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CHILDREN'S ONCOLOGY GROUP

AAML1421

A Phase 1/2 Study of CPX-351 (NSC# 775341; [redacted]) Alone Followed by Fludarabine, Cytarabine, and G-CSF (FLAG) for Children with Relapsed Acute Myeloid Leukemia (AML)

IND Sponsor for CPX-351: COG

Phase 1 (Completed 12/2016): Participation Limited to the COG Phase 1 Consortium and the following institutions:

OH006 / Nationwide Children's Hospital
CA009 / Children's Hospital of Los Angeles
MO024 / Children's Mercy Hospital and Clinics
TX011 / University of Texas – Southwestern Medical Center
MD017 / Johns Hopkins University/Sidney Kimmel Cancer Center
DE008 / Alfred I DuPont Hospital for Children
CO011 / Children's Hospital of Colorado
CA435 / Valley Children's Hospital
MA036 / Dana Farber Cancer Institute
OH033 / Rainbow Babies and Children's Hospital
MS005 / University of Mississippi Medical Center
MN010 / Children's Hospitals and Clinics of Minnesota-Minneapolis
CA078 / Loma Linda University Medical Center

Phase 2: Open to COG Institutions in the USA, Canada, Australia and New Zealand

[redacted]

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 (ie patient eligibility or treatment-related) contact the Study PI of the Lead Protocol Organization.		
For non-clinical questions (ie 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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INDUSTRY SUPPLIED AGENT:
CPX-351, NSC# 775341, Jazz Pharmaceuticals

OTHER AGENTS:
Fludarabine, NSC# 312887, Commercial
Cytarabine, NSC# 63878, Commercial
Filgrastim, NSC# 614629, Commercial

[redacted]
IND SPONSOR: COG

SEE SECTION 14 FOR SPECIMEN SHIPPING ADDRESSES

ABSTRACT

