12 mo: 30 mg/m²/dose	15 & 22	Refer to Section 4.8.4 for admin guidelines.
Cytarabine (ARAC)	IV push or SubQ	Age / Dose < 6 mo: 50 mg/m²/dose ≥ 6 mo & < 12 mo: 56 mg/m²/dose ≥ 12 mo: 75 mg/m²/dose	16-19 & 23-26	Patients should have ANC ≥ 300/µL and platelets ≥ **30,000**/µL to start each 4-day cytarabine block beginning on Days 9, 16, and 23. Refer to Section 4.8.4 for admin guidelines.
Intrathecal Hydrocortisone (IT HC)	IT	Age (yr) / Dose < 1: 12 mg ≥ 1: 16 mg	15	Refer to Section 4.8.4 for admin guidelines.
Intrathecal Cytarabine (IT ARAC)	IT	Age (yr) / Dose < 1: 15 mg ≥ 1: 20 mg	15	Refer to Section 4.8.4 for admin guidelines.

Enter Cycle #: _____ **Ht** _____ **cm** **Wt** _____ **kg** **BSA** _____ **m²**

Date Due	Date Given	Day	DEX ___mg ___mg ___mg	TG ___mg	VCR ___mg	DAUNO ___mg	ARAC ___mg	IT HC ___mg	IT ARAC ___mg	Studies
			Enter calculated dose above and actual dose administered below							
		15	___mg ___mg ___mg	___mg	___mg	___mg		___mg	___mg	a-c, e
		16	___mg ___mg ___mg				___mg			
		17	___mg ___mg ___mg				___mg			
		18	___mg ___mg ___mg				___mg			
		19	___mg ___mg ___mg				___mg			
		20	___mg ___mg ___mg							
		21	___mg ___mg ___mg							
		22			___mg	___mg				b
		23					___mg			
		24					___mg			
		25					___mg			
		26					___mg			
		28								
		29								
		36	Begin Azacitidine EPI Block #4 (Section 4.9) on Day 36 or when all ANC ≥ 500/µL and platelets ≥ 50,000/µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).							

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

Delayed Intensification Pt. 1

4.8.3 Required Observations in DELAYED INTENSIFICATION PART 1

a) History & physical exam (including length, weight, BSA, and performance status (if ≥ 12 months of age))
b) CBC/diff/ platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili
d) Echo or MUGA
e) CSF cell count/diff/cytospin
f) Report asparaginase activity levels for **optional** pegaspargase pharmacodynamic study (Section 14.6).

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.8.4 Treatment Details for DELAYED INTENSIFICATION PART 1

Administer non-intrathecal drug doses based on the age on Day 1 of DI Part 1. Administer intrathecal drug doses based on the age on the day of administration.

Criteria to Start Delayed Intensification Part 1

Begin Delayed Intensification Part 1 on Day 6 following Azacitidine Epigenetic Therapy #3 irrespective of peripheral blood cell counts.

See the Parenteral Chemotherapy Administration Guidelines (CAGs) on the COG website at: https://cogmembers.org/_files/disc/Pharmacy/ChemoAdminGuidelines.pdf for special precautions and suggestions for patient monitoring during the infusions. As applicable, also see the CAGs for suggestions on hydration, or hydrate according to institutional guidelines.

Pegaspargase: Intravenous (IV) over 1-2 hours or Intramuscular (IM)
Day: 1
Dose: Age based dosing

Age	Dose
< 6 months (mo)	1650 International Units/m^2/dose
≥ 6 mo & < 12 mo	1875 International Units/m^2/dose
≥ 12 mo	2500 International Units/m^2/dose

Dexamethasone: Oral (PO), Nasogastric (NG) or Intravenous (IV)
Days: 1-14 and 15-21
Dose: Age based dosing. Administer full dose during Days 1-14 (3 doses per day) and **taper to zero (0) over Days 15-21**.

Age	Dose
< 6 mo	1.3 mg/m^2/dose TID
≥ 6 mo & < 12 mo	1.5 mg/m^2/dose TID
≥ 12 mo	2 mg/m^2/dose TID

Note: Suggested taper: reduce dexamethasone dose by 20-30% of the administered dose every 1-2 days, then discontinue. Consider tapering to BID or once daily schedule as clinically appropriate.

6-Thioguanine: Oral (PO) or Nasogastric (NG) daily
Days: 1-28
Dose: Age based dosing.

Age	Dose
< 6 mo	40 mg/m^2/dose
≥ 6 mo & < 12 mo	45 mg/m^2/dose
≥ 12 mo	60 mg/m^2/dose

Note: VinCRIStine, DAUNOrubicin, and the start of a 4-day cytarabine block on Days 9, 16, and 23 should be delayed and thioguanine interrupted if the ANC falls

to <300/µL or platelets < 30,000/µL. If a 4-day cytarabine block has been started, then cytarabine and thioguanine should not be interrupted.

VinCRIStine: Intravenous (IV) over 1 minute or infusion via minibag as per institutional policy
Days: 1, 8, 15, and 22
Dose: Age based dosing. Max dose 2 mg.

Age	Dose
< 6 mo	1 mg/m^2/dose
≥ 6 mo & < 12 mo	1.1 mg/m^2/dose
≥ 12 mo	1.5 mg/m^2/dose

Note: Round vinCRIStine dose to the nearest 0.01 mg.

Special precautions: FOR INTRAVENOUS USE ONLY.
The container or the syringe containing vincristine must be enclosed in an overwrap bearing the statement "Do not remove covering until moment of injection. For intravenous use only- Fatal if given by other routes."

Medication errors have occurred due to confusion between vinCRIStine and vinBLAStine. VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). The conventional and liposomal formulations are NOT interchangeable; use of the liposomal formulation is not permitted in this trial.

DAUNOrubicin: Intravenous (IV) over 1-15 minutes
Days: 1, 8, 15, and 22
Dose: Age based dosing

Age	Dose
< 6 mo	20 mg/m^2/dose
≥ 6 mo & < 12 mo	23 mg/m^2/dose
≥ 12 mo	30 mg/m^2/dose

Cytarabine: Intravenous (IV) push or Subcutaneous (SubQ)
Days: 2-5, 9-12, 16-19, 23-26
Dose: Age based dosing

Age	Dose
< 6 mo	50 mg/m^2/dose
≥ 6 mo & < 12 mo	56 mg/m^2/dose
≥ 12 mo	75 mg/m^2/dose

Note: Patients should have ANC ≥ 300/µL and platelets ≥ 30,000/µL to start each 4-day cytarabine block beginning on Days 9, 16, and 23.

Hydrocortisone: Intrathecal (IT)
Days: 1 and 15

Age:	Dose:
< 1 year	12 mg
≥ 1 year	16 mg

Cytarabine: Intrathecal (IT)
Days: 1 and 15

Age:	Dose:
< 1 year	15 mg
≥ 1 year	20 mg

See Section 5.0 for Dose Modifications on Toxicities.

Following completion of Delayed Intensification Part. 1, begin Azacitidine Epigenetic Block #4 (EPI#4, Section 4.9) on Day 36 or when ANC ≥ 500/µL and platelets ≥ 50,000/ µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).

4.9 Azacitidine EPI BLOCK #4

4.9.1 Therapy Delivery Map – Azacitidine EPI BLOCK #4 Following DI Pt. 1 and peripheral blood count recovery, azacitidine will be given as a pre-treatment prior to Delayed Intensification Part 2. Azacitidine therapy is 5 days.	_____ Patient COG ID number _____ DOB

Begin Azacitidine Block# 4 on Day 36 or when ANC ≥ 500/µL and Platelets ≥ 50,000/µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).Treatment details and criteria to start are in Section 4.9.3. This Therapy Delivery Map is **two (2)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Azacitidine (AZA) IND # 133688 Do not use commercial supply	IV over 10-40 mins	2.5 mg/**kg**/dose	1-5	Infusion must be completed within 45 minutes of vial reconstitution

Ht _____cm Wt _____kg BSA _____m^2

Date Due	Date Given	Day	Azacitidine _____mg	Studies
			Enter actual dose administered below	
		1	_____mg	a-c
		2	_____mg	
		3	_____mg	
		4	_____mg	
		5	_____mg	
		6	Continue to Delayed Intensification Part 2 (Section 4.10) on Day 6 irrespective of ANC and platelet counts.	

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

4.9.2 Required Observations in Azacitidine EPI BLOCK #4

a) History & physical exam (including length, weight, BSA, and performance status (if ≥ 12 months of age))
b) CBC/diff/ platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.9.3 Treatment Details for Azacitidine EPI BLOCK #4

Azacitidine will be given as pre-treatment prior to Delayed Intensification Part 2 therapy.

Criteria to Start Azacitidine EPI Block #4

Begin Azacitidine EPI Block #4 on Day 36 of DI Part. 1 or when peripheral counts recover with ANC ≥ 500/µL and platelets ≥ 50,000/µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).

Azacitidine: Intravenous (IV) over 10-40 mins
Days: 1-5
Dose: 2.5 mg/**kg**/dose
Note: infusion must be completed within 45 minutes of vial reconstitution
Do not use commercial supply.

See Section 5.0 for Dose Modifications on Toxicities.

Following completion of Azacitidine Epigenetic Therapy Block #4, begin Delayed Intensification Part 2 therapy (Section 4.10) on Day 6, irrespective of ANC and platelet counts.

4.10 Delayed Intensification PART 2

4.10.1 Therapy Delivery Map – DELAYED INTENSIFICATION PART 2

All patients will receive the same Delayed Intensification therapy with regards to agents and schedule. **All drugs are dosed based on age on Day 1 of DI Part. 2 and BSA, as outlined below**. Delayed Intensification Pt. 2 therapy is 3 weeks (21 days) duration.

_____ Patient COG ID number

_____ DOB

Following completion of EPI Block #4, begin on Day 6 irrespective of peripheral blood cell counts. Treatment details and criteria to start are in Section 4.10.3. This Therapy Delivery Map is **two (2)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
6-Thioguanine (TG)	PO or NG	Age / Dose < 6 mo: 40 mg/m²/dose ≥ 6 mo & < 12 mo: 45 mg/m²/dose ≥ 12 mo: 60 mg/m²/dose	1-14	
Cyclophosphamide (CPM)	IV over 15-30 min	Age / Dose < 6 mo: 330 mg/m²/dose ≥ 6 mo & < 12 mo: 375 mg/m²/dose ≥ 12 mo: 500 mg/m²/dose	1 & 15	Refer to Section 4.10.3 for admin guidelines.
Cytarabine (ARAC)	IV push or SubQ	Age / Dose < 6 mo: 50 mg/m²/dose ≥ 6 mo & < 12 mo: 56 mg/m²/dose ≥ 12 mo: 75 mg/m²/dose	2-5 & 9-12	Patients should have ANC ≥ 300/µL and platelets ≥ **30,000**/µL to start the 4-day cytarabine block beginning on Day 9.

Ht _____cm Wt _____kg BSA _____m²

Date Due	Date Given	Day	TG ____mg	CPM ____mg	ARAC _____mg	Studies
			Enter calculated dose above and actual dose administered below			
		1	____mg ↓	mg		a-c
		2			____mg	
		3			____mg	
		4			____mg	
		5			____mg	
		8				
		9			____mg	
		10			____mg	
		11			____mg	
		12			____mg	
		14				
		15		____mg		a-c
		22	Continue to Maintenance (Section 4.11) on Day 22 or when ANC ≥ 500/µL and platelets ≥ 50,000/µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).			

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

4.10.2 Required Observations in DELAYED INTENSIFICATION PART. 2

a) History & physical (including length, weight, BSA, and performance status (if ≥ 12 months of age))
b) CBC/diff/ platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.10.3 Treatment Details for DELAYED INTENSIFICATION PART 2

Administer drug doses based on the age on Day 1 of DI Part 2.

Criteria to Start Delayed Intensification Part 2
Begin Delayed Intensification Part 2 on Day 6 following Azacitidine Epigenetic Block #4 irrespective of peripheral blood cell counts.

See the Parenteral Chemotherapy Administration Guidelines (CAGs) on the COG website at: https://cogmembers.org/_files/disc/Pharmacy/ChemoAdminGuidelines.pdf for special precautions and suggestions for patient monitoring during the infusions. As applicable, also see the CAGs for suggestions on hydration, or hydrate according to institutional guidelines.

6-Thioguanine: Oral (PO) or Nasogastric (NG) daily
Days: 1-14
Dose: Age based dosing

Age	Dose
< 6 mo	40 mg/m^2/dose
≥ 6 mo & < 12 mo	45 mg/m^2/dose
≥ 12 mo	60 mg/m^2/dose

Note: The 4-day cytarabine block beginning on Day 9 should be delayed and thioguanine interrupted if the ANC falls to <300/µL or platelets < 30,000/µL. If a 4-day cytarabine block has been started, then cytarabine and thioguanine should not be interrupted.

Cyclophosphamide: Intravenous (IV) over 15-30 minutes
Days: 1 and 15
Dose: Age based dosing

Age	Dose
< 6 mo	330 mg/m^2/dose
≥ 6 mo & < 12 mo	375 mg/m^2/dose
≥ 12 mo	500 mg/m^2/dose

Cytarabine: Intravenous (IV) push or Subcutaneous (SubQ)
Days: 2-5 and 9-12
Dose: Age based dosing

Age	Dose
< 6 mo	50 mg/m^2/dose
≥ 6 mo & < 12 mo	56 mg/m^2/dose
≥ 12 mo	75 mg/m^2/dose

Patients should have ANC ≥ 300/µL and platelets ≥ **30,000**/µL to start the 4-day cytarabine block beginning on Day 9.

See Section 5.0 for Dose Modifications on Toxicities.

Following completion of Delayed Intensification Part 2, begin Maintenance therapy (Section 4.11) on Day 22 or when ANC > 500/µL and platelets > 50,000/µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).

4.11 Maintenance Cycle 1

4.11.1 Therapy Delivery Map –MAINTENANCE Cycle 1

All patients will receive the same Maintenance therapy with regards to agents and schedule. **Non-IT drugs are dosed based on age on Day 1 of Maintenance Cycle 1 and BSA, as outlined below. IT drugs are dosed based on age on day of administration.** Cycle 1 lasts 12 weeks (84 days).

_____ Patient COG ID number

_____ DOB

Begin Maintenance on Day 22 of DI Pt. 2 or when ANC ≥ 500/µL and platelets ≥ 50,000/ µL and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later) Details and criteria to start are in Section 4.11.3. This Therapy Delivery Map is **two (2)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES≥
Mercaptopurine (MP)	PO or NG	Age: ≥ 6 mo & < 12 mo — Dose: 38 mg/m²/dose; ≥ 12 mo — 50 mg/m²/dose	1-84	Refer to Section 4.11.3 for admin guidelines.
Methotrexate (MTX)	PO	Age: ≥ 6 mo & < 12 mo — Dose: 15 mg/m²/dose; ≥ 12 mo — 20 mg/m²/dose	Once weekly	Omit during weeks when IT MTX is administered. Refer to Section 4.11.3 for admin guidelines.
Intrathecal Methotrexate (IT MTX)	IT	Age (yr): < 1 — Dose: 6 mg; ≥ 1 — 8 mg	1	
.Intrathecal Hydrocortisone (IT HC)	IT	Age (yr): < 1 — Dose: 12 mg; ≥ 1 — 16 mg	1 & 57	
Intrathecal Cytarabine (IT ARAC)	IT	Age (yr): < 1 — Dose: 15 mg; ≥ 1 — 20 mg	57	

Ht_____cm Wt_____kg BSA_____m²

Date Due	Date Given	Day	MP ____mg	MTX ____mg	IT MTX ____mg	IT HC ____mg	IT ARAC ____mg	Studies
			Enter calculated dose above and actual dose administered below					
		1	____mg		mg	mg		a-d
		8		____mg				
		15		____mg				
		22		____mg				
		29		____mg				b
		36		____mg				
		43		____mg				
		50		____mg				
		57		____mg		____mg	____mg	b, d
		64		____mg				
		71		____mg				
		78		____mg				
		84						
		85	Following completion of Maintenance therapy Cycle 1, continue onto Maintenance therapy Cycle 2 on Day 85 per Section 4.12.					

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV and the COG Member website for Supportive Care Guidelines.

4.11.2 Required Observations in MAINTENANCE Cycle 1

a) History & physical exam (including length, weight, BSA, and performance status (if ≥ 12 months of age))
b) CBC/diff/ platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili
d) CSF cell count/diff/cytospin

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.11.3 Treatment Details for MAINTENANCE Cycle 1

Administer drug dosages based on the age on Day 1 of Maintenance Cycle 1.

Criteria to start Maintenance

Begin Maintenance on Day 22 of Delayed Intensification Part 2 or when peripheral counts recover with ANC ≥ 500/µL and platelets ≥ 50,000/µL, and resolution of mucositis and diaper area dermatitis to ≤ Grade 2 (whichever occurs later).

The administration schedule below describes the 12-week cycle of Maintenance therapy Cycle 1.

See the Parenteral Chemotherapy Administration Guidelines (CAGs) on the COG website at: https://cogmembers.org/_files/disc/Pharmacy/ChemoAdminGuidelines.pdf for special precautions and suggestions for patient monitoring during the infusions. As applicable, also see the CAGs for suggestions on hydration, or hydrate according to institutional guidelines.

Mercaptopurine: Oral (PO) or Nasogastric (NG)
Days: Days 1-84
Dose: Age based dosing

Age	Dose
≥ 6 mo & < 12 mo	38 mg/m^2/dose
≥ 12 mo	50 mg/m^2/dose

See Section 5.7 for suggested starting dose based on TPMT and NUDT15 status (if status is known)

It is strongly recommended that mercaptopurine be taken at the same time each day. The liquid or tablet formulation may be used.

Methotrexate: Oral (PO)
Days: Once weekly, omit during week 1.
Dose: Aged based dosing

Age	Dose
≥ 6 mo & < 12 mo	15 mg/m^2/dose
≥ 12 mo	20 mg/m^2/dose

Note: Administer the tablets on an empty stomach (at least 1 hour before or 2 hours after food or milk). Food or milk delays absorption and decreases the peak concentration.

Methotrexate: Intrathecal (IT)
Day: 1
Dose: Aged based dosing

Age:	Dose:
< 1 year	6 mg
≥ 1 year	8 mg

Hydrocortisone: Intrathecal (IT)
Days: 1 & 57
Dose: Aged based dosing

Age:	Dose:
< 1 year	12 mg
≥ 1 year	16 mg

Cytarabine: Intrathecal (IT)
Day: 57
Dose: Aged based dosing

Age:	Dose:
< 1 year	15 mg
≥ 1 year	20 mg

See Section 5.0 for Dose Modifications on Toxicities.

Following the completion of Maintenance therapy Cycle 1, continue onto Maintenance therapy Cycle 2 on Day 85, per Section 4.12.

4.12 Maintenance Cycle 2

4.12.1 Therapy Delivery Map –MAINTENANCE Cycle 2

All patients will receive the same Maintenance therapy with regards to agents and schedule. **Non-IT drugs are dosed based on age on Day 1 of Maintenance Cycle 2 and BSA, as outlined below. IT drugs are dosed based on age on day of administration**. Cycle 2 lasts 12 weeks (84 days).

Patient COG ID number

DOB

Treatment details and criteria to start are in Section 4.12.3. This Therapy Delivery Map is **two (2)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Mercaptopurine (MP)	PO or NG	Age / Dose ≥ 6 mo & < 12 mo: 56 mg/m²/dose ≥ 12 mo: 75 mg/m²/dose	1-84	Refer to Section 4.12.3 for admin guidelines.
Methotrexate (MTX)	PO	Age / Dose ≥ 6 mo & < 12 mo: 15 mg/m²/dose ≥ 12 mo: 20 mg/m²/dose	Once weekly	Omit during weeks when IT MTX is administered. Refer to Section 4.12.3 for admin guidelines.
Intrathecal Methotrexate (IT MTX)	IT	Age (yr) / Dose < 1: 6 mg ≥ 1: 8 mg	15	
.Intrathecal Hydrocortisone (IT HC)	IT	Age (yr) / Dose < 1: 12 mg ≥ 1: 16 mg	15	

Ht ____ cm Wt ____ kg BSA ____ m²

Date Due	Date Given	Day	MP mg	MTX mg	IT MTX mg	IT HC mg	Studies
			Enter calculated dose above and actual dose administered below				
		1	___ mg	___ mg			a-c
		8		___ mg			
		15			___ mg	___ mg	d
		22		___ mg			
		29		___ mg			b
		36		___ mg			
		43		___ mg			
		50		___ mg			
		57		___ mg			b
		64		___ mg			
		71		___ mg			
		78		___ mg			
		84					
		85	Following the completion of Maintenance therapy Cycle 2, continue onto Maintenance Therapy Cycle 3 on Day 85 per Section 4.13.				

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV the COG Member website for Supportive Care Guidelines.

4.12.2 Required Observations in MAINTENANCE Cycle 2

a) History & physical exam (including length, weight, BSA, and performance status (if ≥ 12 months of age))
b) CBC/diff/ platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili
d) CSF cell count/diff/cytospin

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.12.3 Treatment Details for MAINTENANCE Cycle 2

Administer drug dosages based on age on Day 1 of Maintenance Cycle 2.

Criteria to start Maintenance

Begin Maintenance Cycle 2 on Day 85 of Maintenance therapy Cycle 1.

The administration schedule below describes the 12-week cycle of Maintenance therapy Cycle 2.

See the Parenteral Chemotherapy Administration Guidelines (CAGs) on the COG website at: https://cogmembers.org/_files/disc/Pharmacy/ChemoAdminGuidelines.pdf for special precautions and suggestions for patient monitoring during the infusions. As applicable, also see the CAGs for suggestions on hydration, or hydrate according to institutional guidelines.

Mercaptopurine: Oral (PO) or Nasogastric (NG)
Days: 1-84
Dose: Age based dosing

Age	Dose
≥ 6 mo & < 12 mo	56 mg/m^2/dose
≥ 12 mo	75 mg/m^2/dose

See Section 5.7 for suggested starting dose based on TPMT and NUDT15 status (if status is known)

It is strongly recommended that mercaptopurine be taken at the same time each day. The liquid or tablet formulation may be used.

Methotrexate: Oral (PO)
Days: Once weekly. Omit during weeks when IT MTX is administered.
Dose: Age based dosing

Age	Dose
≥ 6 mo & < 12 mo	15 mg/m^2/dose
≥ 12 mo	20 mg/m^2/dose

Note: Administer the tablets on an empty stomach (at least 1 hour before or 2 hours after food or milk). Food or milk delays absorption and decreases the peak concentration.

Methotrexate: Intrathecal (IT)
Day: 15
Dose: Age based dosing

Age:	**Dose:**
< 1 year	6 mg
≥ 1 year	8 mg

Hydrocortisone: Intrathecal (IT)
Days: 15

Age:	Dose:
< 1 year	12 mg
≥ 1 year	16 mg

See Section 5.0 for Dose Modifications on Toxicities.

Following the completion of Maintenance therapy Cycle 2, continue onto Maintenance therapy Cycle 3 on Day 85 per Section 4.13.

4.13 Maintenance Cycle 3 and Subsequent Cycles

4.13.1 Therapy Delivery Map –MAINTENANCE Cycle 3 and Subsequent Cycles

All patients will receive the same Maintenance therapy with regards to agents and schedule. Each cycle of Maintenance will last 12 weeks (84 days), cycles are to be repeated until 2 years have passed from the start of Induction therapy. Use this TDM for the remainder of Maintenance Therapy. Note cycle number below.

_____
Patient COG ID number

_____
DOB

Treatment details and criteria to start are in Section 4.13.3. This Therapy Delivery Map is **two (2)** pages in length.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Mercaptopurine (MP)	PO or NG	Age ≥ 12 mo — Dose 75 mg/m^2/dose	1-84	Refer to Section 4.13.3 for admin guidelines.
Methotrexate (MTX)	PO	Age ≥ 12 mo — Dose 20 mg/m^2/dose	Once weekly	Refer to Section 4.13.3 for admin guidelines.

Enter Cycle #: _____ **Ht _____cm** **Wt _____kg** **BSA _____m^2**

Date Due	Date Given	Day	MP ____mg	MTX ____mg	Studies
			Enter calculated dose above and actual dose administered below		
		1	____mg	mg	a-c
		8		____mg	
		15		____mg	
		22		mg	
		29		mg	b
		36		____mg	
		43		mg	
		50		____mg	
		57		mg	b
		64		mg	
		71		mg	
		78		____mg	
		84			
		85	Continue Maintenance therapy until 2 years from the start of Induction therapy.		

See Section 5.0 for Dose Modifications for Toxicities and Appendix IV the COG Member website for Supportive Care Guidelines.

4.13.2 Required Observations in MAINTENANCE Cycle 3 and Subsequent Cycles

a) History & physical exam (including length, weight, BSA, and performance status (if ≥ 12 months of age))
b) CBC/diff/ platelets
c) Electrolytes/BUN/Cr/AST/ALT/total bili

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.13.3 Treatment Details for MAINTENANCE Cycle 3 and Subsequent Cycles.
Administer drug dosages based on age on Day 1 of each Maintenance cycle.

Criteria to start Maintenance
Begin Maintenance Cycle 3 on Day 85 of Cycle 2.

The administration schedule below describes one 12-week cycle of Maintenance therapy. Follow this schedule for Cycle 3 and all subsequent cycles. Maintenance is to be continued until 2 years from the start of Induction therapy.

See the Parenteral Chemotherapy Administration Guidelines (CAGs) on the COG website at:
https://cogmembers.org/_files/disc/Pharmacy/ChemoAdminGuidelines.pdf for special precautions and suggestions for patient monitoring during the infusions. As applicable, also see the CAGs for suggestions on hydration, or hydrate according to institutional guidelines.

Mercaptopurine: Oral (PO) or Nasogastric (NG)
Days: 1-84
Dose: Administer up to 75 mg/m^2/dose as tolerated.

See Section 5.7 for suggested starting dose based on TPMT and NUDT15 status (if status is known)

It is strongly recommended that mercaptopurine be taken at the same time each day. The liquid or tablet formulation may be used.

Methotrexate: Oral (PO)
Days: Once weekly
Dose: 20 mg/m^2/dose

Note: Administer the tablets on an empty stomach (at least 1 hour before or 2 hours after food or milk). Food or milk delays absorption and decreases the peak concentration.

See Section 5.0 for Dose Modifications on Toxicities.

Maintenance therapy continues until 2 years from the start of Induction therapy.

5.0 DOSE MODIFICATIONS FOR TOXICITIES

5.1 Azacitidine

5.1.1 Definition of Dose-Limiting Toxicity (DLT)

The DLT evaluation period consists of the first 3 courses of azacitidine in combination with chemotherapy (EPI #1 through Delayed Intensification Part 1) and until the patient meets counts requirements to begin the fourth course of azacitidine therapy (EPI #4).

A DLT is defined as any of the following that occurs during the DLT evaluation period:

- Any Grade 5 toxicity.
- Any Grade 3 or 4 toxicity that occurs during the 5 days of azacitidine AND results in:
  - The omission of 2 or more of the 5 planned doses of azacitidine, OR
  - The delay of the start of the first day of chemotherapy for ≥ 72 hours.
- Any Grade 3 or 4 toxicity that occurs during the 5 days of azacitidine and/or a chemotherapy course AND results in:
  - The delay of Consolidation Day 29 chemotherapy for ≥ 4 weeks from the expected start date.
  - The delay of the start of the subsequent course of therapy for ≥ 4 weeks from the expected start date (with the expected start date of EPI #2 as 2 weeks following administration of Consolidation Day 29 chemotherapy), OR
  - The removal of the patient from the planned protocol therapy.

5.1.2 Hematological Toxicity

Patients who experience hematological toxicity during any 5-day course of azacitidine will continue to receive full dose azacitidine. Chemotherapy will begin on Day 6 regardless of peripheral blood cell numbers.

Patients who experience a delay in the start of any azacitidine course of at least 4 weeks from the expected start date will be considered to have dose-limiting toxicity (see definition of DLT as defined above).

Patients with any Grade 3 or greater hematological toxicity that is considered by the local investigator to be immediately life-threatening, regardless of attribution or duration, will have doses of azacitidine withheld.

Missed doses of azacitidine will not be made up. If doses of azacitidine are withheld then azacitidine and/or Day 6 chemotherapy may not resume until the toxicity has resolved to ≤ Grade 2.

If the toxicity that caused the delay or cessation is NOT a DLT (as defined in Section 5.1.1), then azacitidine therapy may resume without a dose reduction with the next scheduled dose.

If the patient experiences a single DLT, then azacitidine will resume with a 30% dose reduction when the patient meets count parameters and for all remaining azacitidine doses in all remaining courses. If the patient experiences a second DLT of any type, the patient will be removed from protocol therapy.

Notify the Study Chair at the time azacitidine doses are withheld, or when a DLT occurs that may result in a dose reduction.

5.1.3 Non-Hematological Toxicity

Patients who experience any Grade 3 or greater toxicity that is of at least 48 hours duration and occurs on days when the patient is receiving scheduled doses of azacitidine (Days 1-5 of any course) will have azacitidine doses omitted until the toxicity has resolved to ≤ Grade 2. Specific exceptions will include febrile neutropenia, infection, constitutional symptoms, mucositis, and diaper area skin ulceration.

Patients with any Grade 3 or greater non-hematological toxicity that is considered by the local investigator to be immediately life-threatening, regardless of attribution or duration, will have doses of azacitidine withheld.

Missed doses will not be made up. If doses of azacitidine are withheld, then azacitidine and/or Day chemotherapy may not resume until the toxicity has resolved to ≤ Grade 2.

If the toxicity that caused the delay or cessation is NOT a DLT (as defined in Section 5.1.1), then azacitidine therapy may resume without a dose reduction with the next scheduled dose.

If the patient experiences a single DLT, then azacitidine will resume with a 30% dose reduction when the patient meets count parameters and for all remaining azacitidine doses in all remaining courses. If the patient experiences a second DLT of any type, the patient will be removed from protocol therapy.

Notify the Study Chair at the time azacitidine doses are withheld, or when a DLT occurs that may result in a dose reduction.

5.2 Cyclophosphamide

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

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

5.3 Cytarabine (ARAC)

Consolidation:
Patients should have ANC ≥ 300/µL and platelets ≥ 30,000/µL to start each 4-day cytarabine block beginning on Days 10, 17, and 24. Once a 4-day block has started, do not interrupt for uncomplicated myelosuppression.

Delayed Intensification Part 1:
Vincristine, daunorubicin, and the start of each 4-day cytarabine block on Days 9, 16, and 23 should be delayed and thioguanine interrupted if the ANC falls to <300/µL or platelets < 30,000/µL. If a 4-day cytarabine block has been started, then cytarabine and thioguanine should not be interrupted.

Delayed Intensification Part 2:
The start of the 4-day cytarabine block on Day 9 should be delayed and thioguanine interrupted if the ANC falls to <300/µL or platelets < 30,000/µL. If a 4-day cytarabine block has been started, then cytarabine and thioguanine should not be interrupted.

Cytarabine Syndrome: Do not withhold cytarabine for fever if it is likely to have been caused by the cytarabine. Obtain blood cultures if a central line is present. For rash or conjunctivitis, withhold for Grade 3-4 toxicity until resolved. Make up missed doses and consider concurrent treatment with hydrocortisone or dexamethasone, and/or with dexamethasone ophthalmic drops for conjunctivitis.

Adequate renal function (defined as creatinine within normal range) is required for the administration of high dose cytarabine. Creatinine Clearance should be measured for patients with elevated creatinine or suspected renal insufficiency. For CrCl < 60 mL/min/1.73 m^2, hold pending recovery and omit if recovery requires > 3 weeks.

5.4 Daunorubicin (Anthracycline)

Cardiac Toxicity: Discontinue for clinical or echocardiographic evidence of cardiomyopathy (SF < 27% or EF < 50%) or Grade 3-4 left ventricular systolic dysfunction (LVSD) per CTCAE version 4.0.

Delayed Intensification Part 1:
Vincristine, daunorubicin, and the start of each 4-day cytarabine block on Days 9, 16, and 23 should be delayed and thioguanine interrupted if the ANC falls to <300/µL or platelets < 30,000/µL.

Note: use the following updated term to report decreases in the EF or SF: *cardiac disorders-others*

Myelosuppression (beyond Induction): If patient has severe infection or severe mucositis (Grade 3-4) and an ANC < 500/µL delay anthracycline during phases other than Induction. During Induction, continue with anthracycline administration. Subsequent doses should be given at full dose.

Hyperbilirubinemia:[31]

Direct Bili	% Dose Reduction
< 1.2 mg/dL	Full dose
1.2 – 3.0 mg/dL	50%
3.1 – 5.0 mg/dL	75%
> 5.0 mg/dL	Withhold dose and administer next scheduled dose if toxicity has resolved. Do not make up missed doses.

Extravasation:
In the event of an extravasation, discontinue the IV administration of the drug and institute appropriate measures to prevent further extravasation and damage according to institutional guidelines. Also see:
https://members.childrensoncologygroup.org/_files/disc/Nursing/extravasationreference.pdf for COG extravasation management reference.

5.5 Intrathecal Methotrexate

Do not withhold dose given on Day 1 of Induction.

Systemic toxicity: The dosage for IT methotrexate will not be reduced for systemic toxicity (myelosuppression, mucositis, etc.). Instead, leucovorin may be used at a dose of 5 mg/m^2/dose every 12 hours x 2 doses, beginning 48 hours after the IT therapy has been delivered. This may reduce the risk of worsening already existent myelosuppression (ANC < 500/µL) or mucositis. Do not administer leucovorin solely to prevent myelosuppression.

Dose modifications following an episode of acute neurotoxicity:
Neurotoxicity has extremely protean manifestations, ranging from transient events, seizures or episodes of acute hemiparesis, to severe necrotizing encephalopathies.[32-34] These toxicities are poorly understood and currently it is impossible to predict who will suffer these complications. In addition, there are no data clearly linking the occurrence of an acute neurotoxic event with an increased risk of long-term neurocognitive dysfunction, nor do changes present on MRI at the time of an acute event clearly correlate with or predict outcome.[34-39] It is clear however, that CNS prophylaxis is a mandatory component of curative therapy for children with ALL. Effective prophylaxis generally takes 2 forms; cranial, or less commonly, craniospinal radiation, with a limited number of doses of IT therapy or prolonged IT therapy with either IT MTX or triple IT therapy (MTX, cytarabine and hydrocortisone). Certain protocols, for example BFM 2000,[40]include fewer doses of IT MTX, with an acceptably low frequency of CNS relapse, but the backbone of the BFM therapies is not the same as those currently used by the Children's Oncology Group. The exclusive use of IT cytarabine has not been studied or described in the context of ALL therapy nor can one demonstrate the safety of omitting multiple doses of IT therapy without concomitant use of cranial irradiation or high dose methotrexate.

The following guidelines are offered for consideration following an acute event, but it must be recognized that there are little data to support these approaches or any others. Thus the treating physician must evaluate the patient and, with the family, make the best possible decision with respect to the relative risk and benefit of continued therapy.

Following an acute neurotoxic event, a history and physical exam should guide the differential diagnosis. A neurology consult may be of value and should be considered.

Seizures and other transient events may be linked to fever, infection, encephalitis, meningitis, hypertension, electrolyte disturbance, hypoglycemia, trauma, intracranial hemorrhage or thrombosis, narcotic withdrawal, or other causes in addition to the direct side effects of chemotherapy. Appropriate laboratory studies may include, but are not limited to, blood cultures, a CBC, electrolytes, including glucose, calcium, magnesium and phosphorus, renal and liver function studies and/or an examination of the CSF. Imaging studies may include a CT scan and/or an MRI. The CT is commonly normal, in the absence of stroke, but if calcifications are present, this finding may be indicative of a more severe mineralizing leukoencephalopathy.[41] MRI abnormalities may be pronounced, but transient. Posterior reversible encephalopathy may be present on MR with extensive diffusion abnormalities, but these do not appear to correlate with subsequent demyelination or gliosis.[42-44] Additional studies, including MR angiography and/or venogram should be considered, if clinically indicated (e.g., focal deficits).

Many acute events, seizures or episodes of transient hemiparesis, are temporally related to the administration of intrathecal therapy, commonly 9 to 11 days after the IT administration.[45] For patients who return to their "pre-event" status, without residual deficits of physical or neurologic exam, there are few data to support or guide therapeutic nterventions. It is reasonable to hold the next dose of IT therapy, or, substitute IT cytarabine for 1 dose of IT MTX, or triple IT therapy. It is also reasonable to include leucovorin rescue at a dose of 5 mg/m^2 q 12 hrs x 2 doses beginning 48 hours after the LP. This pattern of rescue was associated with a clear diminution in the incidence of acute neurotoxicity in one case series.[45] There have been questions about potential interference of leucovorin with the efficacy of the IT MTX, but there are little data to support or refute this position. Moreover, the administration 48 hours later would minimize any potential interference. If the event does not recur, resumption of standard therapy should be considered, following 1 modified or omitted IT dose. In the face of multiply recurrent events, or evidence of progressive encephalopathy, another evaluation is warranted and the treating physician may consider a more prolonged or definitive change in therapy. These decisions are extremely difficult and may hinge on an individual's view of the importance of quality of life versus an increase in the risk of relapse. Since the greatest impact of CNS prophylaxis occurs early in therapy, the timing of these events may also influence clinical decisions. Cranial radiation has been suggested as an alternative to continued IT therapy though much of the literature on long-term neurocognitive dysfunction supports a more deleterious effect from CRT than IT therapy.[46-49] Dramatic deviations from protocol recommended therapy might result in the child being taken off protocol therapy.

The use of dextromethorphan (DM) has been suggested as a neuroprotectant, capable of preventing NMDA mediated neurotoxicity without prohibitive toxicity. Low dose therapy has been recommended, in part, based on data suggesting that DM is concentrated in brain relative to serum. However, the literature on the use of DM supports a tight dose response relationship, with the likelihood of sparing an initially unaffected area, following ischemic damage, linked to dose, in both clinical trials and animal models of CNS ischemia.[50-53] At doses thought to be therapeutic, side effects have included nystagmus, nausea and vomiting, distorted vision, ataxia, and dizziness. In addition, Hollander *et al* [54] have raised concerns about the potential deleterious effects of long-term NMDA receptor blockade on memory because hippocampal long-term potentiation is dependent on the activation of the NMDA receptor. Thus in the absence of a clinical trial there are few data to support the addition of DM.

<u>Hydrocephalus, microcephaly or known abnormality of CSF flow precluding intrathecal chemotherapy via lumbar puncture</u>:

Intraventricular chemotherapy via Ommaya catheter may be used in place of intrathecal therapy delivered by LP. Intraventricular chemotherapy should be given according to the same schedule, but at **50% of the corresponding age-based doses** that would be given by LP. NOTE: Obstruction to CSF flow may be a contraindication to intrathecal and/or intraventricular therapy.

Viral, bacterial, or fungal meningitis: Omit until resolved.

5.6 High Dose Methotrexate (HD MTX) and Leucovorin Rescue

[*Please note that **HD methotrexate** refers to* IV *methotrexate **5g/m^2 given over 24** **hrs***]
Review of methotrexate dosing on BFM-based protocols indicated that excessive methotrexate toxicity has not been encountered in patients larger than 2 m^2 who receive more than 10 grams of methotrexate. The investigator should base the methotrexate on the patient's meter-squared dosing and not cap at 10 grams of methotrexate.

5.6.1 HD Methotrexate Infusion Guidelines

See Appendix III for a flowchart of the High Dose methotrexate / leucovorin guidelines.

Hold trimethoprim-sulfamethoxazole on the days of HD methotrexate infusion and for at least 72 hours after the start of the HD methotrexate infusion and until the methotrexate level is less than 0.1 μM.

Hold any nonsteroidal anti-inflammatory medications, penicillins, proton pump inhibitors or aspirin-containing medications on the day of HD methotrexate infusion and for at least 72 hours after the start of the HD methotrexate infusion and until the methotrexate level is less than 0.1 μM.

Infants receiving therapeutic doses of amphotericin should have that drug withheld on the day HD methotrexate is administered and for the following 24 hours due to the risk of delayed methotrexate excretion, renal dysfunction and resultant toxicity.

Recommended Prehydration with D5 ¼ NS with 30 mEq $NaHCO_3$/L at 125 mL/m^2/hour until urine specific gravity is ≤ 1.010 and pH is ≥ 7.0 and ≤ 8.0. Ringers Lactate may be used as the initial fluid if a bicarbonate containing solution is unavailable. Adjust fluid volume and sodium bicarbonate to maintain urine specific gravity and pH at above parameters. An acetate or bicarbonate bolus (0.5-1 mEq/kg over 15 min) may be given to raise the urine pH relatively quickly, a normal saline bolus may also be helpful in facilitating hydration. Recommend hydration for a minimum of 54 hours after the methotrexate bolus is started for patients who meet expected clearance parameters. In patients with delayed methotrexate clearance, continue hydration and leucovorin as instructed (Appendix III) until the plasma methotrexate concentration is below 0.1 μM.

Hour 0: methotrexate 10 % of the total dose to be administered IV infused over 30 minutes. This is followed, immediately, by the remaining 90% of the methotrexate dose given by continuous IV infusion over 23.5 hours. Be certain that the HD methotrexate infusion is completed in the 24 hour period. Unintentional prolongation to as long as 26 hours though not encouraged is acceptable.

Hours 24, (36), 42 and 48: Draw methotrexate level and serum creatinine; NOTE: 36 hour level is only drawn if needed (see below)

For methotrexate levels that exceed these expected values modify the rescue regimen as noted below and increase hydration to 200 mL/m^2/hr, monitor urine pH to assure a value ≥ 7.0 and monitor urine output to determine if volume is ≥ 80% of the fluid intake, measured every 4 hours. If serum creatinine rises significantly, at any time point, assure appropriate urine pH and urine volume as above and draw a 42 hour level. If urine output fails to continue at 80% of the fluid intake, consider furosemide. Regardless of urine output, also consider glucarpidase (carboxypeptidase G_2) (see below). For patients with delayed clearance during a previous course, begin the following course with the increased hydration (200 mL/m^2/hr). If subsequent course is not associated with delayed clearance, attempt to use standard hydration.

If the 24 hour level is < 150 µM draw the next level at hour 42 and refer to table below.

If the 24 hour level is ≥ 150 µM and/or creatinine > 125% baseline, repeat level if methotrexate contamination is possible. If the value is "real" refer to the changes in hydration, etc. described above and repeat the level with a serum Cr at hour 36. Then refer to the table below.

If the 42 and 48 hour levels are ≤ 1 and 0.4 µM, respectively, give Leucovorin at 15 mg/m^2 IV/PO at 42, 48 and 54 hours post the start of methotrexate loading dose. Continue to check methotrexate level every 12-24 hours and give Leucovorin 15 mg/m^2 IV/PO every 6 hours until the methotrexate level is <0.1µM.

(36 hr MTX level)	42 hr MTX level	48 hr MTX level	Leucovorin Rescue++
Only required if 24 hr level is ≥ 150 µM. See below for guidelines**	1.01 to 9.9 µM	0.41 to 5.9 µM	Continue 15 mg/m^2 q 6h until MTX level < 0.1 µM (draw q12-24 h).
	10 to 19.9 µM	6 to 9.9 µM	Increase to 15 mg/m^2 q 3h until MTX level < 0.1 µM (draw q 6-24 h). Consider glucarpidase.
	20 to 200 µM	10 to 100 µM	Increase to 100 mg/m^2 q 6h until MTX level < 0.1 µM (draw q 6-24 h). Consider glucarpidase.
	> 200 µM	> 100 µM	Increase to 1000 mg/m^2 q 6h until MTX level < 0.1 µM (draw q 6-24 h). Consider glucarpidase.

** **If the 36 hour level exceeds 3** µM, increase hydration to 200 mL/m^2/hr, monitor urine pH to assure a value ≥ 7.0 and monitor urine output to determine if volume is ≥ 80% of the fluid intake, measured every 4 hours. If urine output fails to continue at 80% of the fluid intake, consider furosemide. Regardless of urine output, also **consider glucarpidase if 36 hour methotrexate (MTX) level exceeds 10** µM (see below).

++ If the level is high at hour 36 or 42, but then the patient "catches up" and the level falls to the expected values of ≤ 1 and/or ≤ 0.4 µM at hours 42 and 48, respectively, resume standard leucovorin and hydration as long as urine output remains satisfactory.

Nephrotoxicity: Postpone course if pre-treatment (methotrexate) serum creatinine is > 1.5 x baseline or GFR creatinine clearance < 65 mL/minute/1.73m^2. If renal function does not recover, omit methotrexate. Do not give HD methotrexate to a patient with this degree or renal impairment, assuming that prolonged excretion can be managed with glucarpidase.

NOTE: For patients who have markedly delayed methotrexate clearance secondary to renal dysfunction, consider using glucarpidase (carboxypeptidase G_2, Voraxaze™).[55,56] To obtain supplies of glucarpidase in the US contact the Voraxaze 24-hour Customer Service line at 855-786-7292. Additional information can be found at http://www.btgplc.com/products/specialty-pharmaceuticals/voraxaze regarding product availability through ASD Healthcare, Cardinal, and McKesson. Canadian sites should contact McKesson at (877) 384-7425 for further information. Sites in Australia and New Zealand should contact Hospira at 1300-046-774 (local) or medicalinformationAUS@hospira.com. Patients requiring glucarpidase rescue will remain on protocol therapy.

Liver Dysfunction: Samples for the determination of ALT value must be drawn within 72 hours, PRIOR to a course of intravenous methotrexate. Blood samples for ALT should not be drawn following the start of methotrexate infusions as methotrexate causes significant short term elevation in ALT levels.

ALT	IV MTX
< 10 X ULN	Continue with therapy as scheduled
10 – 20 X ULN	Continue with therapy as scheduled for 1 cycle
10 – 20 X ULN for 2 consecutive cycles	Discontinue TMP/SMX* Hold therapy until ALT < 10 X ULN, then resume at full doses at point of interruption. Do not skip doses.
> 20 X ULN	Hold therapy until ALT < 10 X ULN, then resume at full doses at point of interruption. Do not skip doses.
> 20 X ULN for > 2 weeks	Evaluate with AST, Bili, Alkaline phosphatase, PT, albumin, total protein, and hepatitis A, B, C, CMV, and EBV serologies. Consider liver biopsy before additional therapy given. Notify Study Chair.

* Please see Supportive care Guidelines in Appendix IV

Hold IV methotrexate for direct hyperbilirubinemia of > 2.0 mg/dL.

Mucositis: For Grade 3-4 mucositis, withhold IV methotrexate until resolved. Increase leucovorin rescue following the next course from 3 to 5 doses on a q6 hr schedule. If subsequent course is not associated with Grade 3-4 mucositis, attempt to decrease the leucovorin. If mucositis recurs despite the extended leucovorin, decrease the dose of methotrexate by 25%, increase hydration to 200 mL/m^2/hr and continue increased leucovorin as above. Should subsequent courses be well tolerated, use a stepwise approach to resuming a standard approach to drug delivery. Consider culturing lesions for herpes simplex if mucositis persists or recurs.

Myelosuppression: All chemotherapy should be held for ANC < 750/µL and platelets < 75 000/µL.

5.7 PO Methotrexate (MTX) and 6-Mercaptopurine (MP)

Consolidation

If possible, mercaptopurine therapy should not be interrupted. If a cytarabine block has to be postponed or interrupted, then mercaptopurine should also be interrupted. Omitted mercaptopurine doses should be made up until the planned cumulative total dose 1680 mg/m^2 (60 mg/m^2 x 28 doses) for infants ≥ 12 months, 1260 mg/m^2 (45 mg/m^2 x 28 doses) for infants 6 to < 12months, 1120 mg/m^2 (40 mg/m^2 x 28 doses) for infants < 6 months.

Interim Maintenance:

If Day 8 HD methotrexate is delayed due to toxicity, then interrupt mercaptopurine. Resume mercaptopurine when Day 8 HD methotrexate is given and complete the planned 14 days of mercaptopurine.

Maintenance:

If neutrophil count falls below 500/µL or if platelet count falls below 50 000/µL during Maintenance, mercaptopurine and methotrexate will be held until recovery above these levels. For the first drop in ANC or platelets, resume chemotherapy (both mercaptopurine and methotrexate) at the same dose the patient was taking prior to the episode of myelosuppression. If neutrophil count falls below 500/µL or if platelet count falls below 50 000/µL for a second (or greater) time, discontinue doses of mercaptopurine and methotrexate until ANC is ≥ 750/µL and platelets are ≥ 75 000/µL. Restart both mercaptopurine and methotrexate at 50% of the dose prescribed at the time the medication was stopped. Then continue to increase to 75% and then 100% of the dose prescribed prior to stopping the medication at 2-4 week intervals provided ANC remains ≥ 750/µL and platelets remain ≥ 75 000/µL. May increase both mercaptopurine and methotrexate simultaneously. Consider discontinuing TMP/SMX as per COG Supportive care Guidelines in Appendix IV. If neutrophil count falls below 500/µL or if platelet count falls below 50 000/µL on > 2 occasions during Maintenance, perform thiopurine pharmacology testing as described below. Should therapy be withheld for myelosuppression or elevated transaminase, do not "make up" that week. Resume therapy at the correct point, chronologically.

Dose escalation during Maintenance:

This protocol includes a planned dose escalation of mercaptopurine from 50 mg/m^2/day in Maintenance Cycle 1 to 75 mg/m^2/day in Maintenance Cycle 2. Proceed with the dose escalation in Cycle 2 if ANC ≥ 500/µL and platelets ≥ 50 000/µL.

Beginning in Maintenance Cycle 2, for ANC ≥ 1500/µL on 3 CBC(s) done over 6 weeks or 2 successive monthly CBC(s) alternately increase doses of methotrexate or mercaptopurine by 25%. As a general rule, do not increase doses more often than every 4 weeks. If both methotrexate and mercaptopurine are increased once without a fall in ANC, consider noncompliance as a possibility. Noncompliance can be assessed by obtaining a sample for RBC thioguanine nucleotides (TGNs). Consider observing the administration of an oral dose of methotrexate and checking plasma methotrexate concentration 2-4 hours later. This will document whether or not poor absorption contributes to lack of response and may facilitate discussions about noncompliance.

Mucositis Grade 3-4:

Methotrexate should be reduced to 50% if Grade 3 toxicity develops; withhold in the presence of Grade 4 toxicity until there is a resolution, then resume at 50% of original dose with gradual dose escalation. If mucositis persists or recurs, consider culturing for herpes simplex.

Liver Dysfunction:
For increase in hepatic transaminases (SGPT/ALT or SGOT/AST) to greater than 5x ULN consistent with Grade 3 toxicity, obtain total bilirubin. Monitor SGPT/ALT or SGOT/AST and total bilirubin every 2 weeks during Consolidation and every 4 weeks during Maintenance as long as transaminases remain over 5x ULN.

Continue full dose therapy unless either of the following occurs:

1) Direct bilirubin > 2.0 mg/dL

2) SGPT/ALT or SGOT/AST > 20x ULN (consistent with Grade 4 toxicity) on 2 determinations at least 1 week apart.

If either of these occurs, hold methotrexate and monitor labs as above, weekly. Restart at full dose therapy when the transaminase is less than 5x ULN, if bilirubin is normal. If liver dysfunction persists, consider a trial period with methotrexate but without mercaptopurine, especially if red cell mercaptopurine methylated derivatives are elevated. Also consider liver biopsy.

Exclude infectious hepatitis (A, B, C) for persistent (> 1 month) elevations in SGPT/ALT or SGOT/AST above 5x ULN.

Pharmacology Testing (TPMT and NUDT15) and Dosage Adjustments:
mercaptopurine and 6-TG are methylated directly by thiopurine methyltransferase (TPMT) to an inactive metabolite. TPMT activity varies tremendously among patients, because of a common inherited genetic defect in TPMT. One in 300 patients is completely deficient (homozygous defective) and 10% of the population are moderately deficient in TPMT activity because they have inherited one variant (non-functional) TPMT allele (i.e., heterozygotes). [57-60] Patients with low TPMT form higher concentrations of the 6-thioguanine nucleotides (6-TGN) and are more susceptible to acute thiopurine toxicity (primarily myelosuppression, involving neutropenia, thrombocytopenia, and anemia). Patients with the complete deficiency of TPMT tolerate less than 10% of protocol doses of mercaptopurine (10 to 30 mg/m^2/day 3 days per week). About 35% of heterozygotes require a lower dose of mercaptopurine to avoid dose-limiting myelosuppression. [61]

Recently, germline variants in the gene encoding the nucleoside diphosphate–linked moiety X-type motif 15 (*NUDT15*) were reported in approximately 4% of Hispanic/Native American and nearly 10% of East Asian children with ALL; these polymorphisms are strongly associated with 6-mercaptopurine intolerance.[62] There are now CLIA certified tests for TPMT genotype and phenotype, *NUDT15* polymorphism, and for measurement of thiopurine metabolites (6-methyl mercaptopurine [6-MMP] and 6-TGN) measurements. Only 3 SNPs constitute well over 90% of the inactivating mutations in the gene, based on studies in numerous racial and ethnic groups worldwide. [57,63-66] Thus, the genotyping test has a low false negative rate, and may be preferable to TPMT phenotype testing in cases where a history of red cell transfusions would potentially confound assessments of RBC TPMT activity. When the genotyping result is coupled with a phenotyping test for TPMT or with thiopurine metabolite concentrations in erythrocytes, the reliability of the tests will be even greater. Moreover, metabolite levels can provide an index of patient compliance with thiopurine therapy.

Recommendations for Thiopurine Monitoring and Dosage Adjustments:
When myelosuppression has led to significant delays in therapy (> 2 weeks) or is disproportionate to the therapy, thiopurine testing should be performed:

- For subjects who have received full dose thiopurine therapy during the 2 weeks immediately preceding the test, RBC thiopurine metabolites will likely predict TPMT status and actual thiopurine exposure.
- In the absence of RBC transfusions for 3 months prior, TPMT activity will accurately reflect TPMT status
- TPMT genotyping will be informative in all subjects, if at least 1 mutant allele is identified. If not, and myelosuppression continues, send samples for TPMT activity and/or metabolites since TPMT genotyping will miss 5%-10% of mutants. *Genotyping can be done despite recent transfusions.*

Suggested Dose Adjustments in Subjects With Unacceptable Myelosuppression:

- If the subject is *homozygous deficient* for TPMT or NUDT15, the thiopurine dose should be *reduced to* 10-20 mg/m^2/day 3 days per week. If the subject is *heterozygous for TPMT and* has experienced significant myelosuppression, the thiopurine dose should be reduced by 30%-50%. It is not yet clear how the dose of thiopurine should be adjusted for patients who are heterozygous for NUDT15 but such patients should be monitored carefully while on thiopurines. If a patient is has two polymorphisms in NUDT15 (ie heterozygous for both the R139C and the R139H), they should be treated as if they are homozygous deficient. Gradual dose escalations should be attempted as outlined below.

- Do not increase the dose in response to a high ANC for 4 weeks to allow for achievement of steady state. All other myelosuppressive medications should be delivered at full dose, and the thiopurine dose should be titrated based on blood counts. Further thiopurine pharmacologic measures are not often necessary.

- If the subject is homozygous wild-type (high activity) for TPMT or NUDT15, then discontinue TMP/SMX and use pentamidine or dapsone. For modifications of the oral mercaptopurine and methotrexate see the beginning of this section (5.7).

5.8 Pegaspargase [Asparaginase (Peg-Asparaginase) or Erwinia]

Allergy

Local Allergic Reactions (inflammation at injection site, swelling): Note these recommendations only apply when the asparaginase product is administered intramuscularly. Continue asparaginase administration in the presence of Grade 1 allergy as defined by CTCAE v4.0 (transient flushing or rash; drug fever < 38ºC).

Systemic Allergic Reactions: In the event of Grade 1 reactions, characterized by transient flushing or rash and drug fever < 38ºC, without the need for treatment with antihistamines or steroids, the dose of asparaginase being administered intravenously may be continued with close observation.

Discontinuation is recommended for Grade 2 or higher allergic reactions as defined by CTCAE v4.0, which require medical intervention.

Note: Premedication with antihistamines to decrease the risk of overt allergy symptoms is strongly discouraged since anti-histamine use may mask the appearance of systemic allergy. Systemic allergy is frequently associated with the presence of asparaginase neutralizing antibodies, which render asparaginase therapy ineffective. In the event of

severe systemic or recurrent local allergic reaction, *Erwinia chrysanthemi* asparaginase (Erwinaze®), which is FDA-approved for this indication, should be substituted.

Therapeutic Drug Monitoring (TDM): TDM of asparaginase activity is available as a CLIA approved assay from AIBioTech®. Centers may elect to discontinue pegaspargase and switch to *Erwinia* asparaginase based upon laboratory evidence of silent inactivation of asparaginase activity in the absence of clinical symptoms of hypersensitivity at their discretion.

Anaphylaxis

Discontinue pegaspargase if the patient develops Grade 3 anaphylaxis as defined by CTCAE v4.0 (symptomatic bronchospasm, with or without urticaria, parenteral intervention indicated; allergy-related edema/angioedema; hypotension). If this occurs, Erwinia asparaginase should be substituted.

Erwinia asparaginase has a shorter half-life and is associated with a shorter duration of asparagine depletion than native *E. coli* asparaginase, with "head-to-head" comparisons of *Erwinia* and *E. coli* asparaginase, using the same dose and schedule for both preparations, demonstrating a superior outcome, favoring *E. coli* asparaginase.[67,68] Pegaspargase has a longer half-life and is associated with more prolonged asparagine depletion than native *E. coli* asparaginase, but the largest randomized trial comparing weekly native to bi-weekly pegaspargase wasn't powered to detect a difference in outcome.[69] Current COG trials have adopted pegaspargase as the preparation of choice, based on the results of CCG 1962.[70] COG AALL07P2 showed that *Erwinia* asparaginase was well tolerated and achieved nadir serum asparaginase activity at both 48 and 72 hours after dosing that was similar to that achieved with pegaspargase. Based on these and other data, the FDA initially approved *Erwinia* asparaginase for use following allergy to pegaspargase, with a dose of Erwinia 25,000 International Units/m^2 x 6 doses IM on a Monday/Wednesday/Friday schedule substituted for a single dose of pegaspargase. In December 2014, the FDA expanded its approval to include intravenous as well as intramuscular administration.

The dose modification guidelines for ALL trials recommend the substitution for replacement of Erwinia asparaginase for either native or pegaspargase utilizing the following schedule. For each dose of 2500 International Units/m^2 of pegaspargase, replace with *Erwinia* 25,000 International Units/m^2/dose IM or IV Monday/Wednesday/Friday x6 doses. AALL15P1 includes age based doses of pegaspargase, including doses lower than 2500 International Units/m^2. For infants on this protocol, replace pegaspargase with 6 doses of *Erwinia* asparaginase M/W/F at a ratio of 1:10 International Units (e.g. the replacement dose for 2,000 International Units/m^2 of pegaspargase is 20,000 International Units/m^2/dose of *Erwinia*). If a patient develops a Grade 3 or higher anaphylaxis to *Erwinia*, discontinue future asparaginase therapy. Consider discontinuation for severe Grade 2 or higher allergic reactions.

To replace a dose of intravenous pegaspargase that was discontinued during the infusion due to an allergic reaction, the following recommendations may be used to guide patient care.

In the event that a pegaspargase infusion is discontinued for an allergic reaction, regardless of amount received, substitution with *Erwinia* asparaginase should begin approximately 48 hours after pegaspargase has been discontinued and preferably to coincide with the recommended Monday/Wednesday/Friday administration schedule detailed above in

patients who are clinically stable. Up to 6 doses of *Erwinia* asparaginase may be administered, as tolerated, to replace the incomplete intravenous pegaspargase dose. Of note, *Erwinia* asparaginase is recommended only for pegaspargase hypersensitivity reactions, and not for pancreatitis, hepatitis, coagulation abnormalities, or other non-hypersensitivity toxicities associated with pegaspargase. To best suit the needs of each individual patient, additional modifications to these recommendations may be made at the discretion of the treating physician.

Coagulopathy: If symptomatic, hold asparaginase until symptoms resolve, then resume with the next scheduled dose. Consider factor replacement (FFP, cryoprecipitate, factor VIIa). Do not withhold dose for abnormal laboratory findings without clinical symptoms.

Hyperbilirubinemia: asparaginase may need to be withheld in patients with an elevated direct bilirubin, since asparaginase has been associated with hepatic toxicity. No specific guidelines are available.

Hyperglycemia: Do not modify dose. Treat hyperglycemia as medically indicated.

Hyperlipidemia: Do not modify dose

Ketoacidosis: Hold asparaginase until blood glucose can be regulated with insulin.

Pancreatitis: Discontinue asparaginase in the presence of Grade 3 or 4 pancreatitis. In the case of asymptomatic Grade 2 pancreatitis (enzyme elevation or radiologic findings only), asparaginase should be held until symptoms and signs subside, and amylase/lipase levels return to normal and then resumed. Grade 3 or 4 pancreatitis is a contraindication to additional asparaginase administration.

Thrombosis: Withhold asparaginase until resolved, and treat with appropriate antithrombotic therapy, as indicated. Upon resolution of symptoms consider resuming asparaginase, while continuing LMWH or antithrombotic therapy. Do not withhold dose for abnormal laboratory findings without clinical correlate. For significant thrombosis, which is not catheter-related, consider evaluation for inherited predisposition to thrombosis.

CNS Events (bleed, thrombosis or infarction): Hold asparaginase. Treat with FFP, factors or anticoagulation as appropriate. Consider resuming at full dose when all symptoms have resolved (and evidence of recanalization in case of thrombosis by CT/MRI). Consider evaluation for inherited predisposition to thrombosis.

5.9 Steroids (Dexamethasone and Prednisone)

Hypertension: Dose should not be reduced. Sodium restriction and anti-hypertensives should be employed in an effort to control hypertension. Avoid calcium channel blockers due to their potential prohemorrhagic effect.

Hyperglycemia: Dose should not be reduced for hyperglycemia. Rather, insulin therapy should be employed to control the blood glucose level.

Pancreatitis: Do not modify dose for asymptomatic elevations of amylase and/or lipase. Discontinue steroids, except for stress doses, in the presence of hemorrhagic pancreatitis or severe pancreatitis.

Osteonecrosis (ON): Do not modify corticosteroid therapy for osteonecrosis (also referred to as avascular necrosis) during Induction or Delayed Intensification.

Varicella: Steroids should be held during active infection except during Induction. Do not hold during incubation period following exposure.

Inability to use oral doses:
For dexamethasone, substitute the IV preparation mg for mg. For prednisone, substitute IV methylprednisolone at 80% of the oral prednisone dose. Note that if substituting oral prednisolone for prednisone, the doses are the same; prednisone is converted in the liver to prednisolone.

Severe infection: Do not hold or discontinue steroids during Induction without serious consideration, as this is a critical period in the treatment of ALL. Later in therapy, one may consider holding steroid until patient achieves cardiovascular stability, except for "stress doses."

Severe psychosis: Dexamethasone dose may be reduced by 50% for severe psychosis. If symptoms persist, consider switching to an equivalent dose of prednisone.

5.10 PO-6-Thioguanine (TG)

Delayed Intensification Part 1:
Vincristine, daunorubicin, and the start of each 4-day cytarabine block on Days 9, 16, and 23 should be delayed and thioguanine interrupted if the ANC falls to <300/µL or platelets < 30,000/µL. If a 4-day cytarabine block has been started, then cytarabine and thioguanine should not be interrupted.

Delayed Intensification Part 2:
The start of the 4-day cytarabine block on Day 9 should be delayed and thioguanine interrupted if the ANC falls to <300/µL or platelets < 30,000/µL. If a 4-day cytarabine block has been started, then cytarabine and thioguanine should not be interrupted.

For severe and/or unexpected myelosuppression, evaluate for TPMT activity as described in Section 5.7.

5.11 VinCRIStine

PLEASE USE "BALIS" SCALE FOR GRADING NEUROPATHY (See text box below)

Severe neuropathic pain (Grade 3 or greater):
Hold dose(s). When symptoms subside, resume at 50% previous calculated dose (maximum dose: 1 mg), then escalate to full dose as tolerated. NOTE: neuropathic pain can be not only severe but difficult to treat. However, because vincristine is an important component of curative therapy and the majority of neuropathies are ultimately reversible, vincristine therapy may be given at full dose at investigator discretion. Severe peripheral neuropathies, with or without a positive family history might suggest the need for a molecular diagnostic evaluation to rule out Charcot Marie Tooth Disease (CMT), Type 1A or Hereditary neuropathy with liability to pressure palsies. Drugs such as gabapentin may be of value.

Vocal Cord paralysis:
Hold dose(s). When symptoms subside, resume at 50% previous calculated dose (maximum dose: 1 mg), then escalate to full dose as tolerated. See above for comment on CMT.

Foot Drop, paresis:
Should be Grade 3 to consider holding or decreasing dose. These toxicities are largely reversible but over months to years. Accordingly, holding doses of vincristine and/or lowering the dose may not result in rapid resolution of symptoms and may compromise cure. See above for comment on CMT. Physical therapy may be beneficial to maintain range of motion and provide AFO's and other forms of support. Drugs such as gabapentin may be of value.

Jaw pain: Treat with analgesics; do not modify vincristine dose.

Hyperbilirubinemia[71,72]:

Direct Bili	Dose reduction
< 3.1 mg/dL	Full dose (maximum dose: 2 mg),
3.1- 5.0 mg/dL	50% of calculated dose (maximum dose: 1 mg),
5.1-6.0 mg/dL	75% of calculated dose (maximum dose: 0.5 mg),
> 6.0 mg/dL	Withhold dose and administer next scheduled dose if toxicity has resolved. Do not make up missed doses.

Constipation or ileus (≥ Grade 3) or typhlitis: Hold dose(s); institute aggressive regimen to treat constipation if present. When symptoms abate resume at 50% of calculated dose (maximum dose: 1 mg) and escalate to full dose as tolerated.

Extravasation:
In the event of an extravasation, discontinue the IV administration of the drug and institute appropriate measures to prevent further extravasation and damage according to institutional guidelines. Also see:
https://members.childrensoncologygroup.org/_files/disc/Nursing/extravasationreference.pdf for COG extravasation management recommendations.

Modified ("Balis") Pediatric Scale of Peripheral Neuropathies

Peripheral Motor Neuropathy:

- Grade 1: Subjective weakness, but no deficits detected on neurological exam, other than abnormal deep tendon reflexes.
- Grade 2: Weakness that alters fine motor skills (buttoning shirt, coloring, writing or drawing, using eating utensils) or gait without abrogating ability to perform these tasks.
- Grade 3: Unable to perform fine motor tasks (buttoning shirt, coloring, writing or drawing, using eating utensils) or unable to ambulate without assistance.
- Grade 4: Paralysis.

Peripheral Sensory Neuropathy:

- Grade 1: Paresthesias, pain, or numbness that do not require treatment or interfere with extremity function.
- Grade 2: Paresthesias, pain, or numbness that are controlled by non-narcotic medications (without causing loss of function), or alteration of fine motor skills (buttoning shirt, writing or drawing, using eating utensils) or gait, without abrogating ability to perform these tasks.
- Grade 3: Paresthesias or pain that are controlled by narcotics, or interfere with extremity function (gait, fine motor skills as outlined above), or quality of life (loss of sleep, ability to perform normal activities severely impaired).
- Grade 4: Complete loss of sensation, or pain that is not controlled by narcotics.

5.12 Drug Interactions

Possible Drug Interactions with Antileukemic drugs:
See Section 4.1.1.3 and Appendix II.

Possible Drug Interactions with Methotrexate:
Avoid non-steroidal anti-inflammatory drugs (NSAIDs), trimethoprim/sulfamethoxazole (TMP/SMX), penicillins, probenecid, IV contrast media, proton pump inhibitors, phenytoin and fosphenytoin. Urinary acidifiers can cause methotrexate to precipitate in the urinary tract.

Possible Drug Interactions with High Dose Methotrexate:
Hold *TMP/SMX on the days of high dose methotrexate infusion and for at least 72 hours after the start of the high dose methotrexate infusion and until the methotrexate level is less than 0.1 μM.*

Hold *any NSAIDs, penicillins, proton pump inhibitors, or aspirin-containing medications on the day of high dose methotrexate infusion and for at least 72 hours after the start of the high dose methotrexate infusion and until the methotrexate level is less than 0.1 μM.*

Infants receiving therapeutic doses of amphotericin should have that drug withheld on the day HD methotrexate is administered and for the following 24 hours due to the risk of delayed methotrexate excretion, renal dysfunction and resultant toxicity.

6.0 DRUG INFORMATION

6.1 AZACITIDINE – INJECTION

(VIDAZA ®, 5-azacitidine) NSC# 102816, IND# [redacted]

(09/09/19)

Source and Pharmacology:

Azacitidine (4-amino-1-β-D-ribofuranosyl-s-triazin-2(1H)-one) is an analog of the naturally occurring pyrimidine nucleoside, cytidine. It differs from cytidine in having nitrogen in the 5-position of the heterocyclic ring. This substitution renders the ring chemically unstable and leads to rapid decomposition of the compound in neutral or alkaline solution. Therefore, for SC or IV administration, the drug is supplied as a lyophilized powder to be reconstituted immediately prior to use.

Azacitidine was developed based on its strong in vitro and in vivo antileukemic activity at cytotoxic concentrations, and its differentiation-inducing potential at lower concentrations in hematopoietic and non-hematopoietic cell lines. The cytotoxic effects of azacitidine may be due to inhibition of protein synthesis and activation of DNA damage pathways, through incorporation into ribonucleic acid (RNA) and DNA, respectively. The ability of azacitidine to cause differentiation is attributed to its activity as a hypomethylating agent. Similar to cancer cells, the disturbed maturation of morphologically dysplastic hematopoietic cells in MDS is thought to reflect a block in their differentiation, resulting in accumulation of these precursors in the bone marrow in spite of low peripheral blood counts. This block in maturation (differentiation), with proliferation of preleukemic myeloblasts, provides a rationale for clinical trials of DNA methylation inhibitors (e.g., azacitidine) in the treatment of MDS.

Pharmacokinetic studies using ^{14}C-radiolabeled azacitidine to evaluate drug disposition demonstrated that azacitidine undergoes rapid and complete absorption following SC administration, with SC bioavailability greater than 70% and maximum concentration (Cmax) achieved 0.5 to 2 hours after dosing. The volume of distribution of azacitidine is 76 ± 26 L following IV administration. Its protein binding in human serum is low (< 10% bound). Azacitidine and/or its metabolites are cleared by the kidneys. The plasma elimination half-life ($t_{1/2}$) is 3.4 to 6.2 hours and amount of radioactivity recovered in urine (50% to 98% of administered dose) were similar after IV and SC dosing.

In vitro and in vivo studies have demonstrated that spontaneous hydrolysis of azacitidine is the major pathway in different species, regardless of the route of administration. Azacitidine is not metabolized by cytochrome P450 isozymes (CYPs) and it will not produce clinically relevant PK drug-drug interactions due to CYP enzyme inhibition or induction when coadministered with CYP substrates, inducers, or inhibitors. Azacitidine is not a substrate for P-glycoprotein (P-gp) and is unlikely to produce any clinically relevant interactions as a P-gp substrate or an inhibitor.

Toxicity:

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
Azacitidine (NSC 102816)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited

Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 1800 patients.* Below is the CAEPR for Azacitidine.

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.7, July 30, 2019[1]

Adverse Events with Possible Relationship to Azacitidine (CTCAE 5.0 Term) [n= 1800]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 3)***
	Febrile neutropenia		***Febrile neutropenia (Gr 3)***
CARDIAC DISORDERS			
	Heart failure		***Heart failure (Gr 2)***
	Pericardial effusion		***Pericardial effusion (Gr 2)***
	Sinus tachycardia		***Sinus tachycardia (Gr 2)***
	Supraventricular tachycardia		***Supraventricular tachycardia (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 3)***
	Colitis		***Colitis (Gr 2)***
Constipation			***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 3)***
	Esophagitis		***Esophagitis (Gr 2)***
	Gastrointestinal hemorrhage[2]		
	Mucositis oral		***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 3)***
Vomiting			***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		***Chills (Gr 2)***
	Edema limbs		***Edema limbs (Gr 2)***
Fatigue			***Fatigue (Gr 3)***
Fever			***Fever (Gr 3)***
Injection site reaction			***Injection site reaction (Gr 2)***
IMMUNE SYSTEM DISORDERS			
		Allergic reaction	***Allergic reaction (Gr 2)***
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
Infection[3]			***Infection[3] (Gr 4)***
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Bruising		***Bruising (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 4)***
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr 2)***

Adverse Events with Possible Relationship to Azacitidine (CTCAE 5.0 Term) [n= 1800]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 4)***
	Blood bilirubin increased		***Blood bilirubin increased (Gr 2)***
	GGT increased		***GGT increased (Gr 2)***
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
Neutrophil count decreased			***Neutrophil count decreased (Gr 4)***
Platelet count decreased			***Platelet count decreased (Gr 4)***
	Weight loss		***Weight loss (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 4)***
METABOLISM AND NUTRITION DISORDERS			
	Acidosis		***Acidosis (Gr 2)***
	Anorexia		***Anorexia (Gr 3)***
	Hypokalemia		
		Tumor lysis syndrome	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 2)***
	Back pain		***Back pain (Gr 2)***
	Generalized muscle weakness		***Generalized muscle weakness (Gr 2)***
	Myalgia		***Myalgia (Gr 2)***
	Pain in extremity		***Pain in extremity (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Headache		***Headache (Gr 2)***
	Peripheral motor neuropathy		***Peripheral motor neuropathy (Gr 2)***
	Somnolence		***Somnolence (Gr 2)***
PSYCHIATRIC DISORDERS			
	Anxiety		
	Confusion		***Confusion (Gr 2)***
	Insomnia		
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Bronchopulmonary hemorrhage	
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 4)***
	Epistaxis		***Epistaxis (Gr 2)***
	Pharyngolaryngeal pain		
	Postnasal drip		***Postnasal drip (Gr 2)***
	Respiratory, thoracic and mediastinal disorders - Other (abnormal breath sound)[4]		***Respiratory, thoracic and mediastinal disorders - Other (abnormal breath sound)[4] (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		***Alopecia (Gr 2)***
	Pruritus		***Pruritus (Gr 2)***
	Purpura		***Purpura (Gr 2)***

Adverse Events with Possible Relationship to Azacitidine (CTCAE 5.0 Term) [n= 1800]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Rash maculo-papular		***Rash maculo-papular (Gr 3)***
VASCULAR DISORDERS			
	Hematoma		***Hematoma (Gr 2)***
	Hypotension		***Hypotension (Gr 3)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[3]Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

[4]Abnormal breath sounds include rales and rhonchi.

Adverse events reported on azacitidine trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that azacitidine caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (agranulocytosis); Blood and lymphatic system disorders - Other (lymphadenopathy); Blood and lymphatic system disorders - Other (pancytopenia); Blood and lymphatic system disorders - Other (splenomegaly); Blood and lymphatic system disorders - Other (transfusion: platelets); Bone marrow hypocellular; Hemolysis; Leukocytosis
CARDIAC DISORDERS - Atrial fibrillation; Atrial flutter; Atrioventricular block complete; Cardiac arrest; Cardiac disorders - Other (cardiac valve vegetation); Cardiac disorders - Other (Wolff-Parkinson-White syndrome); Chest pain - cardiac; Myocardial infarction; Palpitations; Pericarditis; Restrictive cardiomyopathy; Sinus bradycardia; Ventricular fibrillation
EAR AND LABYRINTH DISORDERS - Hearing impaired; Tinnitus
EYE DISORDERS - Eye disorders - Other (eye/conjunctival hemorrhage); Eye disorders - Other (retina hemorrhage); Papilledema; Uveitis
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Duodenal ulcer; Dyspepsia; Dysphagia; Enterocolitis; Esophageal pain; Esophageal ulcer; Flatulence; Gastritis; Gastrointestinal disorders - Other (enteritis); Gastrointestinal disorders - Other (inguinal hernia, obstructive); Gastrointestinal disorders - Other (intestinal ischemia); Gastrointestinal disorders - Other (intussusception); Gastrointestinal pain; Hemorrhoids; Pancreatitis; Periodontal disease; Small intestinal obstruction; Visceral arterial ischemia
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Death NOS; Edema face; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (systemic inflammatory response syndrome); Generalized edema; Malaise; Multi-organ failure; Non-cardiac chest pain; Pain; Sudden death NOS

HEPATOBILIARY DISORDERS - Cholecystitis; Hepatic failure; Hepatobiliary disorders - Other (bile duct stone); Hepatobiliary disorders - Other (hepatic cirrhosis)
IMMUNE SYSTEM DISORDERS - Autoimmune disorder; Immune system disorders - Other (GVHD)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Burn; Fall; Fracture; Hip fracture; Injury, poisoning and procedural complications - Other (excoriation); Injury, poisoning and procedural complications - Other (transfusion reaction); Postoperative hemorrhage; Wound dehiscence
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Blood lactate dehydrogenase increased; Creatinine increased; Electrocardiogram QT corrected interval prolonged; INR increased; Investigations - Other (blood urea increased); Investigations - Other (cardiac murmur); Investigations - Other (coagulopathy); Investigations - Other (protein total decreased); Investigations - Other (thrombocytosis); Lipase increased; Lymphocyte count increased; Serum amylase increased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperglycemia; Hyperkalemia; Hyperphosphatemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (gout exacerbation); Metabolism and nutrition disorders - Other (hypovolemia); Metabolism and nutrition disorders - Other (low carbon dioxide)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Bone pain; Chest wall pain; Flank pain; Muscle cramp; Muscle weakness lower limb; Musculoskeletal and connective tissue disorder - Other (chondritis); Musculoskeletal and connective tissue disorder - Other (intervertebral disc protrusion); Musculoskeletal and connective tissue disorder - Other (musculoskeletal stiffness); Neck pain
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Myelodysplastic syndrome; Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (colonic polyp, vaginal polyp); Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (metastases to central nervous system); Treatment related secondary malignancy
NERVOUS SYSTEM DISORDERS - Dysesthesia; Dysgeusia; Hydrocephalus; Intracranial hemorrhage; Lethargy; Memory impairment; Nervous system disorders - Other (head injury); Paresthesia; Peripheral sensory neuropathy; Seizure; Stroke; Syncope
PSYCHIATRIC DISORDERS - Delirium; Depression; Hallucinations; Psychiatric disorders - Other (mental status changes)
RENAL AND URINARY DISORDERS - Chronic kidney disease; Dysuria; Hematuria; Proteinuria; Renal and urinary disorders - Other (bladder distention); Renal and urinary disorders - Other (calculus urinary); Renal calculi; Urinary frequency; Urinary retention
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Erectile dysfunction; Reproductive system and breast disorders - Other (benign prostatic hyperplasia); Uterine hemorrhage; Vaginal hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Atelectasis; Hypoxia; Laryngeal hemorrhage; Nasal congestion; Oropharyngeal pain; Pleural effusion; Pleuritic pain; Pneumonitis; Pneumothorax; Productive cough; Pulmonary edema; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (chronic obstructive pulmonary disease); Respiratory, thoracic and mediastinal disorders - Other (pharyngeal erythema); Rhinorrhea; Sinus pain; Wheezing
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Dry skin; Hyperhidrosis; Palmar-plantar erythrodysesthesia syndrome; Skin and subcutaneous tissue disorders - Other (skin laceration); Skin and subcutaneous tissue disorders - Other (skin lesion); Skin and subcutaneous tissue disorders - Other (skin nodule); Skin and subcutaneous tissue disorders - Other (Sweet's Syndrome); Skin induration; Urticaria
VASCULAR DISORDERS - Flushing; Hypertension; Thromboembolic event; Vascular disorders - Other (pallor); Vascular disorders - Other (poor venous access); Vasculitis

Note: Azacitidine in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated.

Formulation and Stability:

Azacitidine is supplied as a lyophilized powder in single-use vials. Each vial contains 100 mg of azacitidine and 100 mg of mannitol as a freeze-dried cake or powder. The azacitidine vial does not contain any preservative and is for single use. Store unreconstituted vials at 25° C (77° F); excursions permitted to 15°-30° C (59°-86° F).

For IV administration: Reconstitute vial with 10 mL SWFI to form a 10 mg/mL solution;

vigorously shake or roll vial until solution is dissolved and clear. Following reconstitution, further dilute azacitidine in NS or lactated Ringer's injection for infusion to a final concentration between 0.9 mg/mL and 4 mg/mL. The reconstituted product may be stored at 25° C (77° F), but the administration of the intravenous solution must be completed within 45 minutes of reconstitution. **Solutions for IV administration have very limited stability and must be prepared immediately prior to each dose.**

Azacitidine is a cytotoxic drug and caution should be exercised when handling and preparing injectable azacitidine. Procedures for proper handling and disposal of azacitidine (vials or reconstituted azacitidine) should be applied according to standards established at each facility for cytotoxic drugs. If a vial is broken or damaged, dispose of the drug product and do not use.

Azacitidine is incompatible with 5% dextrose solution, Hespan, or solutions that contain bicarbonate. These solutions have the potential to increase the rate of degradation of azacitidine and should therefore be avoided.

Guidelines for Administration: See Treatment and Dose Modification sections of the protocol.

Supplier:

Azacitidine is supplied by Celgene and distributed by the Division of Cancer Treatment and Diagnosis (DCTD), NCI. **Do not use commercial supply.**

Obtaining the Agent

Agent Ordering

NCI supplied agent may be requested by the eligible participating investigator (or their authorized designee) at each participating institution. Sites may order initial agent supplies when a subject is being screened for enrollment onto the study. The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), NIH Biosketch, Agent Shipment Form, and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead participating investigator at that institution.

Submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an “active” account status, and a “current” password, and active person registration status. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time. Refer to the PMB’s website for specific policies and guidelines related to agent management.

Agent Accountability

Agent Inventory Records:

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from

the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status and a "current" password and active person registration status. Questions about IB access may be directed to the PMB IB coordinator via email.

Useful Links and Contacts:

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP/
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

6.2 CYCLOPHOSPHAMIDE INJECTION

(Cytoxan) NSC#26271 (03/13/13)

Source and Pharmacology:

Cyclophosphamide is an alkylating agent related to nitrogen mustard. Cyclophosphamide is inactive until it is metabolized by P450 isoenzymes (CYP2B6, CYP2C9, and CYP3A4) in the liver to active compounds. The initial product is 4-hydroxycyclophosphamide (4-HC) which is in equilibrium with aldophosphamide which spontaneously releases acrolein to produce phosphoramide mustard. Phosphoramide mustard, which is an active bifunctional alkylating species, is 10 times more potent *in vitro* than is 4-HC and has been shown to produce interstrand DNA cross-link analogous to those produced by mechlorethamine. Approximately 70% of a dose of cyclophosphamide is excreted in the urine as the inactive carboxyphosphamide and 5-25% as unchanged drug. The plasma half-life ranges from 4.1 to 16 hours after IV administration.

Toxicity:

	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	Anorexia, nausea & vomiting (acute and delayed)	Abdominal discomfort, diarrhea	Transient blurred vision, nasal stuffiness with rapid administration, arrhythmias (rapid infusion), skin rash, anaphylaxis, SIADH
Prompt: Within 2-3 weeks, prior to the next course	Leukopenia, alopecia, immune suppression	Thrombocytopenia, anemia, hemorrhagic cystitis (L)	Cardiac toxicity with high dose (acute – CHF hemorrhagic myocarditis, myocardial necrosis) (L), hyperpigmentation, nail changes, impaired wound healing, infection secondary to immune suppression
Delayed: Any time later during therapy	Gonadal dysfunction: azoospermia or oligospermia (prolonged or permanent)[1] (L)	Amenorrhea[1]	Gonadal dysfunction: ovarian failure[1] (L), interstitial pneumonitis, pulmonary fibrosis[2] (L)
Late: Any time after completion of treatment			Secondary malignancy (ALL, ANLL, AML), bladder carcinoma (long term use > 2 years), bladder fibrosis
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of cyclophosphamide (alone or in combination with other antineoplastic agents) have been noted in humans. Toxicities include: chromosomal abnormalities, multiple anomalies, pancytopenia, and low birth weight. Cyclophosphamide is excreted into breast milk. Cyclophosphamide is contraindicated during breast feeding because of reported cases of neutropenia in breast fed infants and the potential for serious adverse effects.		

[1] *Dependent on dose, age, gender, and degree of pubertal development at time of treatment.*
[2] *Risk increased with pulmonary chest irradiation and higher doses.*
(L) Toxicity may also occur later.

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

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol.

Cyclophosphamide for Injection:
If the drug will be administered as undiluted drug at the 20 mg/mL concentration, then reconstitute to 20 mg/mL with NS ONLY to avoid a hypotonic solution. If the drug will be further diluted prior to administration, then first reconstitute with NS, SWFI, or Bacteriostatic Water for Injection (paraben preserved only) to a concentration of 20 mg/mL. Following reconstitution further dilute in dextrose or saline containing solutions for IV use.

Supplier: Commercially available from various manufacturers. See package insert for further information.

6.3 CYTARABINE – ALL ROUTES
(Cytosine arabinoside, Ara-C, Cytosar ®) NSC #63878 (07/13/15)

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.

Toxicity: (Intravenous, SubQ, IM)

	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 *With High Dose*: conjunctivitis	Flu-like symptoms with fever, rash	Ara-C syndrome (fever, myalgia, bone pain, occasionally chest pain, maculopapular rash, malaise, conjunctivitis), anaphylaxis, swelling, pain and redness at the site of the medication injection (SubQ or IM injection) *With High Dose:* cardiomyopathies (vasculitis, and pericarditis), cerebral and cerebellar dysfunction including: encephalopathy, aseptic meningitis, ataxia, dysphasia, nystagmus, a decreased level of consciousness, personality changes, somnolence, seizures
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (anemia, thrombocytopenia, leukopenia, megaloblastosis, reticulocytopenia), stomatitis, alopecia	Diarrhea, hypokalemia, hypocalcemia, hyperuricemia *With High Dose*: capillary pulmonary leak syndrome (RDS, pulmonary edema)	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.		

Toxicity: (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 condition			Necrotizing leukoencephalopathy, paraplegia, blindness (in combination with XRT & systemic therapy)

Formulation:
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.

Guidelines for Administration: See Treatment and Dose Modification sections of the protocol.

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 give by IV push injection, by IV infusion, or by continuous infusion.

Low Dose (≤ 200 mg/m²/dose): For administration by IV push, reconstitute to a concentration of 20-100 mg/mL.

High Dose (≥ 1000 mg/m²/dose): Administer steroid eye drops (dexamethasone or predniso(lo)ne), 2 drops each eye q6h 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 q2-4 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.

Subcutaneous or IM:
Dilute with Bacteriostatic Water for Injection or NS to a concentration not to exceed 100 mg/mL. Rotate injection sites for subcutaneous/IM administration.

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.

Patient Age (years)	Recommended volume	10% CSF volume	CSF Volume *
1 – 1.99	5 – 10 mL	5 mL	50 ± 10 mL (babies)
2 – 2.99	5 – 10 mL	8 mL	80 ± 20 mL (younger children)
3 – 8.99	5 – 10 mL	10 mL	100 ± 20 mL (older children)

9 or greater	5 – 10 mL	13 mL	130 ± 30 mL (adults)

*Rieselbach, R.E. et.al. Subarachnoid distribution of drugs after lumbar injection; N Engl J Med. 1962 Dec 20; 267:1273-8

Of Note: Larger volumes approximating at least 10% of the CSF volume, isovolumetric delivery, with the patient remaining prone after the procedure may facilitate drug distribution. These procedures have not been validated in clinical trials. They are allowed but not mandated for patients on COG studies.

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

Supplier: Commercially available from various manufacturers. See package insert for further information.

6.4 DAUNORUBICIN

Daunomycin, rubidomycin, Cerubidine®) NSC #82151 (05/09/11)

Source and Pharmacology:

Daunorubicin is an anthracycline antibiotic isolated from cultures of *Streptomyces coeruleorubidus*. Daunorubicin is closely related structurally to doxorubicin only differing in that the side chain of daunorubicin terminates in a methyl group rather than an alcohol. The cytotoxic effect of daunorubicin on malignant cells and its toxic effects on various organs are similar to those of doxorubicin and are thought to be related to nucleotide base intercalation and cell membrane lipid binding activities. Intercalation inhibits nucleotide replication and action of DNA and RNA polymerases. The interaction of daunorubicin with topoisomerase II to form DNA-cleavable complexes appears to be an important mechanism of cytocidal activity. Daunorubicin cellular membrane binding may affect a variety of cellular functions. Enzymatic electron reduction of daunorubicin by a variety of oxidases, reductases, and dehydrogenases generate highly reactive species including the hydroxyl free radical (OH•) which may lead to DNA damage or lipid peroxidation. Daunorubicin is metabolized more rapidly by aldoketoreductases to the active metabolite, daunorubicinol, than is doxorubicin. Daunorubicin hydrochloride is rapidly and widely distributed in tissues, with the highest levels in the spleen, kidneys, liver, lungs, and heart. Daunorubicin serum decay pattern is multiphasic. The initial $t_{½}$ is approximately 45 minutes followed by a terminal $t_{½}$ of 18.5 hours. By 1 hour after drug administration, the predominant plasma species is daunorubicinol, which disappears with a half-life of 26.7 hours. Twenty five percent of an administered dose of daunorubicin is eliminated in an active form by urinary excretion and an estimated 40% by biliary excretion.

Toxicity:

	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, pink or red color to urine, sweat, tears, and saliva	Hyperuricemia, sclerosis of the vein	Diarrhea, anorexia, abdominal pain, extravasation (rare) but if occurs = local ulceration, anaphylaxis, fever, chills, rash, urticaria, acute arrhythmias
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (leukopenia, thrombocytopenia, anemia), alopecia	Mucositis (stomatitis and esophagitis), hepatotoxicity	Radiation recall reactions, myocarditis-pericarditis syndrome, conjunctivitis and lacrimation
Delayed: Any time later during therapy			Cardiomyopathy[1] (uncommon at cumulative doses ≤ 550 mg/m², 400 mg/m² with mediastinal radiation, 300 mg/m² in children, or 10 mg/kg in children < 2 yrs or 0.5 m²) (L), hyper-pigmentation of nail beds
Late: Any time after completion of treatment		Subclinical cardiac dysfunction	CHF (on long term follow up in pediatric patients), secondary malignancy (in combination regimens)
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of daunorubicin have been noted in animals. It is unknown whether the drug is excreted in breast milk.		

[1] *Risk increases with cardiac irradiation, exposure at a young or advanced age.*
(L) Toxicity may also occur later.

Formulation and Stability:
Daunorubicin is available as red-orange lyophilized powder[1] for injection in 20 mg single dose vials and a preservative free 5 mg/mL solution[2] in 20 mg (4 mL) and 50 mg (10 mL) vials.

[1] *Each vial contains 21.4 mg of daunorubicin hydrochloride (equivalent to 20 mg of daunorubicin) and 100 mg mannitol.*
[2] *Each mL contains 5.3 mg daunorubicin hydrochloride (equivalent to 5 mg of daunorubicin), 9 mg of sodium chloride, sodium hydroxide or hydrochloric acid to adjust pH, and Sterile Water for Injection.*

Powder for Injection:
Store intact, unreconstituted vials at room temperature, 15°-30°C (59°-86°F). Protect from light. Retain in carton until contents are used. Reconstitute a 20 mg vial with 4 mL SWFI to a final concentration of 5 mg/mL. After adding the diluent, the vial should be shaken gently and the contents allowed to dissolve. The reconstituted solution is stable for 24 hours at room temperature and 48 hours refrigerated. Protect from exposure to sunlight.

Aqueous Solution:
Store refrigerated 2°-8°C, (36°-46°F). Protect from light. Retain in carton until contents are used.

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol.

Administer by IV side arm into a rapidly flowing infusion solution. Alternately, daunorubicin may be further diluted in saline or dextrose containing solutions and administered by infusion. Protect final preparation from light. To avoid extravasation, the use of a central line is suggested.

Supplier: Commercially available from various manufacturers. See package insert for further information.

6.5 DEXAMETHASONE

(Decadron®, Hexadrol®, Dexone®, Dexameth®) NSC #34521 **(05/09/11)**

Source and Pharmacology:

Dexamethasone is a synthetic fluorinated glucocorticoid devoid of mineralocorticoid effects. Dexamethasone, 0.75 mg, has potent anti-inflammatory activity equivalent to approximately 5 mg of prednisone. Glucocorticoids produce widespread and diverse physiologic effects on carbohydrate, protein, and lipid metabolism, electrolyte and water balance, functions of the cardiovascular system, kidney, skeletal muscle, and the nervous systems. Glucocorticoids reduce the concentration of thymus-dependent lymphocytes (T-lymphocytes), monocytes, and eosinophils. Glucocorticoids selectively bind to the cortisol receptors on human lymphoid cells which are found in larger numbers on leukemic lymphoblasts. They also decrease binding of immunoglobulin to cell surface receptors and inhibit the synthesis and/or release of interleukins, thereby decreasing T-lymphocyte blastogenesis and reducing expansion of the primary immune response. The specific cellular mechanisms that act to halt DNA synthesis are thought to be related to inhibition of glucose transport or phosphorylation, retardation of mitosis, and inhibition of protein synthesis. Elimination half-lives for the following age groups have been reported to be: infants and children under 2 years of age: 2.3 to 9.5 hours, 8 to 16 years: 2.82 to 7.5 hours, and adults (age not specified): 3 to 6 hours. The biologic half-life is 36-72 hours. It is primarily metabolized in the liver and excreted by the kidneys.

Toxicity:

	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	Insomnia, hyperphagia	Gastritis	Hyperuricemia
Prompt: Within 2-3 weeks, prior to the next course	Immunosuppression, personality changes (mood swings, euphoria, anxiety, depression), pituitary-adrenal axis suppression, acne (L)	Hyperglycemia, facial erythema, poor wound healing, infections (bacterial, fungal, parasitic, viral), edema	Pancreatitis (L), increased intraocular pressure (L), hypertension, psychosis, vertigo, headache
Delayed: Any time later during therapy	Cushing's syndrome (moon facies, truncal obesity)	Striae and thinning of the skin, easy bruising, muscle weakness, osteopenia	Spontaneous fractures (L), growth suppression, peptic ulcer and GI bleeding, pseudotumor cerebri (increased intracranial pressure with papilledema, headache), aseptic necrosis of the femoral and humeral

			heads (L), urolithiasis[1] (L)
Late: Any time after completion of treatment		Cataracts (which may be reversible on discontinuation of dexamethasone in children)	
Unknown Frequency and Timing:	**Fetal and teratogenic toxicities**: dexamethasone crosses the placenta with 54% metabolized by enzymes in the placenta. In animal studies, large doses of cortisol administered early in pregnancy produced cleft palate, stillborn fetuses, and decreased fetal size. Chronic maternal ingestion during the first trimester has shown a 1% incidence of cleft palate in humans. There are no reports of dexamethasone excretion into breast milk in humans; however, it is expected due to its low molecular weight that it would partition into breast milk.		

[1] Mainly reported in pediatric patients with ALL. Howard SC et al. Urolithiasis in pediatric patients with acute lymphoblastic leukemia. Leukemia 2003; 17: 541-6.
(L) Toxicity may also occur later.

Formulation and Stability:
Oral:
Available in 0.5 mg, 0.75 mg, 1 mg, 1.5 mg, 2 mg, 4 mg, and 6 mg tablets; liquid formulations are available in 0.5 mg/5 mL and 1 mg/1 mL concentrations. Inactive ingredients vary depending on manufacturer but tablet formulations may include: calcium or magnesium stearate, corn starch, lactose, and various dyes. Liquid formulations may include: 5%-30% alcohol, benzoic acid, sorbitol, sodium saccharin, glycerin, purified water, and various dyes.

Injection:
Dexamethasone Sodium Phosphate Solution for Injection is available as 4 mg/mL (1 mL, 5 mL, and 30 mL vials) and 10 mg/mL (1 mL and 10 mL vial sizes). Vials are available in multi-dose vials as well as unit of use vials and syringes. Inactive ingredients vary depending on manufacturer but include creatinine, sodium citrate, sodium hydroxide to adjust pH, Water for Injection, sodium sulfite, bisulfite and metabisulfite, methyl and propyl paraben, benzyl alcohol, and EDTA.

Guidelines for Administration: See Treatment and Dose Modifications section of the protocol.

Dexamethasone Sodium Phosphate for Injection may be given IV, or IM undiluted. For IV use, it may be further diluted in dextrose or saline containing solutions. Avoid using benzyl alcohol-containing dexamethasone solutions in neonates. Diluted solutions that contain no preservatives should be used within 24 hours, but maintain stability for at least 14 days in PVC bags at room temperature protected from light.

Supplier:
Commercially available from various manufacturers. See package insert for further information.

6.6 HYDROCORTISONE - INTRATHECAL
(Hydrocortisone sodium succinate, Solu-cortef®) NSC #010483 (07/30/14)

Source and Pharmacology:
Hydrocortisone is a synthetic compound closely related to cortisol. Glucocorticoids produce widespread and diverse physiologic effects on carbohydrate, protein, and lipid metabolism, electrolyte and water balance, functions of the cardiovascular system, kidney, skeletal

muscle, and the nervous systems. Glucocorticoids reduce the concentration of thymus-dependent lymphocytes (T-lymphocytes), monocytes, and eosinophils. Glucocorticoids selectively bind to the cortisol receptors on human lymphoid cells which are found in larger numbers on leukemic lymphoblasts. They also decrease binding of immunoglobulin to cell surface receptors and inhibit the synthesis and/or release of interleukins, thereby decreasing T-lymphocyte blastogenesis and reducing expansion of the primary immune response. The specific cellular mechanisms that act to halt DNA synthesis are thought to be related to inhibition of glucose transport or phosphorylation, retardation of mitosis, and inhibition of protein synthesis. Hydrocortisone is approximately 90% protein bound with a plasma $t_{½}$ of 1-2 hours. The elimination of hydrocortisone from the CNS is prolonged.

Toxicity: (Toxicities for Hydrocortisone Intrathecal and Methotrexate and/or Cytarabine)[1]

	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	Arachnoditis: (headache, fever, vomiting, meningismus and pleocytosis)	Rash, anaphylaxis (L), paresis, bleeding into subarachnoid or subdural space (risk > with platelet counts < 20,000), confusion, fatigue, disorientation, seizures
Prompt: Within 2-3 weeks, prior to the next course			Myelosuppression, somnolence, ataxia, cranial nerve palsy, transient paraplegia,(L) speech disorders
Delayed: Any time later during therapy, excluding the above condition		Cognitive disturbances (L), learning disabilities(L)	Demyelating leukoencephalopathy[1] (L), blindness[1]
Late: Any time after the completion of treatment			Progressive CNS deterioration[2]

[1] *Toxicity for hydrocortisone alone has not been described*
[2] *May be enhanced by systemic therapy such as high dose methotrexate or cytarabine and/or cranial irradiation.*
(L) Toxicity may also occur later.

Formulation and Stability:
For intrathecal administration, use hydrocortisone sodium succinate 100 mg vial sterile powder for injection **WITHOUT** preservative. Do not reconstitute vial with bacteriostatic water for injection.

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol.

Intrathecal Methotrexate and Hydrocortisone:
***Optimal Volume (20 mL)*:** Final concentration of Intrathecal Methotrexate and Hydrocortisone: Methotrexate 1 mg/mL and Hydrocortisone 1 mg/mL
The following is a suggested method for preparing the IT using the 25 mg/mL concentration of preservative free methotrexate. Institutional practices may be used as clinically appropriate.

- Withdraw 0.8 mL (20 mg) of methotrexate 25 mg/mL concentration
- Reconstitute hydrocortisone 100 mg vial with 10 mL of preservative free NS to a concentration of 10 mg/mL. Withdraw 2 mL (20 mg) of hydrocortisone
- Combine 0.8 mL (20 mg) methotrexate and 2 mL (20 mg) hydrocortisone
- QS with 17.2 mL of NS for a total volume of 20 mL

***Alternative Volume (12 mL)*:** Final concentration of Intrathecal Methotrexate and Hydrocortisone: Methotrexate 1.5 mg/mL and Hydrocortisone 1.5 mg/mL
The following is a suggested method for preparing the IT using the 25 mg/mL concentration of preservative free methotrexate. Institutional practices may be used as clinically appropriate.

- Withdraw 0.72 mL (18 mg) of methotrexate 25 mg/mL concentration
- Reconstitute hydrocortisone 100 mg vial with 10 mL of preservative free NS to a concentration of 10 mg/mL. Withdraw 1.8 mL (18 mg) of hydrocortisone
- Combine 0.72 mL (18 mg) methotrexate and 1.8 mL (18 mg) hydrocortisone
- QS with 9.48 mL of NS for a total volume of 12 mL

Intrathecal Cytarabine and Hydrocortisone:
Optimal Volume (20 mL): Final concentration of Intrathecal Cytarabine and Hydrocortisone: Hydrocortisone 1 mg/mL and Cytarabine 2 mg/mL
The following is a suggested method for preparing the IT. Institutional practices may be used as clinically appropriate.

- Reconstitute Hydrocortisone 100 mg vial with 10 mL of preservative free NS to a concentration of 10 mg/mL. Withdraw 2 mL (20 mg) of hydrocortisone
- Reconstitute Cytarabine 100 mg vial with 5 mL of preservative free NS to a concentration of 20 mg/mL. Withdraw 2 mL (40 mg) of cytarabine.
- Combine 2 mL (20 mg) hydrocortisone and 2 mL (40 mg) of cytarabine.
- QS with 16 mL of NS for a total volume of 20 mL.

Alternative Volume (12 mL): Final concentration of Intrathecal cytarabine and hydrocortisone: Hydrocortisone 1.5 mg/mL and Cytarabine 3.0 mg/mL.
The following is a suggested method for preparing the IT. Institutional practices may be used as clinically appropriate.

- Reconstitute Hydrocortisone 100 mg vial with 10 mL of preservative free NS to a concentration of 10 mg/mL. Withdraw 1.8 mL (18 mg) of hydrocortisone
- Reconstitute Cytarabine 100 mg vial with 5 mL of preservative free NS to a concentration of 20 mg/mL. Withdraw 1.8 mL (36 mg) of cytarabine
- Combine 1.8 mL (18 mg) hydrocortisone and 1.8 mL (36 mg) of cytarabine
- QS with 8.4 mL of NS for a total volume of 12 mL

Intrathecal Methotrexate and/or cytarabine and hydrocortisone are stable in preservative free NS for 24 hours at 25ºC but contain no preservative and should be administered as soon as possible after preparation.

Supplier: Hydrocortisone is commercially available. See package insert for further information

6.7 LEUCOVORIN CALCIUM

(LCV, Wellcovorin®, citrovorum factor, folinic acid) NSC #003590 **(05/09/11)**

Source and Pharmacology:
Leucovorin is a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid (THF). The biologically active compound of the mixture is the (-)- l-isomer, known as Citrovorum factor or (-)-folinic acid. Leucovorin does not require reduction by the enzyme dihydrofolate reductase in order to participate in reactions utilizing folates as a source of "one-carbon" moieties. Administration of leucovorin can

counteract the therapeutic and toxic effects of folic acid antagonists such as methotrexate, which act by inhibiting dihydrofolate reductase. In contrast, leucovorin can enhance the therapeutic and toxic effects of fluoropyrimidines used in cancer therapy, such as 5-fluorouracil. Leucovorin is readily converted to another reduced folate, 5,10-methylenetetrahydrofolate, which acts to stabilize the binding of fluorodeoxyuridylic acid (an active metabolite of 5-FU) to thymidylate synthase and thereby enhances the inhibition of this enzyme. Peak serum levels of 5-methyl THF (an active metabolite) were reached at approximately 1.3-1.5 hours (IV/IM) and 2.3 hours for the oral form. The terminal half-life of total reduced folates was approximately 6.2 hours. Following oral administration, leucovorin is rapidly absorbed and expands the serum pool of reduced folates. At a dose of 25 mg, almost 100% of the *l*-isomer (the biologically active form) but only 20% of the *d*-isomer is absorbed. Oral absorption of leucovorin is saturable at doses above 25 mg. The apparent bioavailability of leucovorin was 97% for 25 mg, 75% for 50 mg, and 37% for 100 mg doses. Both oral and parenteral leucovorin raise the CSF folate levels.

Toxicity:

	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			Anaphylaxis, urticaria, seizure
Unknown Frequency and timing:	Fetal toxicities and teratogenic effects of leucovorin in humans are unknown. It is unknown whether the drug is excreted in breast milk.		

Formulation and Stability:
Leucovorin calcium for injection is supplied as a sterile ready to use liquid and a sterile powder for injection. The 10 mg/mL preservative free liquid is available in 50 mL vials containing sodium chloride 400 mg/vial. Store preservative free liquid in the refrigerator at 2°-8°C (36°-46°F) protected from light. The powder for injection is available in 50 mg, 100 mg, 200 mg, and 350 mg vials. Store at room temperature 15°-25°C (59°-77°F) protected from light. Reconstitute the sterile powder with sterile water for injection or bacteriostatic water for injection to a concentration of 10 mg/mL leucovorin calcium. **Do not use diluents containing benzyl alcohol for doses > 10 mg/m² or in infants < 2 years of age or patients with allergy to benzyl alcohol.** When Bacteriostatic Water is used, the reconstituted solution is good for 7 days. If reconstituted with SWFI, use solution immediately as it contains no preservative. One milligram of leucovorin calcium contains 0.004 mEq of leucovorin and 0.004 mEq of calcium.

The oral form of leucovorin is available as 5 mg, 10 mg, 15 mg, and 25 mg tablets. Inactive ingredients vary depending on manufacturer but tablet formulations may include: corn starch, dibasic calcium phosphate, magnesium stearate, pregelatinized starch, lactose, microcrystalline cellulose, and sodium starch glycolate.

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol.

Injection:
Because of the calcium content of the leucovorin solution, no more than 160 mg of leucovorin should be injected intravenously per minute (16 mL of a 10 mg/mL solution per minute). IV leucovorin and sodium bicarbonate are incompatible.

Oral:
Oral leucovorin should be spaced evenly (e.g., every six hours) throughout the day and may be taken without regard to meals. Doses > 25 mg should be given IV due to the saturation of absorption.

Leucovorin should not be administered < 24 hours after intrathecal injections which contain methotrexate unless there are special circumstances.

Supplier: Commercially available from various manufacturers. See package insert for further information.

6.8 MERCAPTOPURINE

(6-MP, Purinethol®, Purixan™, 6-mercaptopurine) NSC #000755 **(11/27/17)**

Source and Pharmacology:
Mercaptopurine is an analogue of the purine bases adenine and hypoxanthine. The main intracellular pathway for MP activation is catalyzed by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) which catalyzes the conversion of MP to several active nucleotide metabolites including thioinosinic acid, a ribonucleotide which can interfere with various metabolic reactions necessary for nucleic acid (RNA and DNA) biosynthesis. It can also cause pseudofeedback inhibition of the first step in de novo purine biosynthesis or convert to another ribonucleotide which can cause feedback inhibition. Mercaptopurine can be incorporated into DNA in the form of TG nucleotides as well and thus produce toxicity. The absorption of an oral dose of MP is incomplete and variable, with only about 16%-50% of an administered dose reaching the systemic circulation secondary to a first pass metabolism in the liver. Food intake and co-administration with cotrimoxazole (TMP/SMX) significantly reduces absorption of MP. After IV administration, MP has a plasma half-life of 21 minutes in children and 47 minutes in adults. Approximately 19% is bound to protein. Mercaptopurine is well distributed into most body compartments except the CSF. (With high dose IV MP the CSF to plasma ratio is 0.15.) MP is metabolized by xanthine oxidase in the liver to 6-Thiouric acid an inactive metabolite. In patients receiving both MP and allopurinol (a xanthine oxidase inhibitor) the dose of MP must be reduced by 50-75%. Since TPMT, 6-thiopurine methyltransferase, is also one of the enzymes involved in the metabolism of MP, those individuals who have an inherited deficiency of the enzyme may be unusually sensitive to the myelosuppressive effects of MP and prone to develop rapid bone marrow suppression following the initiation of treatment. Mercaptopurine is excreted in urine as metabolites and some unchanged drug; about half an oral dose has been recovered in 24 hours. A small proportion is excreted over several weeks.

Toxicity:

Incidence	Toxicities
Common (>20% of patients)	Neutrophil count decreased, white blood cell decreased, anorexia, fatigue
Occasional (4 - 20% of patients)	Diarrhea, nausea, vomiting, malaise, oligospermia, infection, fever, platelet count decreased, anemia, mucositis, stomach pain, ulcerative bowel lesion, skin rash, alanine aminotransferase increased, aspartate aminotransferase increased

Incidence	Toxicities
Rare (≤ 3% of patients)	Urticaria, skin hyperpigmentation, alopecia, hyperuricemia, hepatic failure, hepatic necrosis, blood bilirubin increased, pulmonary fibrosis, secondary malignant neoplasm, renal toxicity, uricosuria, pancreatitis
Pregnancy and Lactation	**Pregnancy Category D** Mercaptopurine can cause fetal harm, including an increased incidence of abortion and stillbirth. Advise women to avoid becoming pregnant while receiving mercaptopurine. Mercaptopurine was embryo-lethal and teratogenic in several animal species (rat, mouse, rabbit, and hamster). It is not known whether mercaptopurine is excreted in human milk; breastfeeding should be avoided.

Formulation and Stability:
Mercaptopurine is available as a 50 mg tablet containing mercaptopurine and the inactive ingredients corn and potato starch, lactose, magnesium stearate, and stearic acid. Store at 15°-25°C (59°-77°F) in a dry place. In the United States, mercaptopurine is also available as an oral suspension in a concentration of 20 mg/mL (2000 mg/100 mL per bottle). The oral suspension is a pink to brown viscous liquid supplied in amber glass multiple-dose bottles with a child resistant closure. It should be stored at 15°-25°C (59°-77°F) in a dry place.

NOTE: the concentration of the commercially available suspension (20 mg/mL) and the compounded suspension (50 mg/mL) are NOT the same; doses should be prescribed in the milligrams required, not mL.

Guidelines for Administration:
See Treatment and Dose Modifications sections of the protocol.
Mercaptopurine should be taken consistently at the same time every day.

If allopurinol is also given, the oral dose of mercaptopurine should be reduced by 67-75%. Patients with severe myelosuppression should have their thiopurine S-methyltransferase (TPMT) status and/or their thiopurine metabolite concentrations evaluated, so that the dose of mercaptopurine can be reduced in patients with a TPMT defect. Patients with the rare homozygous deficient TPMT phenotype may tolerate only 1/10th to 1/20th the average mercaptopurine dose. TPMT testing and thiopurine metabolite measurements are commercially available.

Suspension:
For children unable to swallow the tablets whole, a 50 mg/mL oral suspension can be compounded. The suspension is prepared by crushing 50 mercaptopurine 50 mg tablets in a mortar and adding 8.5 mL sterile water for irrigation. The mixture is triturated to form a smooth paste. Next, 16.5 mL simple syrup (pH=7) are added with continuous mixing and finally cherry syrup (pH=7.1) is added to a total volume of 50 mL. The suspension is stable in amber glass bottles at room temperature (19ºC -23ºC) for up to 5 weeks. The suspension should be shaken well before each use. Procedures for proper handling and disposal of cytotoxic drugs should be used when preparing the suspension. (Aliabadi HM, Romanick M, Desai S et al. Effect of buffer and antioxidant on stability of mercaptopurine suspension. *Am J Heath-Syst Pharm.* 65:441-7, 2008.)

Supplier: Commercially available from various manufacturers. See package insert for further information. **PLEASE NOTE there is a difference in the concentration of the commercially available (20 mg/mL) and extemporaneously compounded (50 mg/mL) oral suspensions.**

6.9 METHOTREXATE – ALL ROUTES

(MTX, amethopterin, Trexall ®, Xatmep ®) NSC#000740 (11/27/17)

Source and Pharmacology:

A folate analogue which reversibly inhibits dihydrofolate reductase, the enzyme that reduces folic acid to tetrahydrofolic acid. Inhibition of tetrahydrofolate formation limits the availability of one carbon fragments necessary for the synthesis of purines and the conversion of deoxyuridylate to thymidylate in the synthesis of DNA and cell reproduction. The polyglutamated metabolites of MTX also contribute to the cytotoxic effect of MTX on DNA repair and/or strand breaks. MTX cytotoxicity is highly dependent on the absolute drug concentration and the duration of drug exposure. MTX is actively transported across cell membranes. At serum methotrexate concentrations exceeding 0.1 µmol/mL, passive diffusion becomes a major means of intracellular transport of MTX. The drug is widely distributed throughout the body with the highest concentration in the kidney, liver, spleen, gallbladder and skin. Plasma concentrations following high dose IV MTX decline in a biphasic manner with an initial half-life of 1.5-3.5 hours, and a terminal half life of 8-15 hours. About 50% is bound to protein. After oral administration, approximately 60% of a 30 mg/m^2 dose is rapidly absorbed from the GI tract, with peak blood levels at 1 hour. At doses > 30 mg/m^2 absorption decreases significantly. Even at low doses absorption may be very erratic, varying between 23% and 95%. The elimination of MTX from the CSF after an intrathecal dose is characterized by a biphasic curve with half-lives of 4.5 and 14 hours. After intrathecal administration of 12 mg/m^2, the lumbar concentration of MTX is ~100 times higher than in plasma. (Ventricular concentration is ~ 10% of lumbar concentration). MTX is excreted primarily by the kidneys via glomerular filtration and active secretion into the proximal tubules. Renal clearance usually equals or exceeds creatinine clearance. Small amounts are excreted in the feces. There is significant entero-hepatic circulation of MTX. The distribution of MTX into third-space fluid collections, such as pleural effusions and ascitic fluid, can substantially alter MTX pharmacokinetics. The slow release of accumulated MTX from these third spaces over time prolongs the terminal half-life of the drug, leading to potentially increased clinical toxicity.

Toxicity:

	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	Transaminase elevations	Nausea, vomiting, anorexia	Anaphylaxis, chills, fever, dizziness, malaise, drowsiness, blurred vision, acral erythema, urticaria, pruritis, toxic epidermal necrolysis, Stevens-Johnson Syndrome, tumor lysis syndrome, seizures[1], photosensitivity
Prompt: Within 2-3 weeks, prior to the next course		Myelosuppression, stomatitis, gingivitis, photosensitivity, fatigue	Alopecia, folliculitis, acne, renal toxicity (ATN, increased creatinine/BUN, hematuria), enteritis, GI ulceration and bleeding, acute neurotoxicity[1] (headache, drowsiness, aphasia, paresis, blurred vision, transient blindness, dysarthria, hemiparesis, decreased reflexes) diarrhea, conjunctivitis
Delayed:		Learning disability[1] (L)	Pneumonitis, pulmonary fibrosis (L), hepatic fibrosis (L),

Any time later during therapy, excluding the above conditions			osteonecrosis (L), leukoencephalopathy[1] (L), pericarditis, pericardial effusions, hyperpigmentation of the nails
Late: Any time after the completion of therapy			Progressive CNS deterioration[1]
Unknown Frequency and Timing:	Methotrexate crosses the placenta. Fetal toxicities and teratogenic effects of methotrexate have been noted in humans. The toxicities include: congenital defects, chromosomal abnormalities, severe newborn myelosuppression, low birth weight, abortion, and fetal death. Methotrexate is excreted into breast milk in low concentrations.		

[1] *May be enhanced by HDMTX and/or cranial irradiation.*
(L) Toxicity may also occur later.

Intrathecal Therapy (Methotrexate Single Agent)
Toxicity:

	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, headache	Arachnoditis: (headache, fever, vomiting, meningismus, nuchal rigidity, and pleocytosis)	Anaphylaxis, vomiting, seizures(L), malaise, confusion, back pain, rash, bleeding into subarachnoid or subdural space (risk > with platelet counts < 20,000),
Prompt: Within 2-3 weeks, prior to the next course			Myelosuppression, ataxia, somnolence, cranial nerve palsy, subacute myelopathy (paraparesis/paraplegia), speech disorders, pain in the legs, bladder dysfunction
Delayed: Any time later during therapy, excluding the above condition		Cognitive disturbances (L)[1], learning disability (L)[1]	Leukoencephalopathy[1] (L)
Late: Any time after the completion of treatment			Progressive CNS deterioration[1]

[1] *May be enhanced by HDMTX and/or cranial irradiation.*
(L) Toxicity may also occur later.

Formulation & Stability:
Methotrexate tablets are available as 2.5 mg, 5 mg, 7.5 mg, 10 mg and 15 mg tablets. Inactive ingredients vary depending on manufacturer but tablet formulations may include: anhydrous lactose, crospovidone, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, pregelatinized starch, sodium carbonate monohydrate, talc and titanium dioxide and various dyes. Store at controlled room temperature 15°-30°C (59°-86°F) and protect from light.

Methotrexate is also available as a clear yellow to orange oral solution (Xatmep®) that contains 2.5 mg of methotrexate per milliliter (equivalent to 2.74 mg of methotrexate sodium/mL) in a 120 mL bottle. Inactive ingredients include purified water, sodium citrate, citric acid, methylparaben sodium, propylparaben sodium, and sucralose. It may also contain sodium hydroxide or hydrochloric acid for pH adjustment. It is packaged in a

high-density polyethylene (HDPE) bottle with a child-resistant cap and tamper-evident seal. Store oral solution under refrigeration (2°C to 8°C/36°F to 46°F) prior to dispensing. Avoid freezing and excessive heat. After dispensing, patients may store methotrexate oral solution at room temperature (20°C to 25°C/68°F to 77°F) for up to 60 days; excursions permitted to 15°C to 30°C (59°F to 86°F).

Methotrexate for Injection is available as a lyophilized powder for injection in 1000 mg vials. The powder for injection contains approximately 7 mEq sodium in the 1000 mg vial. Methotrexate for Injection is also available as a 25 mg/mL solution in 2, 4, 8, 10, and 40 mL preservative free vials and 2 and 10 mL vials with preservative. The 2, 4, 8, 10, and 40 mL solutions contain approximately 0.43, 0.86, 1.72, 2.15, and 8.6 mEq sodium per vial, respectively. The preserved vials contain 0.9% benzyl alcohol as a preservative.

Sterile methotrexate powder or solution is stable at 20º-25ºC (68º-77ºF); excursions permitted to 15º-30ºC (59º- 86 Fº). Protect from light

Guidelines for Administration: See Treatment and Dose Modifications sections of protocol. Leucovorin rescue may be necessary with certain doses of methotrexate.

Oral administration: Food or milk delays absorption and reduces peak concentration. Methotrexate for oral use should preferentially be given on an empty stomach, 1 hour before or 2 hours after food or milk and at the same time each day. Methotrexate injection diluted in water can be used for oral administration if an oral solution formulation is not readily available (Marshall PS, Gertner E. Oral administration of an easily prepared solution of injectable methotrexate diluted in water: a comparison of serum concentrations vs methotrexate tablets and clinical utility. *J Rheumatol* 23:455-8, 1996).

For IM/IV use: Powder for injection: Dilute 1000 mg vial with 19.4 mL of preservative free SWFI, D5W or NS to a 50 mg/mL concentration. The powder for injection may be further diluted in NS or dextrose containing solutions to a concentration of ≤ 25mg/mL for IV use.

The 25 mg/mL solution may be given directly for IM administration or further diluted in Saline or Dextrose containing solutions for IV use. **Do not use the preserved solution for high dose methotrexate administration due to risk of benzyl alcohol toxicity.** Methotrexate dilutions are chemically stable for at least 7 days at room temperature but contain no preservative and should be used within 24 hours. Diluted solutions especially those containing bicarbonate exposed to direct sunlight for periods exceeding 4 hours should be protected from light.

High dose methotrexate requires alkalinization of the urine, adequate hydration and leucovorin rescue. Avoid sulfamethoxazole/trimethoprim, probenecid, penicillins, cephalosporins, aspirin, proton pump inhibitors, and NSAIDS as renal excretion of MTX is inhibited by these agents.

For Intrathecal use: Use **preservative free** 25 mg/mL solution.

For intrathecal administration, dilute with 5-10 mL preservative free NS, lactated Ringer's, or Elliot's B solution as per institutional standard of practice. The volume of CSF removed should be equal to at least half the volume delivered.

Patient Age (years)	Methotrexate dose	Recommended volume	10% CSF volume	CSF Volume *
1–1.99	8 mg	5–10 mL	5 mL	50 ± 10 mL (babies)
2–2.99	10 mg	5-10 mL	8 mL	80 ± 20 mL (younger children)
3–8.99	12 mg	5-10 mL	10 mL	100 ± 20 mL (older children)
9 or greater	15 mg	5-10 mL	13 mL	130 ± 30 mL (adults)

*Rieselbach, R.E. et.al. Subarachnoid distribution of drugs after lumbar injection; N Engl J Med. 1962 Dec 20; 267:1273-8

Of Note: Larger volumes approximating at least 10% of the CSF volume, isovolumetric delivery, with the patient remaining prone after the procedure may facilitate drug distribution. These procedures have not been validated in clinical trials. They are allowed but not mandated for patients on COG studies.

Diluted methotrexate for intrathecal administration is stable for 24 hours at 25ºC but contains no preservative and should be administered as soon as possible after preparation.

Supplier: Commercially available from various manufacturers. See package insert for further information.

6.10 PEGASPARGASE

(PEG-asparaginase, PEGLA, PEG-L-asparaginase, polyethylene glycol-L-asparaginase, Oncaspar®)
NSC #624239 **(06/05/17)**

Source and Pharmacology:

Pegaspargase is a modified version of the enzyme L-asparaginase. L-asparaginase is modified by covalently conjugating units of monomethoxypolyethylene glycol (PEG), molecular weight of 5000, to the enzyme, forming the active ingredient PEG-L-asparaginase. The L-asparaginase (L-asparagine amidohydrolase, type EC-2, EC 3.5.1.1) used in the manufacture of Pegaspargase is derived from *Escherichia coli* which is purchased in bulk from Merck, Sharp and Dohme. L-asparagine is a nonessential amino acid synthesized by the transamination of L-aspartic acid by a reaction catalyzed by the enzyme L-asparagine synthetase. The ability to synthesize asparagine is notably lacking in malignancies of lymphoid origin. Asparaginase depletes L-asparagine from leukemic cells (especially lymphoblasts) by catalyzing the conversion of L-asparagine to aspartic acid and ammonia. In predominately L-asparaginase naive adult patients with leukemia and lymphoma, initial plasma levels of L-asparaginase following intravenous administration of pegaspargase were determined. Apparent volume of distribution was equal to estimated plasma volume. L-asparaginase was measurable for at least 15 days following the initial treatment with Pegaspargase. The approximate $t_{½}$ in adult patients is 5.73 days. The enzyme could not be detected in the urine. The half-life is independent of the dose administered, disease status, renal or hepatic function, age, or gender. In a study of newly diagnosed pediatric patients with ALL who received either a single intramuscular injection of pegaspargase (2500 IU/m^2), *E. coli* L-asparaginase (25000 IU/m^2), or *Erwinia* (25000 IU/m^2), the plasma half-lives for the three forms of L-asparaginase were: 5.73 ± 3.24 days, 1.24 ± 0.17 days, and 0.65 ± 0.13 days respectively. The plasma half-life of pegaspargase is shortened in patients who are previously hypersensitive to native L-asparaginase as compared to non-hypersensitive patients. L-asparaginase is cleared by the reticuloendothelial system and very little is excreted in the urine or bile. Cerebrospinal fluid levels are < 1% of plasma levels.

Toxicity:

	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	Allergic reactions (total likelihood of local, and or systemic reaction especially if previous hypersensitivity reaction to native asparaginase), pain at injection site, weakness, fatigue, diarrhea	Allergic reactions (total likelihood of local, and or systemic reaction if **no** previous hypersensitivity reaction to native asparaginase), rash	Anaphylaxis, hyper/hypotension, tachycardia, periorbital edema, chills, fever, dizziness, dyspnea, bronchospasm, lip edema, arthralgia, myalgia, urticaria, mild nausea/vomiting, abdominal pain, flatulence, somnolence, lethargy, headache, seizures (L), hyperuricemia
Prompt: Within 2-3 weeks, prior to the next course	Hyperammonemia (L), coagulation abnormalities with prolonged PTT, PT and bleeding times (secondary to decreased synthesis of fibrinogen, AT-III & other clotting factors) (L)	Hyperglycemia, abnormal liver function tests, pancreatitis (L), increased serum lipase/amylase	Hemorrhage (L), DIC, thrombosis, anorexia, weight loss, CNS ischemic attacks, edema, azotemia and decreased renal function, mild leukopenia, granulocytopenia, thrombocytopenia, pancytopenia, hemolytic anemia, infections (sepsis with/without septic shock, subacute bacterial endocarditis [SBE], URI), CNS changes including irritability, depression, confusion, EEG changes, hallucinations, coma and stupor,

			paresthesias, hypertriglyceridemia, hyperlipidemia, Parkinson-like syndrome with tremor and increase in muscular tone, hyperbilirubinemia, chest pain
Delayed: Any time later during therapy			Renal failure, urinary frequency, hemorrhagic cystitis, elevated creatinine and BUN, fatty liver deposits, hepatomegaly, liver failure
Unknown Frequency and Timing:	Animal reproduction studies have not been conducted with pegaspargase. It is not known whether pegaspargase can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. However, fetal toxicities and teratogenic effects of asparaginase have been noted in animals. It is unknown whether the drug is excreted in breast milk.		

(L)Toxicity may also occur later.

Formulation and Stability:
Each milliliter of pegaspargase contains: PEG-L-asparaginase 750 IU ± 20%, monobasic sodium phosphate, *USP* 1.20 mg ± 5% dibasic sodium phosphate, *USP* 5.58 mg ± 5%, sodium chloride, *USP* 8.50 mg ± 5% , Water for Injection, *USP* qs to 1 mL. The specific activity of pegaspargase is at least 85 IU per milligram protein. Available in 5 mL vials as Sterile Solution for Injection in ready to use single-use vials, preservative free. Keep refrigerated at 2°-8°C (36°-46°F). Do not use if stored at room temperature for more than 48 hours. **DO NOT FREEZE.** Do not use product if it is known to have been frozen. Freezing destroys activity, which cannot be detected visually.

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol.

For IM administration: the volume at a single injection site should be limited to 2 mL. If the volume to be administered is greater than 2 mL, multiple injection sites should be used.

For IV administration: dilute pegaspargase in 100 mL of NS or D5W and infuse over 1 to 2 hours through a NS or D5W running infusion line. Pegaspargase admixed in 100 mL of NS or D5W is stable for 48 hours at room temperature. Pegaspargase diluted in 100 mL of NS is stable for up to 72 hours refrigerated (4ºC [39°F]) (refrigerated stability data on file with Sigma-Tau). Avoid excessive agitation. DO NOT SHAKE. Do not use if cloudy of if precipitate is present.

Have available during and after the infusion: antihistamine, epinephrine, oxygen, and IV corticosteroids. Observe patient for ONE hour after administration for signs of hypersensitivity reactions.

Supplier: Commercially available. See package insert for further information.

6.11 PREDNISO(LO)NE

(11/16/17)

(Deltasone®, PrediSONE Intensol®, Rayos®, Meticorten®, Liquid Pred, Pediapred®, Millipred®, OraPred ODT®) NSC #010023 (prednisome), NSC# 9151 (prednisolone)

Source and Pharmacology:
Prednisone and prednisolone are a synthetic compounds closely related to hydrocortisone. Glucocorticoids produce widespread and diverse physiologic effects on carbohydrate, protein, and lipid metabolism, electrolyte and water balance, functions of the cardiovascular system, kidney, skeletal muscle, and the nervous systems. Glucocorticoids reduce the concentration of thymus-dependent lymphocytes (T-lymphocytes), monocytes, and eosinophils. Glucocorticoids selectively

bind to the cortisol receptors on human lymphoid cells which are found in larger numbers on leukemic lymphoblasts. They also decrease binding of immunoglobulin to cell surface receptors and inhibit the synthesis and/or release of interleukins, thereby decreasing T-lymphocyte blastogenesis and reducing expansion of the primary immune response. The specific cellular mechanisms that act to halt DNA synthesis are thought to be related to inhibition of glucose transport or phosphorylation, retardation of mitosis, and inhibition of protein synthesis. Peak blood levels occur within 2 hours of oral intake. Prednisone is approximately 75% protein bound with a plasma $t_{½}$ of 3.2 to 4 hours. (Biologic half-life is 12-36 hours.)

Toxicity:

	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	Insomnia, hyperphagia	Gastritis	Hyperuricemia
Prompt: Within 2-3 weeks, prior to the next course	Immunosuppression, personality changes (mood swings, euphoria, anxiety, depression), pituitary-adrenal axis suppression, acne (L)	Hyperglycemia, facial erythema, poor wound healing, infections (bacterial, fungal, parasitic, viral), edema	Pancreatitis (L), electrolyte imbalance (Na retention, hypokalemia, hypocalcemia) (L), increased intraocular pressure (L), hypertension, psychosis, vertigo, headache
Delayed: Any time later during therapy	Cushing's syndrome (moon facies, truncal obesity)	Striae and thinning of the skin, easy bruising, muscle weakness, osteopenia	Spontaneous fractures (L), growth suppression, peptic ulcer and GI bleeding, pseudotumor cerebri (increased intracranial pressure with papilledema, headache), aseptic necrosis of the femoral and humeral heads (L), urolithiasis[1] (L)
Late: Any time after completion of treatment		Cataracts (which may be reversible on discontinuation of prednisone in children)	
Unknown Frequency and Timing:	Fetal and teratogenic toxicities: Corticosteroids cross the placenta (prednisone has the poorest transport). In animal studies, large doses of cortisol administered early in pregnancy produced cleft palate, stillborn fetuses, and decreased fetal size. Chronic maternal ingestion during the first trimester has shown a 1% incidence of cleft palate in humans. Prednisone is excreted into breast milk in humans; however, several studies suggest that amounts excreted in breast milk are negligible with prednisone doses ≤ 20 mg/day.		

[1] Mainly reported in pediatric patients with ALL. Howard SC et al. Urolithiasis in pediatric patients with acute lymphoblastic leukemia. Leukemia 2003; 17: 541-6.
(L) Toxicity may also occur later.

Formulation and Stability:
Prednisone is available in 1 mg, 2.5 mg, 5 mg, 10 mg, 20 mg, and 50 mg tablets. Also available as a solution in 1 mg/1 mL or 5 mg/mL concentrations. Inactive ingredients vary depending on manufacturer but tablet formulations may include calcium or magnesium stearate, corn starch, lactose, erythrosine sodium, mineral oil, sorbic acid, sucrose, talc and various dyes. The solution may include 5-30% alcohol, fructose, sucrose, saccharin, and sorbitol.

Prednisolone is available as 5 mg scored tablets (base) and 10 mg, 15 mg, and 30 mg orally disintergrating tablets (ODT; sodium phosphate [strength expressed as base]). Liquid formulations of prednisolone are available as 15 mg/5 mL oral solution (base); 5 mg/5 mL, 10 mg/5 mL, 15 mg/5

mL, 20 mg/5 mL oral solution (sodium phosphate [strength expressed as base]; and 15 mg/5 mL oral syrup (base). Inactive ingredients vary depending on manufacturer. Tablet formulations may contain dyes and liquid formulations may contain edetate disodium, methylparaben, saccharin sodium.

Guidelines for Administration:
See Treatment and Dose Modifications sections of the protocol.

PredniSONE and prednisolone are equipotent corticosteroids.

Supplier: Commercially available from various sources. See package insert for further information.

6.12 THIOGUANINE

(6-thioguanine, tioguanine, 2-amino-1,7-dihydro-6H-purine-6-thione, WR-1141, Tabloid®, Lanvis®)
NSC #752 **(12/05/16)**

Source and Pharmacology:
Thioguanine is a purine analogue of the nucleic acid guanine with the substitution of a thiol group in place of the hydroxyl group on guanine. The main intracellular pathway for 6-TG activation is catalyzed by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) which catalyzes the conversion of 6-TG to the active nucleotide, 6-thioguanylic acid. The monophosphate nucleotide form of 6-TG inhibits *de novo* purine synthesis and purine interconversion reactions, whereas the nucleotide triphosphate metabolite is incorporated directly into nucleic acids. Incorporation of fraudulent nucleotides into DNA interferes with DNA replication and results in the formation of DNA strand breaks. The net consequence of its action is a sequential blockade of the synthesis and utilization of the purine nucleotides. The relative contribution of each of these actions to the mechanism of cytotoxicity of 6-TG is unclear. The absorption of an oral dose of 6-TG is incomplete and variable, averaging approximately 30% of the administered dose (range: 14% to 46%).

6-TG undergoes deamination by the enzyme guanine deaminase resulting in 6-thioxanthene, which is then oxidized by xanthine oxidase to 6-thiouric acid. In contrast to mercaptopurine, 6-TG is not a direct substrate for xanthine oxidase. Because the inhibition of xanthine oxidase results in the accumulation of 6-thioxanthene, an inactive metabolite, adjustments in 6-TG dosage are not required for patients receiving allopurinol. Since TPMT, 6-thiopurine methyltransferase, is one of the enzymes involved in the deactivation of 6-TG, those individuals who have an inherited deficiency of the enzyme may be unusually sensitive to the myelosuppressive effects of 6-TG and prone to developing rapid bone marrow suppression following the initiation of treatment.

Peak levels occur 2 to 4 hours after oral administration with a median half-life is about 90 minutes (range: 25-240 minutes). Very little unchanged drug is excreted renally.

Toxicity:

	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		Anorexia, nausea, vomiting, diarrhea, malaise	Urticaria, rash, hyperuricemia

Prompt: Within 2-3 weeks, prior to next course	Myelosuppression		Toxic hepatitis (L), increased SGOT (AST)/SGPT (ALT), ataxia, mucositis
Delayed: Anytime later during therapy			Hepatic fibrosis(L), sinusoidal obstruction syndrome (SOS, formerly VOD) (L), hyperbilirubinemia
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of thioguanine have been noted in animals. It is unknown whether the drug is excreted in breast milk.		

(L) Toxicity may also occur later.

Formulation and Stability:
Each greenish-yellow, scored tablet contains 40 mg thioguanine. Store at 15°-25°C (59°-77°F) in a dry place.

For patients unable to swallow tablets, a 20 mg/mL oral suspension may be compounded. Crush fifteen (n=15) 40 mg tablets in a mortar and reduce to a fine powder. Add 10 mL methylcellulose 1% in incremental proportions and mix to a uniform paste. Transfer to a graduated cylinder, rinse mortar with simple syrup, and add quantity of simple syrup sufficient to make 30 mL. Dispense in an amber glass bottle and label "shake well" and "refrigerate". If methylcellulose is not available, substitute 15 mL of Ora-Plus in place of the methylcellulose and qs with Ora-Sweet (in place of simple syrup) to a final volume of 30 mL. Both preparations are stable for 63 days at 19° C – 23° C. (Aliabadi HM, Romanick M, Somayah V, et al. Stability of compounded thioguanine oral suspensions. *Am J Health Syst Pharm* 2011;68:1278. Dressman JB, Poust RI. Stability of Allopurinol and Five Antineoplastics in Suspension. *Am J Hosp Pharm* 1983;40(4):616-8.)

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol. Thioguanine should be taken consistently at the same time every day.

Substantial dosage reductions may be required in patients with an inherited deficiency of the enzyme thiopurine methyltransferase (TPMT) due to accumulation of active thioguanine metabolites resulting in a higher incidence of myelosuppression.

Supplier: Commercially available. See package insert for more detailed information.

6.13 VINCRISTINE SULFATE
(Oncovin®, VCR, LCR) NSC #67574 **(08/16/12)**

Source and Pharmacology:
Vincristine is an alkaloid isolated from Vinca rosea Linn (periwinkle). It binds to tubulin, disrupting microtubules and inducing metaphase arrest. Its serum decay pattern is triphasic. The initial, middle, and terminal half-lives are 5 minutes, 2.3 hours, and 85 hours respectively; however, the range of the terminal half-life in humans is from 19 to 155 hours. The liver is the major excretory organ in humans and animals; about 80% of an injected dose of vincristine sulfate appears in the feces and 10% to 20% can be found in the urine. The p450 cytochrome involved with vincristine metabolism is CYP3A4. Within 15 to 30 minutes after injection, over 90% of the drug is distributed from the blood into tissue, where it remains tightly, but not irreversibly bound. It is excreted in the bile and feces. There is poor CSF penetration.

Toxicity:

	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		Jaw pain, headache	Extravasation (rare) but if occurs = local ulceration, shortness of breath, and bronchospasm
Prompt: Within 2-3 weeks, prior to the next course	Alopecia, constipation	Weakness, abdominal pain, mild brief myelosuppression (leukopenia, thrombocytopenia, anemia)	Paralytic ileus, ptosis, diplopia, night blindness, hoarseness, vocal cord paralysis, SIADH, seizure, defective sweating
Delayed: Any time later during therapy	Loss of deep tendon reflexes	Peripheral paresthesias including numbness, tingling and pain; clumsiness; wrist drop, foot drop, abnormal gait	Difficulty walking or inability to walk; sinusoidal obstruction syndrome (SOS, formerly VOD) (in combination); blindness, optic atrophy; urinary tract disorders (including bladder atony, dysuria, polyuria, nocturia, and urinary retention); autonomic neuropathy with postural hypotension; 8th cranial nerve damage with dizziness, nystagmus, vertigo and hearing loss
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of vincristine (either alone or in combination with other antineoplastic agents) have been noted in humans. The toxicities include: chromosome abnormalities, malformation, pancytopenia, and low birth weight. It is unknown whether the drug is excreted in breast milk.		

Formulation and Stability:
Vincristine is supplied in 1 mL and 2 mL vials in which each mL contains vincristine sulfate 1 mg (1.08 µmol), mannitol 100 mg, SWFI; acetic acid and sodium acetate are added for pH control. The pH of vincristine sulfate injection, *USP* ranges from 3.5 to 5.5. This product is a sterile, preservative free solution. Store refrigerated at 2˚-8˚C or 36˚-46˚F. Protect from light and retain in carton until time of use.
Do not mix with any IV solutions other than those containing dextrose or saline.

Guidelines for Administration: See Treatment and Dose Modifications sections of protocol.

The World Health Organization, the Institute of Safe Medicine Practices (United States) and the Safety and Quality Council (Australia) all support the use of minibag rather than syringe for the infusion of vincristine. The delivery of vincristine via either IV slow push or minibag is acceptable for COG protocols. Vincristine should **NOT** be delivered to the patient at the same time with any medications intended for central nervous system administration. Vincristine is fatal if given intrathecally.

Injection of vincristine sulfate should be accomplished as per institutional policy. Vincristine sulfate must be administered via an intact, free-flowing intravenous needle or catheter. Care should be taken to ensure that the needle or catheter is securely within the vein to avoid extravasation during administration. The solution may be injected either directly into a vein or into the tubing of a running intravenous infusion.

Special precautions: FOR INTRAVENOUS USE ONLY.
The container or the syringe containing vinCRIStine must be enclosed in an overwrap bearing the statement: "Do not remove covering until moment of injection. For intravenous use only - Fatal if given by other routes."

Supplier: Commercially available from various manufacturers. See package insert for more detailed information.

7.0 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable (except where explicitly prohibited within the protocol).

7.1 End of Therapy & Follow-up

STUDIES TO BE OBTAINED	End of Therapy
History	X
Physical Exam with VS	X
Ht, Wt, BSA	X
Performance Status	X
CBC, differential, platelets	X
Urinalysis	X
Electrolytes including Ca^{++}, PO_4, Mg^{++}	X
Creatinine, SGPT, bilirubin	X
Total protein/albumin	X
ECHO	X

See COG Late Effects Guidelines for recommended post treatment follow-up: http://www.survivorshipguidelines.org/

Note: Follow-up data are expected to be submitted per the Case Report Forms (CRFs) schedule.

7.2 Correlative Biology Studies

The pharmacodynamic (PD) assessment of DNA methylation (Section 14.1) is a required correlative study. All other studies in the table below require patient consent.

For patients that consented to cell banking on AALL08B1 or APEC14B1 (if open), submit required samples.

Note: If the volume of blood required for correlative studies exceeds safe limits of blood draw over a 24 hour period, the priority for peripheral blood sampling for infants on this study is as follows:

- Test required for clinical care
- Pharmacodynamics of DNA methylation (Section 14.1)
- Banking for future research (Section 14.3)
- Pharmacokinetics of Azacitidine (Section 14.4)
- (CAR) T cell expansion (Section 14.5)

SAMPLES TO BE OBTAINED	Diagnosis	Induction	EPI #1	Consolidation	EPI #2	IM	DI PT. 1	Relapse
PD Assessment of DNA Methylation: Peripheral Blood Section 14.1			X		X			
MRD Results Reporting Section 14.2		X		X		X		
Banking for Future Research Section 14.3	X	X		X		X		X
PK of Aza: Peripheral Blood Appendix VIII			X					
Assessment of Infant T cell Proliferative Capacity: Peripheral Blood Section 14.5	X	X		X				
PD of Peg: Report Results Section 14.6		X				X	X	

7.3 Shipping Information for MRD Flow Cytometry Laboratories

For a list of COG-approved Flow Cytometry Labs, see:
https://members.childrensoncologygroup.org/_files/admin/mrdflowlabs.pdf

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Removal from Protocol Therapy

a) Patients without *KMT2A*-R leukemia following Induction and prior to EPI Block #1.
b) Patients with *KMT2A*-R leukemia who do not meet the criteria to start EPI Block #1 by Day 64.
c) Two or more dose limiting toxicities (DLTs) in an individual patient.
d) Failure to achieve M1 marrow status following completion of Consolidation therapy.
e) Relapse at any site after achieving remission.
f) Adverse Events/ Side Effects/ Complications.
g) Refusal of further protocol therapy by patient/parent/guardian.
h) Completion of planned therapy.
i) Physician determines it is in patient's best interest.
j) Development of a second malignancy.
k) Repeat eligibility studies (if required) are outside the parameters required for eligibility (see Section 3.2).
l) Inevaluable.
m) Patients with *KMT2A*-R leukemia who meet criteria to start EPI Block #1, following the treatment of 6 patients with azacitidine, but prior to completion of interim toxicity analysis.

Patients who are off protocol therapy are to be followed until they meet the criteria for Off Study (see below). Follow-up data will be required unless patient is taken off study.

8.2 Off Study Criteria

a) Death
b) Lost to follow-up
c) Patient enrollment onto another COG study with tumor therapeutic intent (e.g., at recurrence).
d) Withdrawal of consent for any further data submission.
e) The fifth anniversary of the date the patient was enrolled on this study.

9.0 STATISTICAL CONSIDERATIONS

9.1 Sample Size and Study Duration

This study will accrue infant patients with ALL for approximately 1.5 to 3.0 years (3.0 if there is a dose reduction). Based on review of infant patient accrual onto COG protocol AALL0631, and since this protocol will be open group-wide, we expect an enrollment rate of approximately 40 patients/year. The study will enroll a minimum of 4 and maximum of up to 116 patients. A minimum of 2 and a maximum of 58 infants with ALL will be enrolled at the first dose level (DL1; Azacitidine 2.5 mg/kg). If a dose reduction is required, then a minimum of 2 and maximum of 58 infants would be enrolled on the reduced dose level (DL0; Azacitidine 1.8 mg/kg).

The maximum accrual of 116 is to ensure the goal of at least 30 evaluable *KMT2A*-R patients for a given dose level. This accounts for the assumptions of 80% *KMT2A*-R, 90% eligibility, and up to 25% drop out before conclusion of the evaluability period.

Amendment # 3
The study was temporarily closed to enrollment on October 30, 2018, when the accrual goal was met for dose level 1 (DL1).

With this amendment the study accrual is increased to allow enrollment of an additional 20 subjects at DL1 to reach accrual of 30 evaluable subjects to ensure fulfillment of the primary study aim. If we use the actual study rates of 71% KMT2A-r rate, 90% eligibility rate, and 43% drop-out rate, 20 additional subjects will result in about 7 additional evaluable subjects. Duration of extended accrual is expected to be 6 months.

9.2 Study Design

9.2.1 Primary Endpoint

To assess tolerability of azacitidine in combination with Interfant-06 standard chemotherapy in 30 evaluable infant patients with newly diagnosed ALL with *KMT2A* gene rearrangement (*KMT2A*-R).

9.2.2 Secondary Endpoint

To evaluate the biologic activity of azacitidine by pharmacodynamic assessment of global DNA methylation in peripheral blood mononuclear cells (PBMCs) of infants treated with azacitidine.

9.2.3 Exploratory Endpoints

9.2.3.1 To determine the 5 year event free survival (EFS) of infants with *KMT2A*-R treated with azacitidine in addition to Interfant-06 standard chemotherapy.

9.2.3.2 To correlate minimal residual disease with outcome in the context of the protocol therapy.

9.2.3.3 To perform pharmacokinetic (PK) testing of azacitidine in infants.

9.2.3.4 To test the expansion of infant T lymphocytes by stimulation with artificial antigen presenting cells identical to those used in CART-19 production.

9.2.3.5 To collect pharmacodynamic (PD) data for asparaginase activity following Pegaspargase administration in infants.

9.2.4 Analysis for Primary Endpoint

A single dose level design, with continuous enrollment, will be used for dose level tolerability and biologic activity determination. Enrollment will begin on DL1, with reduction to DL0 only if DL1 is deemed too toxic. Up to 58 patients will be enrolled at DL1, with the goal of evaluating 30 patients with *KMT2A*-R for tolerability and biologic activity (see Section 9.5 for definition of evaluability for primary endpoint). If dose reduction to DL0 is required, then up to 58 additional

patients will be enrolled at DL0, with the goal of evaluating 30 patients with *KMT2A*-R for tolerability and biologic activity.

With Amendment #3, the study accrual is increased to allow enrollment of an additional 20 subjects at DL1 (total 78 patients) to reach accrual of 30 evaluable subjects to ensure fulfillment of the primary study aim. If we use the actual study rates of 71% KMT2A-r rate, 90% eligibility rate, and 43% drop-out rate, 20 additional subjects will result in about 7 additional evaluable subjects.

DL1 and/or DL0 will be considered intolerable at any time if the number of patients with a DLT meets or exceeds pre-determined stopping boundaries. In the event that DL1 is deemed too toxic, any patient with remaining doses of azacitidine planned at DL1 will instead receive DL0. If neither dose level is tolerable, then the investigators will review the biologic correlates to determine if there is sufficient evidence of epigenetic modification to propose amending the study to test a lower DL.

After the 6th evaluable patient, DLTs will be monitored continuously using Pocock stopping bounds as described by Ivanova *et al*.[77] As detailed in Section 5.1.1, a DLT is defined such that we expect chemotherapy alone would result in a DLT rate of approximately 10% (including a < 10% rate of delays in subsequent courses and a toxic death rate of approximately 3%). The stopping bounds for DLTs are designed to stop the study early with probability of 5% when the true DLT rate is 10%. The dose level stopping bounds that will deem a dose level excessively toxic are based on the number of evaluable patients and the number of patients with DLTs at any given time (Table 2). Table 2 assumes evaluation of 6 patients prior to initiation of stopping boundaries. The intent of this design is to allow the trial to progress in the event of a spuriously high rate of DLT during early enrollment, while maintaining safety by creating boundaries that become more conservative as the study progresses. With 30 evaluable patients, the probabilities of declaring the dose level as too toxic are approximately 82.5%, 40.1%, and 4.3% when the true DLT rates are 30%, 20%, and 10%, respectively.

Table 2. Continuous monitoring table for DLT

n	b(n)
≤6	3
7-10	4
11-16	5
17-21	6
22-28	7
29-30	8

n = number of evaluable patients treated on any single dose level
b(n) = toxicity boundary (If the number of patients with at least one DLT is ≥ b(n) on any single dose level, then that dose level will be deemed excessively toxic)

Toxic deaths will be considered DLT events (see definition of DLT in Section 5.1) and will also be monitored independently of DLT. Toxic death will be defined as any Grade 5 toxicity that occurs as a first event during protocol therapy. The expected toxic death rate during the DLT evaluation period is 3%. The study will

be stopped for toxicity and safety review if 2 of the first 15 or any 3 infants experience a toxic death during the evaluation period. Using 3% as the planning rate, the study will have a 75.8% probability of stopping if the true death rate is 12% and a 10.0% chance of stopping for review if the true death rate is 3%. Pending the outcome of the toxicity and safety review, the study may progress with further enrollment, with amendments if necessary.

An informal interim analysis of toxicity and safety will be conducted after 3 patients complete EPI #1 and Consolidation. Prior to the completion of this interim analysis, no more than 6 patients will be exposed to azacitidine (i.e., if a 7th patient is due to receive the first dose of azacitidine prior to the interim analysis of the first 3 patients, this patient will be removed from protocol therapy). The study committee will convene to review the toxicity data for each of these 3 patients, and will formulate an adjusted plan for further enrollment, if necessary.

9.2.5 Analysis for Secondary Endpoint

LINE-1 global methylation assessment of peripheral blood mononuclear cells (PBMCs) on Day 1 prior to azacitidine and on Day 5 of the first two courses of azacitidine will be performed. We will observe for relative changes in methylation in infant PBMCs. We will calculate the mean LINE-1 methylation for all patients before and after azacitidine and perform paired t-test analysis to determine if there is significant demethylation in the study population for the tested dose level.

9.2.6 Analysis for Exploratory Endpoints

Five year EFS will be estimated for infants with *KMT2A*-R treated with azacitidine in addition to Interfant-06 standard chemotherapy. With a sample size of 30 we would be able to estimate this with a maximum standard error of 9.1%.

The following are all optional studies and hence sample size availability for analyses cannot be accurately assessed. Statistical analyses will be primarily descriptive. Minimal Residual Disease evaluations are optional studies; hence it is not possible to estimate how many patients will be evaluable for this endpoint. Descriptive analysis will be conducted to correlate MRD with the EFS for the *KMT2A*-R patients. Pharmacokinetic (PK) testing and analysis of azacitidine in infants will be performed by Covance as described in Appendix VIII. Optional biological study participation will explore the feasibility of T-cell collection for the purposes of chimeric antigen receptor (CAR) T-cell production from the peripheral blood in infants with ALL as described in Section 14.5. Pharmacodynamic (PD) data for asparaginase activity following pegaspargase administration in infants will be collected and correlated with EFS for the *KMT2A*-R patients (see Section 14.6).

9.3 Methods of Analysis

Toxicity monitoring will be performed using Pocock continuous stopping bounds as described by Ivanova *et al*.[77] Toxic deaths will also be monitored with guidelines in place for toxicity and safety review as detailed above. Paired t-tests will be used to test demethylation levels before and after the first course of azacitidine. Five year EFS estimation will be calculated from time of enrollment. Standard errors and confidence intervals for EFS will be calculated using Peto's method.[78]

9.4 Evaluability for Response

Response will be evaluated at the end of Induction. All patients on study at end of Induction will be evaluated for response.

9.5 Evaluability for Toxicity

The presence or absence of a DLT will be determined for each patient during each of the first 3 courses of azacitidine in combination with chemotherapy and until the patient meets count requirements to begin the fourth course of azacitidine therapy. Course 4 will not be included in the DLT evaluation period because it includes a repeat of drug combinations used in the prior blocks. Patients who do not experience a DLT but go off protocol therapy prior to completion of Course 3 of azacitidine will be inevaluable for DLT and will be replaced.

In addition to monitoring for DLT, distinct stopping rules will be in place for an unexpectedly high rate of deaths due to toxicity during the evaluation period (see Section 5.1.1).

9.6 Gender and Minority Accrual Estimates

The gender and minority distribution of the study population is expected to be:

DOMESTIC PLANNED ENROLLMENT					
Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	1	1	0	0	**2**
Asian	4	1	0	0	**5**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	7	2	0	0	**9**
White	47	31	14	15	**107**
More Than One Race	1	0	1	0	**2**
Total	**60**	**35**	**15**	**15**	**125**

This distribution was derived from enrollment distributions on AALL0631 with known Ethnic/Race categories.

INTERNATIONAL (including Canadian participants) PLANNED ENROLLMENT					
Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	0	1	0	0	**1**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	0	0	0	0	**0**
White	4	6	0	0	**10**
More Than One Race	0	0	0	0	**0**
Total	**4**	**7**	**0**	**0**	**11**

This distribution was derived from enrollment distributions on AALL0631 with known Ethnic/Race categories.

10.0 EVALUATION CRITERIA

10.1 Common Terminology Criteria for Adverse Events (CTCAE)

This study will utilize version 4.0 of the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). Additionally, toxicities are to be reported on the appropriate case report forms.

Please note: 'CTCAE v4.0' is understood to represent the most current version of CTCAE v4.0 as referenced on the CTEP website (i.e., v4.02 and all subsequent iterations prior to version 5.0).

10.2 Response Criteria for Patients with Leukemia

See definitions in Section 3.4

11.0 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Purpose

Adverse event (AE) data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Certain adverse events must be reported in an expedited manner to allow for timelier monitoring of patient safety and care. The following sections provide information about expedited reporting.

11.2 Determination of reporting requirements

Reporting requirements may include the following considerations: 1) whether the patient has received an investigational or commercial agent; 2) the characteristics of the adverse event including the *grade* (severity), the *relationship to the study therapy* (attribution), and the *prior experience* (expectedness) of the adverse event; 3) the Phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

An investigational agent is a protocol drug administered under an Investigational New Drug Application (IND). In some instances, the investigational agent may be available commercially, but is actually being tested for indications not included in the approved package label.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. The NCI, rather than a commercial distributor, may on some occasions distribute commercial agents for a trial.

When a study includes both investigational and commercial agents, the following rules apply.

- *Concurrent administration*: When an investigational agent is used in combination with a commercial agent, the combination is considered to be investigational and expedited reporting of adverse events would follow the guidelines for investigational agents.
- *Sequential administration*: When a study includes an investigational agent and a commercial agent on the same study arm, but the commercial agent is given for a period of time prior to starting the investigational agent, expedited reporting of adverse events that occur prior to starting the investigational agent would follow the guidelines for commercial agents. Once therapy with the investigational agent is initiated, all expedited reporting of adverse events follow the investigational agent reporting guidelines.

11.3 Expedited Reporting Requirements – Serious Adverse Events (SAEs)

To ensure compliance with these regulations/this guidance, as IND/IDE sponsor, NCI requires that AEs be submitted according to the timeframes in the AE reporting tables assigned to the protocol, using the CTEP Adverse Event Reporting System (CTEP-AERS).

Any AE that is serious qualifies for expedited reporting. An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. A Serious Adverse Event (SAE) is any adverse drug event (experience) occurring at any dose that results in ANY of the following outcomes:

1) Death.
2) A life-threatening adverse drug experience.
3) An adverse event resulting in inpatient hospitalization or prolongation of existing hospitalization (for ≥ 24 hours). This does not include hospitalizations that are part of routine medical practice.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

11.4 Special Situations for Expedited Reporting

11.4.1 SAEs Occurring More than 30 Days After Last Dose of Study Drug

Any Serious Adverse Event that occurs more than 30 days after the last administration of the investigational agent/intervention **and** has an attribution of a possible, probable, or definite relationship to the study therapy must be reported according to the CTEP-AERS reporting tables in this protocol.

11.4.2 Persistent or Significant Disabilities/Incapacities

Any AE that results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions (formerly referred to as disabilities), congenital anomalies or birth defects, must be reported via CTEP-AERS if it occurs at any time following treatment with an agent under a NCI IND/IDE since these are considered to be serious AEs.

11.4.3 Death

Reportable Categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: Newborn death occurring during the first 28 days after birth.
- Sudden Death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as Grade 5 "*Disease progression*" in the system organ class (SOC) "*General disorders and administration site conditions"*. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Any death occurring ***within 30 days*** of the last dose, regardless of attribution to the investigational agent/intervention requires expedited reporting within 24 hours.

Any death occurring ***greater than 30 days*** after the last dose of the investigational agent/intervention requires expedited reporting within 24 hours **only if** it is possibly, probably, or definitely related to the investigational agent/intervention.

11.4.4 Secondary Malignancy

A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A metastasis of the initial neoplasm is not considered a secondary malignancy.

The NCI requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy
- Myelodysplastic syndrome
- Treatment related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) must also be reported via the routine reporting mechanisms outlined in this protocol.

11.4.5 Second Malignancy

A ***second malignancy*** is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting via CDUS unless otherwise specified.

11.5 Reporting Requirements for Specialized AEs

11.5.1 Baseline AEs

Although a pertinent positive finding identified on baseline assessment is not an AE, when possible it is to be documented as "Course Zero" using CTCAE terminology and grade. An expedited AE report is not required if a patient is entered on a protocol with a pre-existing condition (e.g., elevated laboratory value, diarrhea). The baseline AE must be re-assessed throughout the study and reported if it fulfills expedited AE reporting guidelines.

a. If the pre-existing condition worsens in severity, the investigator must reassess the event to determine if an expedited report is required.
b. If the AE resolves and then recurs, the investigator must re-assess the event to determine if an expedited report is required.
c. No modification in grading is to be made to account for abnormalities existing at baseline.

11.5.2 Persistent AEs

A persistent AE is one that extends continuously, without resolution between treatment cycles/courses.

ROUTINE reporting: The AE must be reported only once unless the grade becomes more severe in a subsequent course. If the grade becomes more severe the AE must be reported again with the new grade.
EXPEDITED reporting: The AE must be reported only once unless the grade becomes more severe in the same or a subsequent course.

11.5.3 Recurrent AEs

A recurrent AE is one that occurs and resolves during a cycle/course of therapy and then reoccurs in a later cycle/course.
ROUTINE reporting: An AE that resolves and then recurs during a subsequent cycle/course must be reported by the routine procedures.
EXPEDITED reporting: An AE that resolves and then recurs during a subsequent cycle/course does not require CTEP-AERS reporting unless:

1) The grade increases OR
2) Hospitalization is associated with the recurring AE.

11.6 Exceptions to Expedited Reporting

11.6.1 Specific Protocol Exceptions to Expedited Reporting (SPEER)

SPEER: Is a subset of AEs within the Comprehensive Adverse Events and Potential Risks (CAEPR) that contains a list of events that are considered expected for CTEP-AERS reporting purposes. (Formerly referred to as the Agent Specific Adverse Event List (ASAEL).

AEs listed on the SPEER should be reported expeditiously by investigators to the NCI via CTEP-AERS ONLY if they exceed the grade of the event listed in parentheses after the event. If the CAEPR is part of a combination IND using multiple investigational agents and has an SAE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

11.6.2 Special Situations as Exceptions to Expedited Reporting

An expedited report may not be required for a specific protocol where an AE is listed as expected. The exception or acceptable reporting procedures will be specified in the protocol. The protocol specific guidelines supersede the NCI Adverse Event Reporting Guidelines. These special situations are listed under the CTEP-AERS reporting Table A for this protocol.

11.7 Reporting Requirements – Investigator Responsibility

Clinical investigators in the treating institutions and ultimately the Study Chair have the primary responsibility for AE identification, documentation, grading, and assignment of attribution to the investigational agent/intervention. It is the responsibility of the treating physician to supply the medical documentation needed to support the expedited AE reports in a timely manner.

Note: All expedited AEs (reported via CTEP-AERS) must also be reported via routine reporting. Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database.

11.8 General Instructions for Expedited Reporting via CTEP-AERS

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

An expedited AE report for all studies utilizing agents under an NCI IND/IDE must be submitted electronically to NCI via CTEP-AERS at: https://eapps-ctep.nci.nih.gov/ctepaers.

In the rare situation where Internet connectivity is disrupted, the 24-hour notification is to be made to the NCI for agents supplied under a CTEP IND by telephone call to (301) 897-7497.
In addition, once Internet connectivity is restored, a 24-hour notification that was phoned in must be entered into the electronic CTEP-AERS system by the original submitter of the report at the site.

- Expedited AE reporting timelines are defined as:
  - **24-Hour; 5 Calendar Days -** The AE must initially be reported via CTEP-AERS within 24 hours of learning of the event, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
  - **7 Calendar Days -** A complete expedited report on the AE must be submitted within 7 calendar days of the investigator learning of the event.

- Any event that results in a persistent or significant incapacity/substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect, or is an IME, which based upon the medical judgment of the investigator may jeopardize the patient and require intervention to prevent a serious AE, must be reported via CTEP-AERS **if the event occurs following investigational agent administration.**

- Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an NCI IND/IDE requires expedited reporting **within 24 hours**.

- Any death occurring greater than 30 days of the last dose with an attribution of possible, probable, or definite to an agent/intervention under an NCI IND/IDE requires expedited reporting **within 24 hours**.

CTEP-AERS Medical Reporting includes the following requirements as part of the report: 1) whether the patient has received at least one dose of an investigational agent on this study; 2) the characteristics of the adverse event including the *grade* (severity), the *relationship to the study therapy* (attribution), and the *prior experience* (expectedness) of the adverse event; 3) the Phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

Any medical documentation supporting an expedited report (e.g., H & P, admission and/or notes, consultations, ECG results, etc.) MUST be faxed within 48-72 hours to the NCI. NOTE: English is required for supporting documentation submitted to the numbers listed below in order for the NCI to meet the regulatory reporting timelines.

Fax supporting documentation **for AEs related to investigational agents supplied under a CTEP IND** to: (301) 897-7404.

Also: Fax or email supporting documentation to COG for **all** IND studies (Fax # (310) 640-9193; email: COGAERS@childrensoncologygroup.org; Attention: COG AERS Coordinator).

- **ALWAYS include the ticket number on all faxed documents.**
- **Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.**

11.9 Reporting Table for Late Phase 2 and Phase 3 Studies – Table A

Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:
1) Death.
2) A life-threatening adverse event.
3) Any AE that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours. This does not include hospitalizations that are part of routine medical practice.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6.)

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	**7 Calendar Days**			**24-Hour Notification 5 Calendar Days**
Not resulting in Hospitalization ≥ 24 hrs	**Not Required**		**7 Calendar Days**	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR. Additional Special Situations as Exceptions to Expedited Reporting are listed below.

Expedited AE reporting timelines are defined as: "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour notification. "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.
[1]SAEs that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows: **Expedited 24-hour notification followed by complete report within 5 calendar days for:** • All Grade 4, and Grade 5 AEs **Expedited 7 calendar day reports for:** • Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization • Grade 3 adverse events

11.10 Protocol Specific Additional Instructions and Reporting Exceptions

- **Grades 1-4 myelosuppression (anemia, neutropenia, thrombocytopenia) do not require expedited reporting.**
- **Grades 1-2 AST/ALT elevations do not require expedited reporting.**

11.11 Reporting of Adverse Events for commercial agents – CTEP-AERs abbreviated pathway

The following are expedited reporting requirements for adverse events experienced by patients on study who have not received any doses of an investigational agent on this study. Commercial reporting requirements are provided in Table B.

COG requires the CTEP-AERS report to be submitted **within 7 calendar days** of learning of the event.

Table B
Reporting requirements for adverse events experienced by patients on study who have NOT received any doses of an investigational agent on this study.

CTEP-AERS Reporting Requirements for Adverse Events That Occur During Therapy With a Commercial Agent or Within 30 Days[1]

Attribution	**Grade 4**		**Grade 5**
	Unexpected	Expected	
Unrelated or Unlikely			CTEP-AERS
Possible, Probable, Definite	CTEP-AERS		CTEP-AERS
[1]This includes all deaths within 30 days of the last dose of treatment with a commercial agent, regardless of attribution. Any death that occurs more than 30 days after the last dose of treatment with a commercial agent that can be attributed (possibly, probably, or definitely) to the agent and is not due to cancer recurrence must be reported via CTEP-AERS.			

11.12 Routine Adverse Event Reporting

Note: The guidelines below are for routine reporting of study specific adverse events on the COG case report forms and do not affect the requirements for CTEP-AERS reporting.

Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database. For this study, routine reporting will include all toxicities reported via CTEP-AERS, non-hematologic Grade 4 and higher Adverse Events, Grade 3 and higher Infectious Events, Adverse Events resulting in Dose Limiting Toxicities (DLTs, Section 5.1), as well as the following specific toxicities identified by the ALL Toxicity Reporting Task Force to be collected on all future COG ALL trials::

1. CNS hemorrhage requiring medical intervention (Grade 2 or 3)
2. GI bleed requiring operative or interventional radiology intervention (Grade 3)
3. Pancreatitis requiring medical intervention (Grade 2 or 3)
4. Osteonecrosis interfering with function (Grade 2 or 3)
5. Transient ischemic attacks (All grades)
6. Stroke (All grades)
7. Encephalopathy (Grade 3)
8. Neuropathy; motor or sensory, interfering with ADL (Grade 3)
9. Seizure (Grade 2 or 3)
10. Allergic reaction (Grade 3)
11 Ileus (Grade 3)
12. Mucositis/stomatitis; functional (Grade 3)
13. Bilirubin (Grade 3)
14. Thrombosis (Grade 3)

11.13 Syndrome Reporting

Unless otherwise specified in this protocol, syndromes should be reported as a single event using the CTCAE term for the composite syndrome, and not as the individual events that make up the syndrome. For example, Tumor Lysis Syndrome should be reported under the composite definition rather than reporting the component events (hyperkalemia, hyperphosphatemia, hypocalcemia, hyperuricemia) separately.

12.0 STUDY REPORTING AND MONITORING

The Case Report Forms and the submission schedule are posted on the COG web site with each protocol under "*Data Collection/Specimens*". A submission schedule is included.

12.1 CDUS

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31. **CDUS** reporting is not a responsibility of institutions participating in this trial.

12.2 Data and Safety Monitoring Committee

To protect the interests of patients and the scientific integrity for all clinical trial research by the Children's Oncology Group, the COG Data and Safety Monitoring Committee (DSMC) reviews reports of interim analyses of study toxicity and outcomes prepared by the study statistician, in conjunction with the study chair's report. The DSMC may recommend the study be modified or terminated based on these analyses.

Toxicity monitoring is also the responsibility of the study committee and any unexpected frequency of serious events on the trial are to be brought to the attention of the DSMC. The study statistician is responsible for the monitoring of the interim results and is expected to request DSMC review of any protocol issues s/he feels require special review. Any COG member may bring specific study concerns to the attention of the DSMC.

The DSMC approves major study modifications proposed by the study committee prior to implementation (e.g., termination, dropping an arm based on toxicity results or other trials reported, increasing target sample size, etc.). The DSMC determines whether and to whom outcome results may be released prior to the release of study results at the time specified in the protocol document.

12.3 CRADA/CTA

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

12.3.1 Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

12.3.2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it

will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

12.3.3 Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator: (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

12.3.4 When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

12.3.5 Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

12.3.6 Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13.0 PATHOLOGY GUIDELINES AND SPECIMEN REQUIREMENTS

No pathology review is planned for this study.

14.0 SPECIAL STUDIES SPECIMEN REQUIREMENTS

14.1 Pharmacodynamic Assessment of DNA Methylation (Required)

14.1.1 Timing of Pharmacodynamic Sampling

Collect peripheral blood from patients just prior to the dose of azacitidine on Day 1 and again on Day 5, for the first 2 courses of azacitidine. Please refer to Appendix VI for additional details.

- EPI Block #1
  - Day 1
  - Day 5

- EPI Block #2
  - Day 1
  - Day 5

14.1.2 Sample Collection and Processing

- Collect 3-4 mL of peripheral blood in a sodium heparin tube (green top).
- Samples can be stored for up to 48 hours at room temperature.

14.1.3 Sample Labeling and Shipping

- Label specimens with study #, COG patient ID#, BPC #, time point (course and day), and date/time of collection.
- Ship the specimens overnight to the Brown Laboratory at room temperature. Please email Dr. Patrick Brown before shipping.
- Do not batch the specimens.
- Deliveries are accepted on weekend and holiday, please contact Pat Brown prior to shipping. If shipping on a Saturday, please indicate on delivery sheet.
- Please include the transmittal form.
- See the AALL15P1 Correlative Studies Shipment sheet on the AALL15P1 web page for the shipping account number.

Dr. Patrick Brown
Johns Hopkins Oncology
Cancer Research Building I, Room 262
1650 Orleans Street
Baltimore, MD 21231
Email:pbrown2@jhmi.edu
Phone:(410) 955-8688
Fax: (410) 955-8897

14.2 Minimal Residual Disease (MRD) Results Reporting (Optional)

14.2.1 Reporting of MRD Results

MRD by flow cytometry in a COG-approved laboratory is required with bone marrow evaluations at the end of Induction, Consolidation, and Interim Maintenance. If the patient consents, enter MRD data into RAVE. MRD results will be correlated with disease outcome.

14.3 Banking for Future Research (Optional)

14.3.1 Timing of Molecular profiling sampling

For patients who consent, collect a bone marrow aspirate from all time points when a bone marrow evaluation is clinically indicated (at diagnosis, at the end of Induction, at the end of Consolidation, at the end of Interim Maintenance, and at the time of relapse). If apheresis is performed pre-treatment, please submit the apheresis sample in addition to the bone marrow sample (if performed).

14.3.2 Sample Collection and Processing

- Collect 3-5 mLs of fresh bone marrow in shipping media (SM) tube.
- If the absolute circulating blast count is at least 2,500/µL, peripheral blood can be substituted for diagnostic and relapse samples.
  - At least 2 mL of blood may be substituted for each 1 mL of required bone marrow.
- If apheresis is performed for clinical purposes, also submit an apheresis sample.
  - If there is a small volume of pheresate, mix 3 mL of SM with every 1 mL of pheresis sample. Use multiple SM tubes as needed.
  - If there is a large volume of pheresate or exchange transfusion product, process as above with a maximum of 10 SM tubes of pheresis sample.
- If samples cannot be shipped on the day of collection, please store at 4◦C until shipment on the next business day.

14.3.3 Sample Labeling and Shipping

- Label specimens with study #, COG patient ID#, BPC #, patient name/initials and date of birth, collection date, time point (course and day), and specimen type (bone marrow, blood, pheresis).
- **Do not** batch the specimens.
- Samples may be shipped Monday through Friday. If shipped on a Friday, mark Saturday delivery on the air bill and email the laboratory prior to shipment.
- Please include the transmittal form.
- Each samples should be prepared in accordance with IATA regulations.
- Ship samples via FedEx priority overnight. Utilize the COG Federal Express account found on the members site:

https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf

Shipping Media (SM)
To request prepared and pre-packaged sample shipping tubes, order tubes through the Biopathology Center Kit Management system. Select AALL08B1 or APEC14B1 (if available for ALL patients).

https://ricapps.nationwidechildrens.org/KitManagement/

Laboratory Contact Information:
Molecular Genetics Laboratory
mglab@nationwidechildrens.org
Phone: (614) 722-2866
Fax: (614) 722-2887

Shipping Address:
COG ALL Molecular Reference Laboratory
Nationwide Children's Hospital
575 Children's Crossroads, Room WB2255
Columbus, OH 43215

14.4 Pharmacokinetics of azacitidine (Optional)

When a patient consents to this study, contact Clinical Logistics for a sample collection kit containing the supplies required for blood collection. Please refer to the PK of Aza information packet on the AALL15P1 study webpage for details related to kit ordering. Peripheral blood samples will be collected during the first course of azacitidine. Please refer to Appendix VIII for details

14.4.1 Timing of pharmacokinetic sampling

- Please refer to Appendix VIII for details.

14.4.2 Sample Collection and Processing

Azacitidine is unstable in blood and therefore, all blood samples must be processed and plasma harvested immediately. Please refer to Appendix VIII.

14.4.3 Sample Labeling and Shipping

- Refer to Appendix VIII for sample preparation details.
- See the PK of Aza information packet on the AALL15P1 study webpage for the shipping account number and details.

Lab Contacts:

Karen Yazell
Project Manager, Bioanalytical
Email: Karen.yazell@covance.com
Phone: (317) 273-5218

Ryan Hill
Email: ryan.hill1@covance.com
Phone: (317) 273-7409

BioA Sample Management (ATTN: Ryan Hill)
Covance Bioanalytical Services, LLC
8211 SciCor Drive, D19 (Suite B)
Indianapolis, IN 46214

14.5 Assessment of Infant T cell Proliferative Capacity (Optional)

AALL15P1 includes an optional biological correlative study testing the feasibility of T-cell collection for the purposes of chimeric antigen receptor (CAR) T-cell production from the peripheral blood in infants with ALL.

T-cells from the peripheral blood will be enumerated and stimulated with artificial antigen presenting cells as they would be in the production phase of CART 19. Measured outcome will be a positive test expansion defined as an increase in cell number > 5 fold within 10 days from diagnosis and end-Induction. Secondary outcomes will assess the ability to expand to a potential number for CART-19 manufacturing and the ALC associated with potential expansion. Please refer to Appendix VII for additional details.

14.5.1 Timing of (CAR) T-cell sampling

For consenting patients, collect peripheral blood samples at the following time points:

- Pretreatment/Diagnosis
- End of Induction (pre-azacitidine)
- End of Consolidation

14.5.2 Sample Collection and Processing

- 5-10 mL of peripheral blood is required
- Peripheral blood should be collected in purple top tubes
- Refrigerate at 4°C for no more than 72 hours

Note: Exchange transfusion leukemia rich peripheral blood collected in purple top tubes may be substituted. No more than 2mL/kg should be drawn, with the exception of peripheral blood removed for exchange that would have been discarded.

14.5.3 Sample Labeling and Shipping

- Label tubes with study #, COG patient ID #, BPC #, source (peripheral or exchange), and timing (diagnosis, end of Induction, or end of Consolidation).
- Submit CBC including differential closest to time point of study lab draw
- Do not batch specimens
- Ship on a cold pack via overnight courier
- Ship specimens Tuesday through Thursday
  Note: If samples are collected on a Friday, store until Monday for shipping
- Do not deliver on a weekend or Holiday.
- Please include the transmittal form.
- See the AALL15P1 Correlative Studies Shipment sheet on the AALL15P1 web page for the shipping account number.

Contact laboratory before specimens are shipped.

Contact: Jessica Perazelli
Email: HULITTJ@email.chop.edu

Phone: (267) 426-5692
Fax: (215) 590-3770

Shipping Address:

David Barrett, M.D., Ph.D.
Colket Translational Research Building, Lab 3100
Children's Hospital of Philadelphia
3501 Civic Center Blvd.
Philadelphia, PA, 19104

14.6 Pharmacodynamics of Pegaspargase (Optional)

14.6.1 Timing of pharmacodynamic sampling

It is recommended that asparaginase activity levels are measured on the 7th day following the administration of pegaspargase during Induction, Interim Maintenance, and Delayed Intensification part I.

Based on the activity level results and/or clinical findings of hypersensitivity or other adverse reaction to pegaspargase (per each institution's standard practice), investigators may substitute *Erwinia* asparaginase.

For those patients who consent, institutional data will be submitted via RAVE and correlated with outcome.

14.7 Bone Marrow: Central review of Cytogenetics/FISH (Required)

Local bone marrow evaluation must be completed to confirm *KMT2A*-R to remain on AALL15P1. Both standard cytogenetic studies and FISH analysis must be performed at a COG-approved cytogenetics lab, and results submitted for central review. The local institution obtains the COG cytogenetics report forms and original karyotypes (not copies) from 2 different cells from each abnormal clone from the approved laboratory and sends them by email to Dr Carroll (Western Division) or Dr Nyla Heerema (Eastern Division) per the address information below (please refer to map). The COG FISH reporting forms and representative FISH images of all FISH analyses should also be included at this time. The cytogenetics data must be submitted for review on time (**by Induction Day 10**) in order for the information to be used for proper post-Induction therapy.

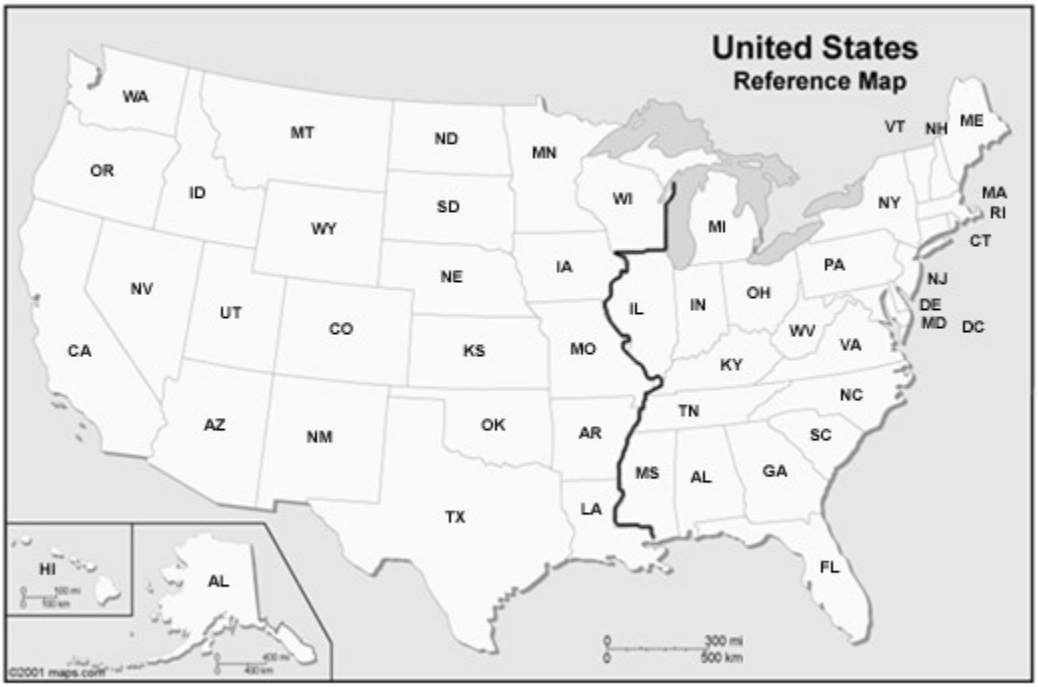
IT IS ESSENTIAL THAT *KMT2A* FISH DATA FOR INFANTS ENROLLED ON AALL15P1 BE ENTERED BY THE LOCAL INSTITUTION IN RAVE BY DAY 10 OF INDUCTION. **If there is any discrepancy between local cytogenetic results and COG-approved Laboratory review results, local institutions should contact the appropriate COG-approved Laboratory Director or the Study Chairs in order to resolve discrepancies.**

Please refer to Appendix X for information regarding specimens, recommended assays, and entry of date into the RAVE system.

Please see the following link for a list of COG approved cytogenetics labs: https://www.cogmembers.org/uploadedFiles/Site/Admin/Uploaded_Documents/Cytogenetics_Approved_Labs.pdf

EASTERN DIVISION
Nyla A Heerema, PhD
Ohio State University
Director of Cytogenetics
1645 Neil Avenue
Hamilton Hall, Room 129
Columbus, OH 43210-1228
Phone: (614) 292-7815
Fax: (614) 293-9919
E-mail: nyla.heerema@osumc.edu

WESTERN DIVISION
Andrew J Carroll, PhD
University of Alabama at Birmingham
Department of Genetics
1720 20TH St. South
Kaul Bldg, Room 314B
Birmingham, AL 35294-2050
Phone: (205) 934-0665
Fax: (205) 934-1078
E-mail: acarroll@uab.edu



15.0 IMAGING STUDIES REQUIRED AND GUIDELINES FOR OBTAINING

No imaging studies are planned for this study.

16.0 RADIATION THERAPY GUIDELINES

No radiation therapy is planned for this study.

APPENDIX I: CTEP AND CTSU REGISTRATION PROCEDURES CTEP INVESTIGATOR REGISTRATION PROCEDURES

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all investigators participating in any NCI-sponsored clinical trial to register and to renew their registration annually.

To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN, RAVE, or TRIAD or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR ***Help Desk*** by email at RCRHelpDesk@nih.gov.

CTSU REGISTRATION PROCEDURES

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Site registration forms may be downloaded from the AALL15P1 protocol page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Either enter the protocol # in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand
- Click on the COG link to expand, then select trial protocol #AALL15P1

Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided.

Requirements for AALL15P1 Site Registration:

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)

Submitting Regulatory Documents:

Submit required forms and documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab →Regulatory Submission

When applicable, original documents should be mailed to:
CTSU Regulatory Office
1818 Market Street, Suite 3000
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

Checking Your Site's Registration Status:

You can verify your site registration status on the members' section of the CTSU website. (Note: Sites will not receive formal notification of regulatory approval from the CTSU Regulatory Office.)

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab at the top of your screen
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead Network. It does not reflect

compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

Data Submission / Data Reporting

Data collection for this study will be done exclusively through the Medidata Rave clinical data management system. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP-IAM account (check at https://ctepcore.nci.nih.gov/iam) and the appropriate Rave role (Rave CRA, Read-Only, CRA (Lab Admin, SLA or Site Investigator) on either the LPO or participating organization roster at the enrolling site. To the hold Rave CRA role or CRA Lab Admin role, the user must hold a minimum of an AP registration type. To hold the Rave Site Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave. If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

APPENDIX II: CYP3A4/5 SUBSTRATES, INHIBITORS AND INDUCERS

This is not an all-inclusive list. Because the lists of these agents are constantly changing, it is important to regularly consult frequently-updated medical references.

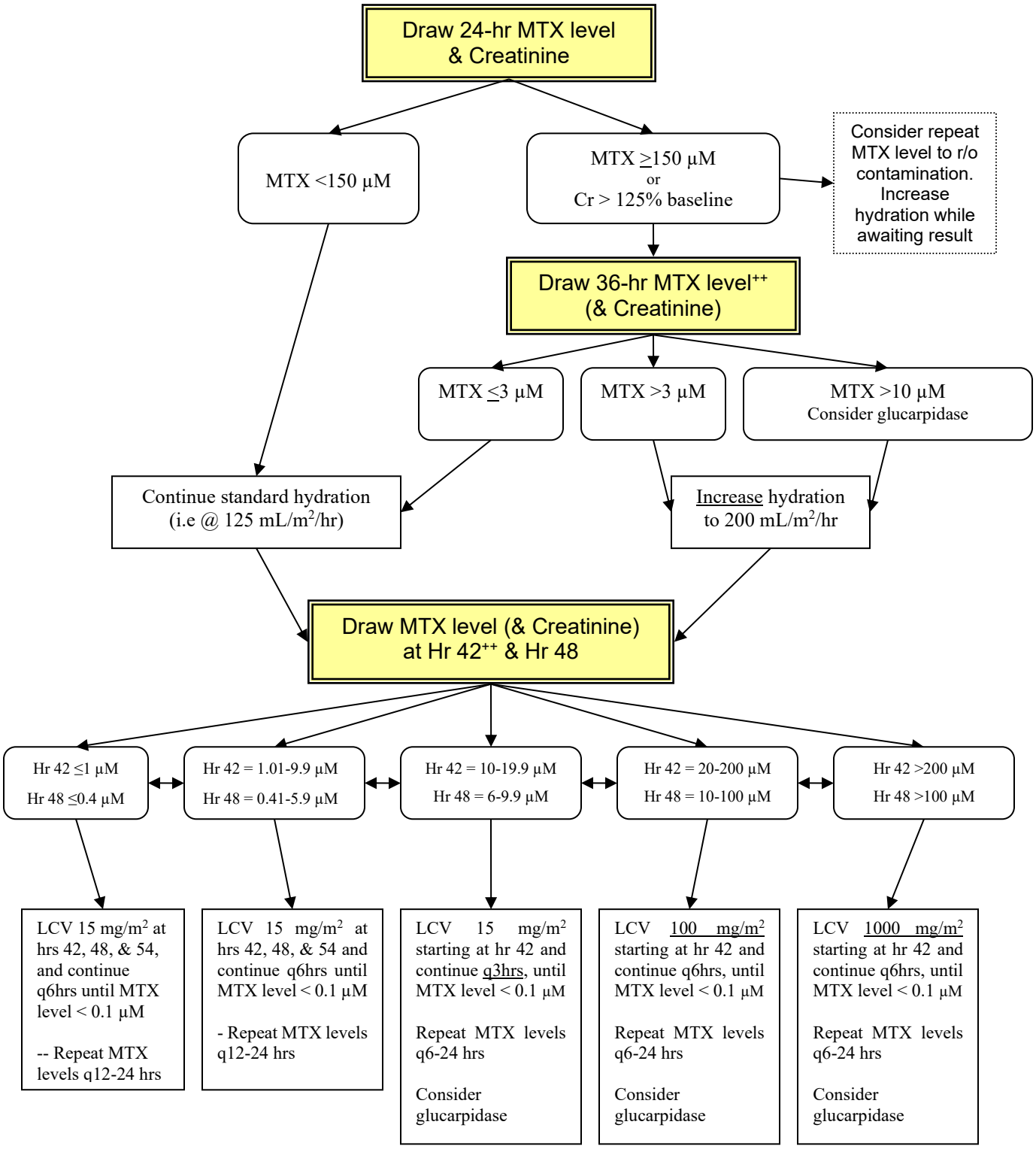
CYP3A4 substrates	Strong Inhibitors[1]	Moderate Inhibitors	Strong Inducers	Moderate Inducers
acalabrutinib[5] alfentanil[4,5] amiodarone[4] aprepitant/fosaprepitant atorvastatin axitinib bortezomib bosutinib[5] budesonide[5] buspirone[5] cabozantinib calcium channel blockers cisapride citalopram/escitalopram cobimetinib[5] conivaptan[5] copanlisib crizotinib cyclosporine[4] dabrafenib dapsone darifenacin[5] darunavir[5] dasatinib[5] dexamethasone[2] diazepam dihydroergotamine docetaxel doxorubicin dronedarone[5] eletriptan[5] eplerenone[5] ergotamine[4] erlotinib estrogens etoposide everolimus[5] felodipine[5] fentanyl[4] gefitinib haloperidol ibrutinib[5] idelalisib imatinib indinavir[5] irinotecan	atazanavir boceprevir clarithromycin cobicistat darunavir delavirdine graperfruit[3] grapefruit juice[3] idelalisib indinavir itraconazole ketoconazole lopinavir/ritonavir nefazodone nelfinavir posaconazole ritonavir saquinavir telaprevir telithromycin voriconazole	aprepitant conivaptan crizotinib diltiazem dronedarone erythromycin fluconazole fosamprenavir grapefruit[3] grapefruit juice[3] imatinib idelalisib mifepristone nilotinib verapamil	barbiturates carbamazepine enzalutamide fosphenytoin phenobarbital phenytoin primidone rifampin St. John's wort	bosentan dabrafenib efavirenz etravirine modafinil nafcillin rifapentin

isavuconazole[5] itraconazole ivacaftor ketoconazole lansoprazole lapatinib losartan lovastatin[5] lurasidone[5] macrolide antibiotics maraviroc[5] medroxyprogesterone methadone midazolam[5] midostaurin[5] modafinil nefazodone nilotinib olaparib ondansetron osimertinib paclitaxel palbociclib pazopanib quetiapine[5] quinidine[4] regorafenib romidepsin saquinavir[5] sildenafil[5] simvastatin[5] sirolimus[4,5] sonidegib sunitinib tacrolimus[4,5] tamoxifen telaprevir temsirolimus teniposide tetracycline tipranavir[5] tolvaptan[5] triazolam[5] trimethoprim vardenafil[5] vemurafenib venetoclax[5] vinca alkaloids zolpidem				

[1] Certain fruits, fruit juices and herbal supplements (star fruit, Seville oranges, pomegranate, gingko, goldenseal) may inhibit CYP 3A4 isozyme, however, the degree of that inhibition is unknown.
[2]Refer to Section 6.5 and Section 6.6 regarding the use of corticosteroids.

[3]The effect of grapefruit juice (strong vs moderate CYP3A4 inhibition) varies widely among brands and is concentration-, dose-, and preparation-dependent.
[4]Narrow therapeutic range substrates
[5]Sensitive substrates (drugs that demonstrate an increase in AUC of ≥5-fold with strong inhibitors)

APPENDIX III: HIGH-DOSE METHOTREXATE FLOWCHART



++ If the level is high at hour 36 or 42, but then the patient "catches up" and the level falls to the expected values of ≤1 and/or ≤ 0.4 µM at hours 42 and 48, respectively, resume standard leucovorin and hydration as long as urine output remains satisfactory.

APPENDIX IV: SUPPORTIVE CARE GUIDELINES

General

These are provided for institutional consideration. Investigator discretion should be used, and individual considerations made for specific patient situations and institutional practices. Study Chairs must be notified of any Serious Adverse Events, an Investigator's decision to deviate in a major way from protocol directed therapy, or a patient taken off study.

Aggressive supportive care improves outcome, particularly in high-risk patient populations receiving very intensive therapy as incorporated in this trial. The following guidelines are intended to give general direction for optimal patient care and to encourage uniformity in the treatment of this study population.

The Children's Oncology Group Supportive Care Guidelines may be found at https://childrensoncologygroup.org/index.php/cog-supportive-care-endorsed-guidelines.

Hydration/Allopurinol/Rasburicase (recombinant urate oxidase)

All infants should be placed on allopurinol (150-300 mg/m^2/day or 10 mg/kg/day in 2-3 divided doses) when the diagnosis of leukemia is made or strongly suspected. In some situations it may be appropriate to use rasburicase (recombinant urate oxidase). Prior to instituting Induction therapy, all patients should be well hydrated. Potassium should not be added to the hydration fluids. Urine alkalinization is NOT necessary for TLS prophylaxis. There is paucity of evidence demonstrating benefit of urine alkalinization and it can potentially lead to calcium phosphate precipitation and/or metabolic acidosis. If the patient has oliguria or severe renal dysfunction, consider the use of rasburicase at 0.1 to 0.2 mg/kg/dose (or as per institutional guidelines) and obtaining a nephrology consult. In patients with a prior history of glucose-6-phosphate dehydrogenase (G-6PD) deficiency, rasburicase is contraindicated and allopurinol should be utilized instead of rasburicase. Evidence of severe tumor lysis syndrome should be stabilized prior to the institution of therapy. In patients with extremely elevated WBC (> 300,000/µL), exchange transfusion is strongly recommended.

Venous Access

Due to the need for frequent blood sampling, intensive chemotherapy, nutritional support and vesicant use, it is **essential** that all patients have a central venous catheter placed.

Blood Components

Blood products should be irradiated following current FDA guidelines found at:
http://www.fda.gov/OHRMS/DOCKETS/98fr/981218g2.pdf

Investigators in Canadian institutions need to follow the CSA standards for Blood and Blood Components CAN/CSA-Z902-10 issued in February 2010 and available at: http://www.shopcsa.ca

Red blood cells (RBCs)

Transfusion with RBCs is indicated to correct severe or symptomatic anemia or acute blood loss. In the setting of extreme hyperleukocytosis investigators should be mindful that packed RBC transfusion may contribute to hyperviscosity.

Platelets

Transfusion with platelets is indicated to correct bleeding manifestations and may be indicated for severe thrombocytopenia without bleeding particularly in the setting of an invasive procedure.

Nutrition
Protein-calorie malnutrition due to chemotherapy induced loss of appetite, nausea, vomiting, mucositis, and sepsis is a major concern. Aggressive nutritional support should be instituted when patient's weight/height ratio ÷ median weight/median height ratio for age and sex falls below 80% **or** when the serum albumin is less than 3 mg/dL.

Caution is advised with the use of early feeding / tube feeding in patients with difficult early courses or extensive mucositis/diaper area skin ulceration. Necrotizing enterocolitis and intestinal perforation have been observed in such infants. Total parenteral nutrition (TPN) should be strongly considered in such infants until it is certain there is no risk to the gut.

Fever and Neutropenia
Aggressively manage episodes of fever (≥ 100.5ºF or 38.0ºC), particularly during Induction, Consolidation, Interim Maintenance, and Delayed Intensification, or when the patient is neutropenic with an ANC ≤ 1000. The risk of life threatening infection is particularly high during the first 4-6 weeks of therapy or when patients are neutropenic with an ANC ≤ 1000. It is strongly advised that patients with fever and neutropenia (ANC < 1000) not be managed with an outpatient antibiotic regimen. It is mandatory that patients with an ANC < 500 and fever be hospitalized with immediate institution of broad spectrum IV antibiotics adjusted appropriately for the causative organism.

See COG endorsed Fever and Neutropenia Guidelines at http://childrensoncologygroup.org/downloads/COG_SC_FN_Guideline_Document.pdf for empiric treatment of febrile neutropenia.

Infants with ALL are also at high risk for life-threatening viral infections, particularly RSV (see RSV Prophylaxis and Treatment sections below), during respiratory season.

Use of filgrastim (G-CSF)
Filgrastim or biosimilar may be used for severe infections with neutropenia, but routine use is discouraged. Filgrastim should not be given concurrently with azacitidine or chemotherapy and it must be discontinued at least 48 hours prior to the start of an azacitidine or chemotherapy course.

Infection Prophylaxis and Treatment
Bacterial and fungal infections are prevalent and severe among infants with ALL. It is strongly recommended that patients remain hospitalized during Induction therapy until there is evidence of marrow recovery and that all potential bacterial infections be treated promptly with empiric antibiotic therapy with broad coverage for BOTH gram-positive and gram-negative organisms. As the predominant pathogenic bacteria are gram-positive organisms including viridians streptococci, the empiric gram-positive coverage should include vancomycin, clindamycin or a drug appropriate for the treatment of viridians streptococci.

<u>Intravenous Immunoglobulin</u>
All patients are to receive intravenous immunoglobulin (IVIG) at a dose of 400 mg/kg if serum IgG level is below 500 mg/dL. Doses should be repeated every 4 weeks as needed to keep IgG level at 500 mg/dL or greater.

<u>Antibiotic and Antifungal Prophylaxis</u>
Infection related mortality is high during all phases prior to Maintenance. Interfant-06 mandates antibiotic and antifungal prophylaxis and hospitalization until count recovery for all patients during and after the intensive chemotherapy courses (Induction, Consolidation, Interim Maintenance, and Delayed Intensification). There are insufficient data to make specific recommendations regarding the choice of

antibiotic and antifungal prophylaxis for infants enrolled on AALL15P1. Azole antifungal agents (i.e., fluconazole, itraconazole, voriconazole) given concurrently with vincristine may increase the risk of neurotoxicity. Investigator caution is advised if azole antifungals are used.

The following are strongly recommended for infants enrolled on AALL15P1, during the intensive phases of therapy (Induction, Consolidation, Delayed Intensification, and Interim Maintenance).

1) Monitor the patient in the hospital from the start of the respective treatment phase until he/she shows signs of bone marrow recovery (specifically evidence that the absolute neutrophil count is rising for 2 consecutive days after the nadir) and the patient is afebrile and clinically stable.
2) Consider antibiotic prophylaxis against gram-positive and gram-negative organisms.
3) Consider antifungal prophylaxis. Options include an echinocandin such as caspofungin or micafungin, or azoles. Concomitant administration of an azole with vincristine should be avoided.

Pneumocystis Prophylaxis

PCP prophylaxis should be started as soon as possible after the diagnosis of ALL is confirmed and continued until 6 months after all therapy is completed.

1. The drug of choice is trimethoprim-sulfamethoxazole at a dosage of 150 mg TMP/m^2/day in 2 divided doses on 2 or 3 consecutive days per week in infants > 4 weeks of age.
    a. Trimethoprim-sulfamethoxazole must be held on the days of HD MTX infusion and for at least 72 hours after the start of the HD MTX infusion and until the MTX level is less than 0.1 μM.
2. Second line options include: Dapsone 2 mg/kg/day or 4mg/kg once a week in infants ≥ 1 month of age, IV pentamidine for infants ≥ 5 months of age, Aerosolized pentamidine for all ages.

RSV Prophylaxis

Palivizumab 15 mg/kg IM every month should be initiated at the start of the RSV season and terminated at the end of the RSV season.

RSV Treatment

All RSV infections (upper and lower respiratory) should be treated. Follow institutional guidelines for RSV treatment in high risk patients.

Additionally, palivizumab 15 mg/kg IM should be administered, if not already given as prophylaxis.

Influenza Immunization

Infants ≥ 6 months of age should receive 2 doses of the influenza immunization per CDC guidelines. Household contacts and out-of-home caregivers should also receive the influenza immunization.

Varicella Infection and Prophylaxis

Varicella-Zoster Virus (VZV) immunization should be avoided until at least 3 months following the end of Maintenance chemotherapy. Patients with exposure to VZV should be treated with Varicella Zoster Immunoglobulin (VZIG) within 10 days of exposure, preferably within the first 96 hours. If VZIG is not available, patients should be treated with IVIG 400 mg/kg. Consideration may be given to oral acyclovir prophylaxis, beginning 7 to 10 days after exposure and continuing for 7 days. Patients with symptoms of

VZV infection should be treated promptly with intravenous acyclovir, and monitored closely for the development of invasive systemic disease. Consultation with an infectious disease specialist is advised for the treatment of infants with VZV infection. The use of oral acyclovir for prophylaxis is recommended after completion of treatment for VZV infection.

Empiric Management of Pulmonary Infiltrates

Pulmonary infiltrates should be evaluated in the context of the patient's clinical and laboratory profile as well as institutional infection pattern. If the patient is not neutropenic and the pulmonary lesions on CT scan are not particularly suggestive of a mold infection (Aspergillus, Mucor), consider coverage with broad spectrum antibiotics and evaluate for viral causes of pulmonary infiltrates including RSV, influenza, and CMV. 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), more aggressive diagnostic measures should be undertaken. Pulmonary infiltrates may be evaluated with bronchoscopy and biopsy, lavage, or open lung biopsy. If a procedure cannot be tolerated and/or if there is high clinical suspicion of fungal disease, begin empiric treatment with amphotericin B, given the high likelihood of fungal disease. Empiric coverage should consider gram-negative and positive bacteria, fungi, RSV, influenza, CMV, Pneumocystis, and Legionella. If fungal pulmonary disease is documented, surveillance radiographic imaging studies of the sinuses, abdomen/pelvis, and brain are indicated. Surgical excision should be considered and is at the discretion of the treatment physician. Treatment of fungal infections with amphotericin B and/or other antifungal agents will be at the discretion of the treating physician. Azole antifungal agents (i.e. fluconazole, voriconazole) given concurrently with vincristine may INCREASE the risk of neurotoxicity. Caution is advised if azole antifungals are used. It is advisable to seek an infectious disease consult during the evaluation and treatment of an infant with pulmonary infiltrates and/or suspected invasive fungal infection.

Stress Steroid Support

If serious illness (particularly a potentially life-threatening infection) should occur in close proximity to the completion of the Induction or Delayed Intensification, consider additional "stress steroid" support.

Mucositis

Moderate (Grade 3) or severe (Grade 4) mucositis requires vigorous treatment including IV fluids, hyperalimentation, and strong consideration of broad spectrum antibiotics if febrile or ill appearing. Antifungal and antiviral therapy should be considered based on culture results and clinical evaluation. Daily oral antifungal prophylaxis with fluconazole should be strongly considered in patients not receiving vincristine. DO NOT PROCEED WITH FURTHER HD MTX or DAUNORUBICIN UNTIL MUCOSITIS BEGINS TO HEAL.

Perineal Irritation

There is a high risk of Grade 3-4 diaper area skin ulceration with daunorubicin and HD MTX. Placement of a Foley catheter for 48-72 hours during administration/urinary excretion of these drugs may dramatically reduce this diaper area skin ulceration. Use of a strong barrier technique is also recommended. If unable to place a Foley catheter, frequent diaper changes are advised during HD MTX/daunorubicin administration. If severe skin ulceration occurs, manage skin care aggressively and strongly consider antibiotic coverage until skin heals. DO NOT PROCEED WITH FURTHER HD MTX/DAUNORUBICIN UNTIL SKIN BEGINS TO HEAL.

Antiemetic Protection

Azacitidine is moderately emetogenic and antiemetics are strongly advised on Days 1 to 5 of every azacitidine course. Antiemetics should be given as needed during all phases of chemotherapy. The routine use of corticosteroids as antiemetics is discouraged.

APPENDIX V: POSSIBLE DRUG INTERACTIONS

The lists below do not include everything that may interact with chemotherapy. Study Subjects and/or their Parents should be encouraged to talk to their doctors before starting any new medications, using over-the-counter medicines, or herbal supplements and before making a significant change in diet. Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.

Some drugs, food, and supplements may interact with cyclophosphamide. Examples include:

Drugs that may interact with cyclophosphamide
• Allopurinol • Amiodarone • Carbamazepine • Cyclosporine • Digoxin • Efavirenz • Etanercept • Hydrochlorothiazide • Lumacaftor • Mifepristone • Pentostatin • Rifampin • Ritonavir • Warfarin

Food and supplements that may interact with cyclophosphamide
• St. John's Wort • Drinks, food, supplements, or vitamins containing "flavonoids" or other "antioxidants"

Some drugs, food, and supplements may interact with <u>daunorubicin</u>. Examples include:

Drugs that may interact with daunorubicin
• Some antibiotics and antifungals (clarithromycin, erythromycin, itraconazole, ketoconazole, rifampin) • Some antiepileptics (carbamazepine, phenobarbital, phenytoin, fosphenytoin) • Some antiretrovirals (lapatinib, lopinavir; nelfinavir, ritonavir, saquinavir, telaprevir, tipranavir) • Some heart medications (amiodarone, carvedilol, digoxin, dronedarone, quinidine, propafenone, verapamil) • Some chemotherapy (be sure to talk to your doctor about this)   ○ Ado-trastuzumab emtansine, bevacizumab, trastuzumab, taxane derivatives) •

- Other agents, such as atorvastatin, clozapine, cyclosporine, dexamethasone, ivacaftor, leflunomide, lumacaftor, natalizumab, nefazodone, progesterone, ranolazine, rifampin, tacrolimus, tofacitinib, and trazodone

Food and supplements that may interact with daunorubicin

- Echinacea
- Grapefruit, grapefruit juice, Seville oranges, star fruit
- St. John's Wort
- Drinks, food, supplements, or vitamins containing "flavonoids" or other "antioxidants"

Some drugs, food, and supplements may interact with <u>dexamethasone</u>. Examples include:

Drugs that may interact with dexamethasone

- Antibiotics
  - Ciprofloxacin, levofloxacin, moxifloxacin, clarithromycin, erythromycin, nafcillin, rifabutin, rifampin, telithromycin
- Antidepressants and antipsychotics
  - Aripiprazole, buproprion, citalopram, clozapine, escitalopram, fluvoxamine, lurasidone, nefazodone, quetiapine
- Antifungals
  - Caspofungin, fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole
- Arthritis medications
  - Leflunomide, tofacitinib
- Anti-rejection medications
  - Cyclosporine, sirolimus, tacrolimus
- Antiretrovirals and antivirals
  - Atazanavir, darunavir, delaviridine, efavirenz, etravirine, fosamprenavir, indinavir, lopinavir, nelfinavir, nevirapine, rilpivirine, ritonavir, saquinavir, Stribild, telaprevir, tipranavir
- Anti-seizure medications
  - Carbamazepine, oxcarbazepine, phenobarbital, phenytoin, primidone
- Heart medications
  - Amiodarone, amlodipine, dronedenarone, verapamil
- Some chemotherapy (be sure to talk to your doctor about this)
- Some oral contraceptives or birth control medications
- Many other drugs, including the following:
  - Aprepitant, artemether/lumefantine, aspirin, deferasirox, ibuprofen, ivacaftor, lomitapide, mifepristone, natalizumab, nimodipine, praziquantel, warfarin

Food and supplements that may interact with dexamethasone

- Echinacea
- St. John's Wort
- Grapefruit, grapefruit juice, Seville oranges, star fruit

Some drugs, food, and supplements may interact with <u>leucovorin</u>. Examples include:

Drugs that may interact with leucovorin
○ Glucarpidase ○ Some antiepileptics (fosphenytoin, phenobarbital, phenytoin, primidone) ○ Trimethoprim

Food and supplements that may interact with leucovorin
• Folic acid

Some drugs, food, and supplements may interact with mercaptopurine. Examples include:

Drugs that may interact with mercaptopurine
○ Artiritis medications: leflunomide, tofacitinib ○ Other medications, such as adalimumab, allopurinol, azathioprine, certolizumab pegol, clozapine, etanercept, febuxostat, golimumab, infliximab, natalizumab, olsalazine, sulfasalazine, warfarin

Food and supplements that may interact with mercaptopurine
• Echinacea

Some drugs, food, and supplements may interact with <u>methotrexate (by mouth or by vein)</u>.
Examples include:

Drugs that may interact with methotrexate
• Some antibiotics (amoxicillin, chloramphenicol, ciprofloxacin, penicillin, piperacillin, tetracycline, trimethoprim/sulfamethoxazole) • Some anti-inflammatory drugs (aspirin, ibuprofen, naproxen, ketorolac, sulfasalazine, sulindac) • Some heartburn medications (esomeprazole, lansoprazole, omeprazole, pantoprazole) • Several other specific agents, including the following: amiodarone, clozapine, cyclosporine, eltrombopag, fosphenytoin, gemfibrozil, leflunomide, phenytoin, pimecrolimus, probenecid, pyrimethamine, ranolazine, retinoids, teriflunomide, theophylline, tolvaptan, warfarin

Food and supplements that may interact with methotrexate
• Alcohol • Echinacea • Some vitamins, including those that contain folic acid or high doses of vitamin C

Some drugs, food, and supplements may interact with pegaspargase. Examples include:

Drugs that may interact with pegaspargase
○ Leflunomide, natalizumab, pegloticase, tofacitinib

Food and supplements that may interact with pegaspargase
• Echinacea

Some drugs, food, and supplements may interact with prednisone or prednisolone. Examples include:

Drugs that may interact with prednisone or prednisolone
• Arthritis medications ○ Leflunomide, tofacitinib • Antidiabetic medications • Antiretrovirals and antivirals ○ Ritonavir, telaprevir • Anti-seizure medications ○ Phenobarbital, phenytoin, primidone • Growth hormones • Heart medications ○ Diltiazem, verapamil • Some chemotherapy (be sure to talk to your doctor about this) • Some oral contraceptives or birth control medications • Many other drugs, including the following: ○ Aprepitant, aripiprazole, aspirin, cyclosporine, deferasirox, desirudin, ibuprofen, itraconazole, mifepristone, natalizumab, pimecrolimus, rifampin, warfarin

Food and supplements that may interact with prednisone
• Echinacea

Some drugs, food, and supplements may interact with thioguanine. Examples include:

Drugs that may interact with thioguanine
○ Arthritis medications: leflunomide, tofacitinib ○ Other medications, such as adalimumab, allopurinol, azathioprine, certolizumab pegol,clozapine, etanercept, golimumab, infliximab, natalizumab, olsalazine, sulfasalazine

Food and supplements that may interact with thioguanine
• Echinacea

Some drugs, food, and supplements may interact with vincristine. Examples include:

Drugs that may interact with vincristine
• Antibiotics ○ Clarithromycin, erythromycin, nafcillin, rifabutin, rifampin, telithromycin • Antifungals ○ Fluconazole, itraconazole, isavuconazole, ketoconazole, posaconazole, voriconazole • Arthritis medications ○ Leflunomide, tocilizumab, tofacitinib • Anti-rejection medications ○ Cyclosporine • Antiretrovirals and antivirals ○ Atazanavir, darunavir, delaviridine, efavirenz, etravirine, fosamprenavir, indinavir, lapatinib, lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, Stribild, telaprevir, tenofovir, tipranavir • Anti-seizure medications ○ Carbamazepine, fosphenytoin, phenobarbital, phenytoin, primidone • Heart medications ○ Amiodarone, carvedilol, diltiazem, dronedenarone, propafenone, quinidine, ranolazine, verapamil • Some chemotherapy (be sure to talk to your doctor about this) • Many other drugs, including the following: ○ Aprepitant, bosentan, cobicistat, conivapatan, deferasirox, fosnetupitant, ivacaftor, mifepristone, modafinil, natalizumab, nefazodone, netupitant

Food and supplements that may interact with vincristine
• Echinacea • St. John's Wort • Grapefruit, grapefruit juice, Seville oranges, star fruit

APPENDIX VI: ADDITIONAL INFORMATION FOR PHARMACODYNAMIC ASSESSMENT OF DNA METHYLATION

a) Rationale

Preclinical studies of methylation patterns in infant ALL cell lines and patient samples have identified DNA hypermethylation as an important pathway driving leukemogenesis and is a potential target for testing in infant ALL models.[7,9,10] Kostadinov *et al*., reported the critical role of subclonal methylation changes in chemoresistance and relapse of infant ALL.[6] Azacitidine has established efficacy as a demethylating agent in myelodysplastic syndrome and demonstrated a direct cytotoxic effect *in vitro* against pre-B ALL cells.[11] Decitabine and zebularine are similar DNA demethylation inhibitors that have demonstrated preclinical efficacy in the treatment of *KMT2A*-R ALL cells. Bhatla *et al*., described reversal of the gene methylation signature and cytotoxicity of the combination of decitabine with vorinostat in B-ALL patient samples and *KMT2A*-R cell lines.[12] Stumpel *et al*., further demonstrated that infant ALL cells with *KMT2A* -R are susceptible to demethylating agents.[5] In a study of the methylation patterns of infant ALL, Schafer *et al.* identified significantly more promoter hypermethylation in *KMT2A*-R patient-derived cells, compared with *KMT2A*-wild type infant ALL cells.[7] Genes with promoter hypermethylation correlated with down-regulated or silenced expression. Treatment with decitabine preferentially led to cytotoxicity of *KMT2A*-R cell lines, compared with *KMT2A*-wild type cell lines, supporting the role for demethylating agents in inducing cytotoxicity and chemosensitivity in the treatment of infant *KMT2A*-R ALL. There is growing clinical experience with DNMTis in combination with chemotherapy in acute leukemia in children, but no specific treatment experience in infants. Thus, a pilot study in infants to determine the safety of this combination with chemotherapy is warranted. For this trial in a highly vulnerable infant population, azacitidine has been chosen for study, based upon its availability and preferred profile of safety and tolerability in prior pediatric studies.

A secondary endpoint of this trial is to determine if there is biologic activity of azacitidine at a tolerable dose level. Dosing of azacitidine and the metabolism of azacitidine may be altered in infants, and the starting dose level is reduced from the typical adult BSA-based dosing, so we feel that it is important to directly assess for evidence of DNA demethylation at each dose level tested.

This pilot study does not seek to define maximum tolerated dose or to escalate doses to evaluate for clinical efficacy. Rather, DL1 (and DL0 if necessary) will be evaluated for tolerability and evidence of biologic epigenetic activity in combination with chemotherapy. If both DL1 and DL0 are too toxic, but show evidence of biologic activity, then consideration will be given to amending the study to test lower doses. If no biologic activity is seen, the strategy will not be pursued further. If DL1 is tolerable but shows no biologic activity, then consideration will be given to amending the study to test higher doses.

b) Hypotheses and specific aims

We hypothesize that the dose levels of azacitidine to be tested on this trial will lead to reduction of global DNA methylation (i.e., 5-methylcytosine content) in peripheral blood mononuclear cells when comparing cells collected just prior to the first dose of azacitidine and cells collected after 5 consecutive days of azacitidine dosing.

- **Specific Aim 1:** To determine, for each patient and for the population of patients as a whole, the total DNA 5-methylcytosine (5mC) content in peripheral blood mononuclear cells (PBMCs) isolated from samples of peripheral blood

collected on Day 1 prior to the first dose of azacitidine and on Day 5 of the first two courses of azacitidine.

c) Methods

Refer to Section 14.1 for specimen collection and shipment. All blood samples will be diluted in HBSS and centrifuged over Lymphocyte Separation Medium (LSM) to isolate mononuclear cells, then suspended in culture medium. Viable cells will be counted by trypan blue exclusion. For yields $\leq 5e^6$, all cells will be used for immediate DNA extraction. For yields $> 5e^6$, excess cells will be suspended in cryoprotectant solution and viably cryopreserved in liquid nitrogen in aliquots of up to $5e^6$ cells for subsequent batched assays as needed.

Using the freshly isolated PBMCs, total cellular DNA will be extracted using Qiagen commercial kits according to manufacturer's protocol. DNA quantity and quality will be assessed using NanoDrop. DNA will be stored at -80 C for subsequent batched assays.

Batched DNA will be thawed and assessed for global DNA methylation using a commercial ELISA LINE-1 kit which measures 5-methylcytosine (5mC) content in the long interspersed nucleotide element 1 (LINE-1) repeat elements, which is a validated surrogate of global DNA methylation. Each sample will be assayed in triplicate using 200 ng of starting DNA for each of the three replicates. The endpoint with the assay is a point estimate of the %5-mC in each replicate, ranging from 0-100%.

The Brown laboratory routinely processes blood specimens from cooperative group clinical trials and performs a wide variety of assays using freshly isolated and/or viably cryopreserved cells. Samples are generally received in the laboratory within 48 hours of collection and processed immediately. Cells are sufficiently stable in transport under these conditions for the proposed assays.

d) Analysis and Statistical Considerations

The mean and standard error of the %5-mC triplicates will be calculated for each sample.

The difference between the mean %5-mC of each paired pre- and post-treatment sample will be calculated and expressed as ΔM (change in methylation). A positive value will represent a decrease in methylation after exposure to azacitidine.

Each set of paired pre- and post-treatment %5-mC values will be assessed for statistically significant change in methylation using paired t-tests.

Each patient will have 2 sets of pre- and post-treatment samples, one for each of the first two courses of azacitidine, so each patient will have two ΔM values (ΔM1 for course 1, and ΔM2 for course 2).

There are two possible dose levels that patients will receive on this pilot study. The patients treated at DL1 and DL0 will be analyzed separately.

We will first describe the distribution of all ΔM values for the entire cohort for each dose level. The anticipated result is that the mean ΔM for the entire cohort will be positive and the paired t-test will demonstrate statistical significance, reflecting the expected reduction in DNA methylation upon exposure to azacitidine.

We will use t-tests to compare the distributions of ΔM1 and ΔM2 within each dose level to assess for evidence of changes in pharmacodynamics between the first and second courses of azacitidine. While we do not anticipate a significant difference, it is possible that changes in drug clearance between the first and second courses may be reflected by a detectable change in biologic activity.

We will use t-tests to compare the ΔM distributions for DL1 and DL0 to assess for evidence of a dose-response relationship in terms of demethylation, if both dose levels have been tested in the pilot. We anticipate that there will be a detectable dose-response relationship.

In published clinical trials where LINE-1 methylation has been measured in PBMCs before and after DNMTi treatment, the reduction in %5-mC has been variable, with most studies showing an average reduction in the 3-10% range at standard doses. Given the relative paucity of data available and the wide variability, we will simply describe the observed differences in %5-mC before and after azacitidine treatment in our patient population, as described above. A strict decision rule in terms of the magnitude of %5-mC reduction will not be pre-determined. Similarly, we do not have sufficient data to guide formal power calculations. We anticipate that for each dose level, at least 30 patients will provide a full set of 4 samples (pre- and post-treatment for each of the first two courses of azacitidine). We anticipate that this sample size will be sufficient to detect clinically significant biologic activity of azacitidine.

APPENDIX VII: ADDITIONAL INFORMATION FOR T CELL PROLIFERATION CAPACITY CORELATIVE BIOLOGY STUDY

a) Rationale

Adoptive immunotherapy with CART-19 has had great early success in pediatric patients with acute lymphoblastic leukemia (ALL)[79-81]. In order for patients to receive this therapy, we must be able to harvest functional T cells from the patient. Not all patients who undergo apheresis have successful collections or ex vivo T cell expansions, and this corresponds with the absence of early memory T cell subsets in the peripheral blood of these patients[82]. This can be directly related to chemotherapy, but is also true at diagnosis in lymphoma and solid tumor patients. Of the first 70 pediatric patients treated with CART19, only one was less than 3 years old. Prospective data on the feasibility of CAR T cell manufacture indicates that very young children (<3 years old) quickly lose T cell proliferative capacity after successive chemotherapy cycles, much more quickly and completely than older children. At diagnosis, however, >80% of children under age 3 had excellent T cell proliferative capacity similar to the rate for children of all ages (81.2%). After 2 cycles of chemotherapy, however, only 16% of children under age 3 had suitable T cells for cellular therapy compared with 75% of all ages. Only one infant ALL patient has been able to be analyzed in any of these studies from a single institution.

This trial includes a correlative study testing the feasibility of T-cell collection for the purposes of chimeric antigen receptor (CAR) T-cell production from the peripheral blood in infants with ALL. For those patients who consent, peripheral blood samples will be collected prior to therapy initiation, at the end of Induction, and at the end of Consolidation therapy. Lymphocyte subsets will be quantified, and functional studies will be performed to assess the ability of the lymphocytes to expand in response to artificial antigen presenting cell stimulation ex vivo. The ability to expand in response to this stimulus is a critical determinant of the feasibility of adoptive immunotherapy, specifically for CART-19.

b) Hypotheses and specific aims

The central hypothesis is that infants and very young children with ALL have highly proliferative T cells available for collection at diagnosis, and that these cells are rapidly destroyed by cycles of chemotherapy.

- **Specific Aim 1**: Determine the lymphocyte subset and phenotype of T cells from infants with ALL.
- **Specific Aim 2**: Determine the feasibility of CART19 manufacture from infants with ALL with T cells collected at diagnosis or after Induction and Consolidation therapy.

c) Methods

Sample receipt and processing. Please refer to Section 14.5 for detail.

The Barrett laboratory routinely processes blood and marrow specimens from cooperative group clinical trials and performs a wide variety of assays using fresh and viably cryopreserved cells. Samples are generally received in the laboratory within 48-72 hours of collection and processed immediately. T cells are sufficiently stable in transport under these conditions for the proposed assays. The flow cytometric and T cell expansion assays are also routinely performed in the Barrett

laboratory.

Multiparameter flow cytometric measurement of lymphocyte subsets and phenotype
Blood samples will be separated by density centrifugation (Ficoll) the day of receipt. White blood cells will be isolated from the buffy coat and plated on plastic overnight at 37◦C to remove adherent cells. Cells will then be stained with isotype controls and the following antibody panels, and analyzed in triplicate by flow cytometry.

- o For lymphocyte subsets: CD3, CD4, CD8, CD19 (to exclude leukemia), CD45RO, CD45RA, CCR7, CD62L, CD95, PD-1, Lag-3, Tim-3, VISTA, and TIGIT.

The **endpoint** for this assay will be the relative percentage of each of the following:

- o Naïve T-cells
- o Memory T-cells (stem central memory vs central vs. effector)
- o Terminal effector T cells
- o Number of negative checkpoint regulators (NCRs) on each subset and in total

T cell proliferative capacity
Small scale manufacture of CART19 replicates the potential of clinical scale manufacturing. Samples from 1-4 mL of peripheral blood subjected to the same manufacturing conditions using CD3/CD28 Dynabeads (called a test expansion) accurately predict the success of clinical CART19 manufacture. T cells from the sample will be counted and plated with CD3/CD28 beads at a ratio of 3:1, and followed for cell size (by Coulter Counter), number and change in T cell subset composition by multiparameter flow cytometry. A cutoff of a 5-fold expansion or higher within 14 days is used to determine successful potential for CART19 manufacture.

The **endpoint** for this assay will be pass (≥5 fold expansion) or fail (< 5 fold).

Rationale for selection of methods and laboratories
The methods described above are performed routinely in the Barrett lab, who published the first and most comprehensive analysis of T cell subsets and CART19 potential in children with B cell malignancies. Thus use of this lab is likely to optimize the chances that the proposed studies will be successful.

d) Analysis and Statistical Considerations

Since we are proposing to use standard assays (flow cytometric quantification of marrow cell subsets) in a novel population, we do not have *a priori* knowledge of the expected range of observed values. As such, these studies are exploratory and definitive power calculations are not possible.

Final cell counts from the test expansions will be used to estimate the volume of blood necessary for making clinical scale CART19 for patients of this population. In addition, the test expansions will be used to judge feasibility of CART19 manufacture from infants at the early time points in their therapy. This knowledge will be used to guide future cell therapy studies in this high risk population.

APPENDIX VIII: PHARMACOKINETIC OF AZACITIDINE

Pharmacokinetic sampling will be done on Days 3, 4 and 5 of EPI Block #1 for determination of plasma azacitidine concentrations. A total of 7 samples (7 mL of blood) will be collected from each patient. Azacitidine is unstable in blood, therefore all blood samples will be processed and plasma harvested **immediately** per the instructions provided. Sample collection kits and supplies will be provided by Clinical Logistics. Kit ordering details and shipment instructions are provided in the PK of Aza information packet found on the AALL15P1 protocol webpage.

To obtain accurate measurements of azacitidine levels in plasma, it is **very important** that tetrahydrouridine (THU) be added to blood collection tubes **immediately** after obtaining PK blood samples. THU inhibits the activity of cytidine deaminase, an enzyme responsible for the breakdown of azacitidine. **The following preparations should occur in advance of PK blood collections.**

Preparation of Tetrahydrouridine

Supplies provided by Clinical Logistics:

- (3) vials containing 0.3 mL THU-Solution A in 10 mL polypropylene capped vial

NOTE: Clinical Logistics will ship the THU- Solution A frozen, in single-use vials, separately from the collection kits. The THU-Solution A must be stored at -20°C immediately upon receipt. Use one vial of THU-Solution A for each PK sampling visit.

1. **THU-Solution A:**
   a. THU-Solution A will be provided in single-use vials at a concentration of 5 mg/mL in methanol.

   b. One the day of use remove one single-use vial of THU-Solution A from the -20°C freezer.

   c. Mix vigorously and thoroughly using a vortex mixer or sonicator after thawing,

2. **THU-Solution B - must be prepared on each PK day:**
   a. Transfer 5 mL of distilled water into the thoroughly mixed, single-use vial of THU-Solution A to prepare THU-Solution B.

   b. Mix thoroughly.

   c. The final concentration of THU-Solution B is 0.3 mg/mL.

   d. Store THU-Solution B at 4°C.

   e. Label the tube "**THU-Solution B**" with the "date of preparation" and the "**expiration date**" (i.e., **expiration date** = date of preparation + 1 day). Clinical Logistics will provide a label.

   f. Discard any remaining THU-Solution B at the end of each day. Prepare a fresh batch of THU-Solution B at the beginning of each PK sampling visit.

Blood Collection and Processing

Containers:
Use 1 x 1.2 mL K2EDTA Monovette tube and lavender top EDTA tube per time point. Refer to Appendix IX for instructions regarding the Monovette drawing system and use.
2 x 2 mL polypropylene cryovial, screw cap without ring sterile per time point
1 x 1 mL Tuberculin syringe per time point
1 polypropylene tube containing pre-made THU solution B per visit

Blood Collection:

i. Pre-chill blood collection tubes on ice for at least 15 minutes prior to blood draw.

ii. Load tuberculin syringes (one for each PK blood collection tube) with 0.05 mL (50 microliters) of THU-Solution B/syringe. Store at 4°C (i.e., refrigerator or wet ice).

iii. Collect blood samples at the appropriate time points (see below) and place into the appropriately labeled 1.2 mL K2EDTA Monovette tube.

iv. **NOTE:** Record the actual collection dates and times on the worksheet for entry into the CRF. Immediately after PK blood collection, add 0.05 mL of THU-Solution B to the blood sample. Use one syringe per collection tube.

v. Invert the collection tube 8-10 times and place on wet ice.

Sample Names (Note Primary or Back-Up for each time point):
SMI/PK PRE-DOSING ≤ 1 HOUR DAY 3
SM2/PK PRE-DOSING ≤ 1 HOUR DAY 4
SM3/PK POST-DOSE 5 MIN
SM4/PK POST-DOSE 30 MIN
SM5/PK POST-DOSE 1 HOUR
SM6/PK POST-DOSE 4 HOURS
SM7/PK POST-DOSE 6 HOURS

Sampling Schedule
Blood samples (1 mL per time point) will be collected from each patient by in-dwelling catheter or peripheral IV, into sample collection tubes.

Days and timing of PK blood sampling will be as follows:

Pharmacokinetic Sampling Schedule			Acceptable Deviation Window
EPI Block #1 Day 3	**EPI Block #1 Day 4**	**EPI Block #1 Day 5**	
Prior to dosing (≤1 hour)	Prior to dosing (≤1 hour)	Not applicable	≤ 60 min
		Postdose 5 minutes	± 1 min
		Postdose 30 minutes	± 3 min
		Postdose 1 hour	± 3 min
		Postdose 4 hours	± 3 min
		Postdose 6 hours	± 3 min

Blood Processing:

i. **Blood must be processed within 30 minutes of collection.**

ii. Separate plasma from blood cells by centrifugation at 2000xg for 10 minutes at 4°C.

iii. Transfer two aliquots of plasma into 2 cryovials. Transfer approximately equivalent volume into the tube designated Primary and into the tube designated Back-Up (BU).

iv. Freeze immediately on dry ice or at -70°C. Store at -70°C until shipment.

v. Ship Primary PK plasma samples frozen on dry ice to Covance CLS within 1 month of collection.

vi. Ship BU PK samples frozen on dry ice to Covance CLS upon sponsor's request.

Shipment instructions are provided in the PK of Aza information packet found on the AALL15P1 protocol webpage.

Pharmacokinetics Analysis

A validated high-performance liquid chromatography/tandem mass spectrometric method (LC-MS/MS) will be used for azacitidine plasma concentration analysis by the bioanalytical laboratory.

The PK population will consist of all patients who had sufficient concentration time data to enable the calculation of PK parameters for azacitidine. For patients that did not receive adequate azacitidine dose, or for patients with incomplete data, a decision as to their inclusion in the analysis will be made on a case-by-case basis.

Pharmacokinetic parameters of azacitidine will be calculated from plasma concentration-time profiles using non-compartmental methods, though compartmental analysis may be employed if appropriate. Plasma PK parameters will include, but not be limited to:

- C_{max}: observed maximum plasma concentration
- T_{max}: observed time to maximum plasma concentration
- AUC_t: area under the plasma concentration-time curve from time zero to the last quantifiable time point, calculated by the linear trapezoidal rule
- AUC_{inf}: area under the plasma concentration-time curve from time zero to infinity, calculated by the linear trapezoidal rule and extrapolated to infinity will be calculated according to the following equation:

  $$AUC_{inf} = AUC_t + (C_t/\lambda_z), \text{ where } C_t \text{ is the last quantifiable concentration}$$

- λ_z: terminal phase rate constant, determined by linear regression of the terminal points of the log-linear plasma concentration-time curve
- $t_{½}$: terminal phase half-life, will be calculated according to the following equation:

  $$t_{½} = 0.693/\lambda_z$$

- CL: total clearance, calculated as $Dose/AUC_{inf}$
- V_z: volume of distribution will be calculated according to the equation: $V_z = (CL)/\lambda z$

A listing of PK blood sample collection times, derived sampling time deviations, and PK parameters will be provided for each patient. Azacitidine plasma concentrations and resulting PK parameters will be summarized using descriptive statistics (N, arithmetic mean, standard deviation, minimum, median, maximum, percent coefficient of variation, geometric mean, and geometric percent coefficient of variation).

Figures of mean azacitidine concentration-time data will also be illustrated. Individual azacitidine patient concentration-time data will be graphically presented on linear and semi-logarithmic scales.

Pharmacokinetic parameters will be derived using WinNonlin® Professional Version 6.3, or higher, (Pharsight Corp., Mountain View, California). All PK computations and graphics will be performed using WinNonlin Professional Version 6.3, or higher; Excel 2007, or higher (Microsoft Corp., Seattle, Washington); SAS® Version 9.2, or higher (SAS Institute, Inc., Cary, North Carolina); or R statistical software (R Foundation for Statistical Computing; Vienna, Austria; http://www.R-project.org).

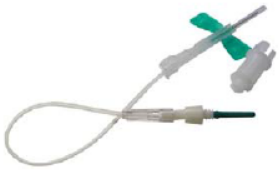
APPENDIX IX: PROCEDURES FOR SARSTEDT MONOVETTE DRAWING SYSTEM

PROCEDURE FOR SARSTEDT MONOVETTE® DRAWING SYSTEM

Monovette® Tube

Monovette® tubes are collection containers that behave similarly to vacuum tubes. However, they have a unique appearance and differences in handling such as: vacuum must be manually created, an adaptor must be used, and Monovette tubes use a turn and lock system.

Items needed to perform blood draw using a butterfly needle and Monovette Tube



Butterfly assembly with multi-adapter

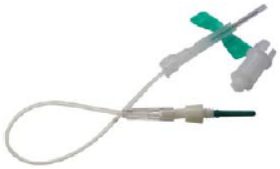
Monovette® tube

1. Prepare the Monovette® tubes by pulling back the plunger until it firmly locks into place and an audible click is heard. Break off the plunger. This creates the vacuum for the tubes.
2. Attached the Monovette® adapter by screwing it onto the butterfly needle.
3. For small volume Monovette tubes, Covance will provide a clear discard tube. After performing the venipuncture, insert and push the clear Monovette® discard tube onto the adapter and turn clockwise to lock. Allow the line to fill with blood. It is not necessary to fill the discard tube. This step is simply needed to prime the butterfly line. Once the line is primed, remove the tube by twisting it counterclockwise. Discard this tube according to your sites' biohazard procedures.
4. Fill the remaining Monovette® tubes as directed.

Non-Monovette® Tube

Monovette® tubes can be used with other vacuum tubes in a single stick. The transition between these tubes is quick and easy. This process is described below.

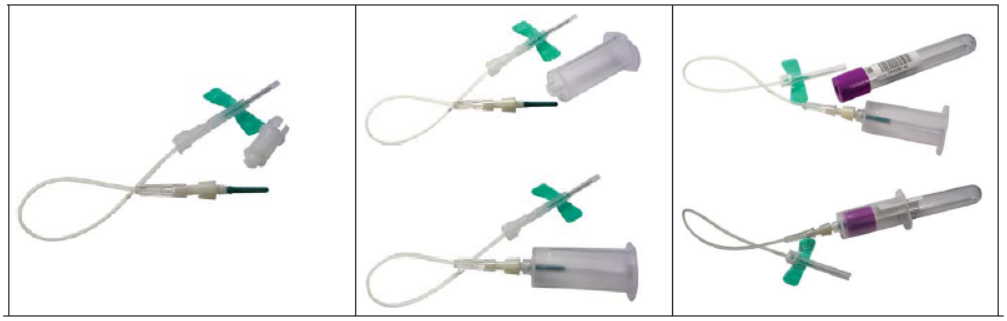
Items needed to perform blood draw using a butterfly needle and non-Monovette® Tube.



Butterfly assembly with multi-adapter

Vacutainer Holder

Non-Monovette® Tube



1. Unscrew the adapter from the butterfly needle.
2. Screw the needle of the butterfly assembly into the standard needle holder.
3. Insert non-Monovette® tube(s) into holder; blood will flow into the tube until the vacuum is filled.

English_Adaptation Procedure for Vacutainer and Monovette_Update: 20150715

APPENDIX X: FISH TESTING: INFORMATION REGARDING SPECIMENS, RECOMMENDED ASSAYS AND ENTRY OF DATA INTO RAVE SYSTEM

FLUORESCENCE IN SITU HYBRIDIZATION (FISH) TESTING FOR COG AALL15P1

Specimens to be submitted to COG-approved Cytogenetic Laboratory:

- 5 mL of BM aspirate obtained prior to initiation of systemic anti-leukemia therapy is optimal.
- If an adequate marrow specimen cannot be aspirated, then a BM biopsy specimen may be substituted at the discretion of the cytogenetic laboratory; contact cytogenetic laboratory for questions regarding specimen handling.
- If an adequate marrow specimen cannot be aspirated, then PB may be substituted for BM if there are at least 1,000 circulating blasts/µL (i.e., a WBC count of 10,000/µL with 10% blasts or a WBC count of 5,000/µL with 20% blasts). If only PB is submitted, please obtain and send **twice** the volume of PB as the recommended BM volume specified in the tables. As long as there are at least 1,000/µL PB blasts, institutions are encouraged to submit PB in addition to BM samples to make sure that adequate material is available to perform the required studies.

Technical Aspects for Cytogenetic Laboratory Directors

Probes:
BCR-ABL1 dual fusion preferred, extra signal acceptable, ***single fusion not acceptable***
ETV6-RUNX1 (TEL-AML1)
KMT2A breakapart.
Centromeres for chromosomes 4 and 10. Simultaneous (i.e., 2-color) evaluation preferred.

Technique:

- Samples must be unstimulated.
- All probes should be validated prior to use.
- Follow manufacturer's directions.
- *KMT2A* (*MLL*) break-apart should be scored positive if the 5' and 3' signals are split OR if the 3' signal is deleted with retention of 5' signal. Loss or translocation of both the 5' and 3' *KMT2A* signals is NOT indicative of an *KMT2A* (*MLL*) rearrangement and should NOT be scored as positive.
- Evaluate/count signal patterns in 200 cells. If less than 100 cells are analyzed for any 1 of the probes, please call Andrew Carroll (West) at (205) 934-0665 or Nyla Heerema (East) at (614) 292-7815 to discuss.

Fill out COG FISH form with results:
KMT2A (*MLL)* results must be returned to your CRA and entered into RAVE by Day 10.
Remaining FISH results must be returned to your CRA by Day 21.

THE FORM INCLUDED ON THE FOLLOWING PAGES WILL PROVIDE THE COG INSTITUTION WITH ALL OF THE DATA NEEDED FOR RAVE ENTRY. THE FIRST PAGE SHOULD BE FILLED OUT BY THE CRA AND THE SUBSEQUENT PAGES BY THE CYTOGENETICS LABORATORY.

COG AALL15P1 DIAGNOSTIC SPECIMEN TESTING

Specific FISH tests are REQUIRED for patients on AALL15P1. The results of these tests must be entered in RAVE by the treating institution.

Results for *KMT2A* (*MLL*) (11q23) for infants MUST be entered in the RAVE by Day 10 of treatment for the infant patients enrolled on AALL15P1.

To be completed by the submitting institution – send/FAX to Cytogenetic Lab:

Patient Name: _____ (last) _____ (first)

COG Registration Number: _____ Date of birth: _____

Treating Institution: _____

Contact Person: _____ Phone Number: _____

Fax Number: _____

Email: _____

Cytogenetic Testing Laboratory: _____

Specimen Type: _____ Bone Marrow (% blasts if available_____)

_____ Peripheral Blood (WBC _____ % blasts if available _____)

Date specimen collected: _____

Results are needed by Day 10 for *KMT2A* (*MLL*):

_____

Patient Name _____ COG Registration No. _____

To be completed by the Cytogenetic Laboratory (Return form to contact person, see front page):

t(9;22)(q34;q11.2) ____ Fusion Positive _____ % of cells positive
BCR-ABL1 ____ Fusion Negative
____ Unsatisfactory

t(12;21)(p13;q22) ____ Fusion Positive _____ % of cells positive
ETV6-RUNX1 ____ Fusion Negative
(TEL-AML1) ____ Unsatisfactory

***KMT2A* rearrangement – Positive if 5' and 3' signals are split OR 3' signal is deleted with retention of 5' signal. Loss or translocation of both the 5' and 3' *KMT2A* signals is NOT considered positive for this purpose.**

____ Positive _____ % of cells positive
____ Negative
____ Unsatisfactory

Double trisomy (DT) – best tested by SIMULTANEOUS detection of both centromere probes (orange & green)

Trisomy 4

____ Positive _____% of cells with 3 or more signals
____ Negative
____ Unsatisfactory

Trisomy 10

____ Positive _____% of cells with 3 or more signals
____ Negative
____ Unsatisfactory

Does this case meet the definition of double trisomy? – Yes/No

Definition of double trisomy:
For a case to be "positive" for trisomy 4 & 10 (double trisomy, DT), it must be positive for both trisomy 4 and trisomy 10, and the percentage of one trisomy cannot be ≥ 2X the percentage of the other. Examples: If trisomy 4 in 20% and trisomy 10 in 40%, the case is not DT. If trisomy 4 in 21% and trisomy 10 in 40%, the case is DT.

In addition to completing this form, the cytogenetic laboratory **must submit all cytogenetics results and karyotypes** to Dr Nyla Heerema (Eastern institutions) or Dr Andrew Carroll (Western institutions) prior to Day 10 for *KMT2A,* and Day 21 for all other results. Please see Section 14.7.

REFERENCES

1. Dreyer ZE, Hilden JM, Jones TL, et al: Intensified chemotherapy without SCT in infant ALL: Results from COG P9407 (Cohort 3). Pediatr Blood Cancer 62:419-26, 2015
2. Pieters R, Schrappe M, De Lorenzo P, et al: A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. Lancet 370:240-50, 2007
3. Salzer WL, Jones TL, Devidas M, et al: Decreased induction morbidity and mortality following modification to induction therapy in infants with acute lymphoblastic leukemia enrolled on AALL0631: a report from the Children's Oncology Group. Pediatr Blood Cancer 62:414-8, 2015
4. Salzer WL, Jones TL, Devidas M, et al: Modifications to induction therapy decrease risk of early death in infants with acute lymphoblastic leukemia treated on Children's Oncology Group P9407. Pediatr Blood Cancer 59:834-9, 2012
5. Stumpel DJ, Schneider P, van Roon EH, et al: Absence of global hypomethylation in promoter hypermethylated Mixed Lineage Leukaemia-rearranged infant acute lymphoblastic leukaemia. Eur J Cancer 49:175-84, 2013
6. Kostadinov R, Scharpf, R., Brown, P.: Identifying Sublonal Epigenetic Changes Driving Chemoresistance in Infant MLL-r Acute Lymphoblastic Leukemias. Blood Abstract, 2015
7. Schafer E, Irizarry R, Negi S, et al: Promoter hypermethylation in MLL-r infant acute lymphoblastic leukemia: biology and therapeutic targeting. Blood 115:4798-809, 2010
8. Stumpel DJ, Schneider P, van Roon EH, et al: Specific promoter methylation identifies different subgroups of MLL-rearranged infant acute lymphoblastic leukemia, influences clinical outcome, and provides therapeutic options. Blood 114:5490-8, 2009
9. Stumpel DJ, Schotte D, Lange-Turenhout EA, et al: Hypermethylation of specific microRNA genes in MLL-rearranged infant acute lymphoblastic leukemia: major matters at a micro scale. Leukemia 25:429-39, 2011
10. Nishi M, Eguchi-Ishimae M, Wu Z, et al: Suppression of the let-7b microRNA pathway by DNA hypermethylation in infant acute lymphoblastic leukemia with MLL gene rearrangements. Leukemia 27:389-97, 2013
11. Pfeiffer MM, Burow H, Schleicher S, et al: Influence of Histone Deacetylase Inhibitors and DNA-Methyltransferase Inhibitors on the NK Cell-Mediated Lysis of Pediatric B-Lineage Leukemia. Front Oncol 3:99, 2013
12. Bhatla T, Wang J, Morrison DJ, et al: Epigenetic reprogramming reverses the relapse-specific gene expression signature and restores chemosensitivity in childhood B-lymphoblastic leukemia. Blood 119:5201-10, 2012
13. Krug U, Koschmieder A, Schwammbach D, et al: Feasibility of azacitidine added to standard chemotherapy in older patients with acute myeloid leukemia--a randomised SAL pilot study. PLoS One 7:e52695, 2012
14. Muller-Tidow C, Tschanter P, Rollig C, et al: Azacitidine in combination with intensive induction chemotherapy in older patients with acute myeloid leukemia: The AML-AZA trial of the study alliance leukemia. Leukemia 30:555-61, 2016
15. Singal R, Ramachandran K, Gordian E, et al: Phase I/II study of azacitidine, docetaxel, and prednisone in patients with metastatic castration-resistant prostate cancer previously treated with docetaxel-based therapy. Clin Genitourin Cancer 13:22-31, 2015
16. Karon M, Sieger L, Leimbrock S, et al: 5-Azacytidine: a new active agent for the treatment of acute leukemia. Blood 42:359-65, 1973
17. Look AT, Dahl GV, Kalwinsky D, et al: Effective remission induction of refractory childhood acute nonlymphocytic leukemia by VP-16-213 plus azacitidine. Cancer Treat Rep 65:995-9, 1981

18. Kalwinsky D, Mirro J, Jr., Schell M, et al: Early intensification of chemotherapy for childhood acute nonlymphoblastic leukemia: improved remission induction with a five-drug regimen including etoposide. J Clin Oncol 6:1134-43, 1988
19. Steuber CP, Krischer J, Holbrook T, et al: Therapy of refractory or recurrent childhood acute myeloid leukemia using amsacrine and etoposide with or without azacitidine: a Pediatric Oncology Group randomized phase II study. J Clin Oncol 14:1521-5, 1996
20. Matei D, Fang F, Shen C, et al: Epigenetic resensitization to platinum in ovarian cancer. Cancer Res 72:2197-205, 2012
21. Fang F, Balch C, Schilder J, et al: A phase 1 and pharmacodynamic study of decitabine in combination with carboplatin in patients with recurrent, platinum-resistant, epithelial ovarian cancer. Cancer 116:4043-53, 2010
22. Glasspool RM, Brown R, Gore ME, et al: A randomised, phase II trial of the DNA-hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in combination with carboplatin vs carboplatin alone in patients with recurrent, partially platinum-sensitive ovarian cancer. Br J Cancer 110:1923-9, 2014
23. Muller-Tidow C, Tschanter P, Rollig C, et al: Azacitidine in combination with intensive induction chemotherapy in older patients with acute myeloid leukemia: The AML-AZA trial of the study alliance leukemia. Leukemia, 2015
24. George RE, Lahti JM, Adamson PC, et al: Phase I study of decitabine with doxorubicin and cyclophosphamide in children with neuroblastoma and other solid tumors: a Children's Oncology Group study. Pediatr Blood Cancer 55:629-38, 2010
25. Benton CB, Thomas DA, Yang H, et al: Safety and clinical activity of 5-aza-2'-deoxycytidine (decitabine) with or without Hyper-CVAD in relapsed/refractory acute lymphocytic leukaemia. Br J Haematol 167:356-65, 2014
26. Sun W GP, Sposto R, et al.: A Phase 1 Study of Azacitidine (AZA) in Combination with Fludarabine and Cytarabine in Relapse/Refractory Childhood Leukemia: A Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) Study. Blood 124:3764, 2014
27. Burke MJ BP, 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. Blood 124:3650, 2014
28. Relling MV, Pui CH, Sandlund JT, et al: Adverse effect of anticonvulsants on efficacy of chemotherapy for acute lymphoblastic leukaemia. Lancet. 356(9226):285-90, 2000
29. Bermudez M, Fuster JL, Llinares E, et al: Itraconazole-related increased vincristine neurotoxicity: case report and review of literature. J Pediatr Hematol Oncol 27:389-92, 2005
30. Ariffin H, Omar KZ, Ang EL, et al: Severe vincristine neurotoxicity with concomitant use of itraconazole. J Paediatr Child Health 39:638-9, 2003
31. Brenner DE, Wiernick PH, Wesley M, et al: Acute doxorubicin toxicity. Relationship to pretreatment liver function, response, and pharmacokinetics in patients with acute nonmlumphocytic leukemia. Cancer 53:1042-1048, 1984
32. Langer T, Martus P, Ottensmeier H, et al: CNS late-effects after ALL therapy in childhood. Part III: neuropsychological performance in long-term survivors of childhood ALL: impairments of concentration, attention, and memory. Med Pediatr Oncol 38(5):320-8, 2002
33. Mulhern RK, Palmer SL: Neurocognitive late effects in pediatric cancer. Curr Probl Cancer 27(4):177-97, 2003
34. Harila-Saari AH, Paakko EL, Vainionpaa LK, et al: A longitudinal magnetic resonance imaging study of the brain in survivors in childhood acute lymphoblastic leukemia. Cancer 83(12):2608-17, 1998
35. Asato R, Akiyama Y, Ito M, et al: Nuclear magnetic resonance abnormalities of the cerebral white matter in children with acute lymphoblastic leukemia and malignant lymphoma during and after central nervous system prophylactic treatment with intrathecal methotrexate. Cancer 70(7):1997-2004, 1992

36. Iuvone L, Mariotti P, Colosimo C, et al: Long-term cognitive outcome, brain computed tomography scan, and magnetic resonance imaging in children cured for acute lymphoblastic leukemia. . Cancer 95(12):2562-70, 2002
37. Paakko E, Vainionpaa L, Pyhtinen J, et al: Minor changes on cranial MRI during treatment in children with acute lymphoblastic leukaemia. Neuroradiology 38(3):264-8, 1996
38. Biti GP, Magrini SM, Villari N, et al: Brain damage after treatment for acute lymphoblastic leukemia. A report on 34 patients with special regard to MRI findings. Acta Oncol 28(2):253-6, 1989
39. Wilson DA, Nitschke R, Bowman ME, et al: Transient white matter changes on MR images in children undergoing chemotherapy for acute lymphocytic leukemia: correlation with neuropsychologic deficiencies. Radiology 180(1):205-9, 1991
40. Schrappe M, Reiter A, Ludwig WD, et al: Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. German-Austrian-Swiss ALL-BFM Study Group. Blood 95(11):3310-22, 2000
41. Brouwers P, Riccardi R, Fedio P, et al: Long-term neuropsychologic sequelae of childhood leukemia: correlation with CT brain scan abnormalities. J Pediatr Hematol/Oncol 106(5):723-8, 1985
42. Rollins N, Winick N, Bash R, et al: Acute methotrexate neurotoxicity: findings on diffusion-weighted imaging and correlation with clinical outcome. AJNR Am J Neuroradiol 25(10):1688-95, 2004
43. Laitt RD, Chambers EJ, Goddard PR, et al: Magnetic resonance imaging and magnetic resonance angiography in long term survivors of acute lymphoblastic leukemia treated with cranial irradiation. Cancer 76(10):1846-52, 1995
44. Kramer JH, Norman D, Brant-Zawadzki M, et al: Absence of white matter changes on magnetic resonance imaging in children treated with CNS prophylaxis therapy for leukemia. Cancer 61(5):928-30, 1988
45. Winick NJ, Bowman WP, Kamen BA, et al: Unexpected acute neurologic toxicity in the treatment of children with acute lymphoblastic leukemia. J Natl Cancer Inst. 84(4):252-6, 1992
46. Bleyer WA: Neurologic sequelae of methotrexate and ionizing radiation: a new classification. Cancer Treat Rep 65 89-98, 1981
47. Meadows AT, Gordon J, Massari DJ, et al: Declines in IQ scores and cognitive dysfunctions in children with acute lymphocytic leukaemia treated with cranial irradiation. Lancet 2(8254):1015-8, 1981
48. Williams JM, Ochs J, Davis KS, et al: The subacute effects of CNS prophylaxis for acute lymphoblastic leukemia on neuropsychological performance: a comparison of four protocols. Arch Clin Neuropsychol 1(2):183-92, 1986
49. Mulhern RK, Fairclough D, Ochs J: A prospective comparison of neuropsychologic performance of children surviving leukemia who received 18-Gy, 24-Gy, or no cranial irradiation. J Clin Oncol. 9(8):1348-56, 1991
50. Steinberg GK, Kunis D, DeLaPaz R, et al: Neuroprotection following focal cerebral ischaemia with the NMDA antagonist dextromethorphan, has a favourable dose response profile. Neurol Res. 15(3):174-80, 1993
51. Steinberg GK, Bell TE, Yenari MA: Dose escalation safety and tolerance study of the N-methyl-D-aspartate antagonist dextromethorphan in neurosurgery patients. J Neurosurg 84(5):860-6, 1996
52. Blin O, Azulay JP, Desnuelle C, et al: A controlled one-year trial of dextromethorphan in amyotrophic lateral sclerosis. Clin Neuropharmacol 19(2):189-92, 1996
53. Gredal O, Werdelin L, Bak S, et al: A clinical trial of dextromethorphan in amyotrophic lateral sclerosis. Acta Neurol Scand 96(1):8-13, 1997
54. Hollander D, Pradas J, Kaplan R, et al: High-dose dextromethorphan in amyotrophic lateral sclerosis: phase I safety and pharmacokinetic studies. Ann Neurol. 36(6):920-4, 1994
55. Wiedemann BC, Balis FM, Murphy RF, et al: Carboxypeptidase-G2, thymidine, and leucovorin rescue in cancer patients with methotrexate-induced renal dysfunction. J Clin Oncol 15(5):2125-34, 1997
56. DeAngelis LM, Tong WP, Lin S, et al: Carboxypeptidase G2 rescue after high-dose methotrexate. J Clin Oncol 14(7):2145-9, 1996

57. Weinshilboum R. M, Sladek S. L: Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. Am.J.Hum.Genet 32:651-662, 1980
58. Bostrom B, Erdmann G: Cellular pharmacology of 6-mercaptopurine in acute lymphoblastic leukemia. Am.J.Pediatr.Hematol.Oncol. 15:80-86, 1993
59. Weinshilboum R: Thiopurine pharmacogenetics: clinical and molecular studies of thiopurine methyltransferase. Drug Metab Dispos 29:601-605, 2001
60. Lennard L, Lewis I. J, Michelagnoli M, et al: Thiopurine methyltransferase deficiency in childhood lymphoblastic leukaemia: 6-mercaptopurine dosage strategies. Med.Pediatr.Oncol. 29:252-255, 1997
61. Relling M. V, Hancock M. L, Rivera G. K, et al: Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. J.Natl.Cancer Inst 91:2001-2008, 1999
62. Yang JJ LW, Yang W, Liu C, Hageman L, Cheng C, Pei D, Chen Y, Crews KR, Kornegay N, Wong FL, Evans WE, Pui CH, Bhatia S, Relling MV.: Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. J Clin Oncol. 33:1235-1242, 2015
63. Yates C. R, Krynetski E. Y, Loennechen T, et al: Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann.Intern.Med 126:608-614, 1997
64. Otterness D, Szumlanski C, Lennard L, et al: Human thiopurine methyltransferase pharmacogenetics: gene sequence polymorphisms. Clin.Pharmacol.Ther 62:60-73, 1997
65. Hon Y. Y, Fessing M. Y, Pui C.-H, et al: Polymorphism of the thiopurine S-methyltransferase gene in African Americans. Hum.Mol.Genet 8:371-376, 1999
66. Krynetski E. Y, Evans W. E: Genetic polymorphism of thiopurine S-methyltransferase: molecular mechanisms and clinical importance. Pharmacology 61:136-146, 2000
67. Duval M, Suciu S, Ferster A, et al: Comparison of Escherichia coli-asparaginase with Erwinia-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer-Children's Leukemia Group phase 3 trial. Blood 99:2734-9, 2002
68. Silverman LB, Levy DE, Dalton VK, et al: Outcome of Dana-Farber cancer institute (DFCI) consortium protocol 95-01 for children with newly diagnosed acute lymphoblastic leukemia (ALL). ASH Annual Meeting Abstracts 104(11):679, 2004
69. Silverman LB, Gelber RD, Dalton VK, et al: Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood 97:1211-8, 2001
70. Avramis VI, Sencer S, Periclou AP, et al: A randomized comparison of native Escherichia coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. Blood 99:1986-94, 2002
71. Van den Berg HW, Desai ZR, Wilson R, et al: The pharmacokinetics of vincristine in man: reduced drug clearance associated with raised serum alkaline phosphatase and dose-limited elimination. Cancer Chemother Pharmacol 8(2):215-9, 1982
72. Sanjuan-Pla A, Bueno C, Prieto C, et al: Revisiting the biology of infant t(4;11)/MLL-AF4+ B-cell acute lymphoblastic leukemia. Blood 126:2676-85, 2015
73. This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.,
74. Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal

hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.,
75. Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.,
76. Abnormal breath sounds include rales and rhonchi.,
77. Ivanova A, Qaqish BF, Schell MJ: Continuous toxicity monitoring in phase II trials in oncology. Biometrics 61:540-5, 2005
78. Peto R, Pike MC, Armitage P, et al: Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. Br J Cancer 35:1-39, 1977
79. Porter DL, Levine BL, Kalos M, et al: Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 365:725-33
80. Grupp SA, Kalos M, Barrett D, et al: Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 368:1509-18, 2013
81. Maude SL, Frey N, Shaw PA, et al: Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 371:1507-17, 2014
82. Singh N, Perazzelli J, Grupp SA, et al: Early memory phenotypes drive T cell proliferation in patients with pediatric malignancies. Sci Transl Med 8:320ra3, 2016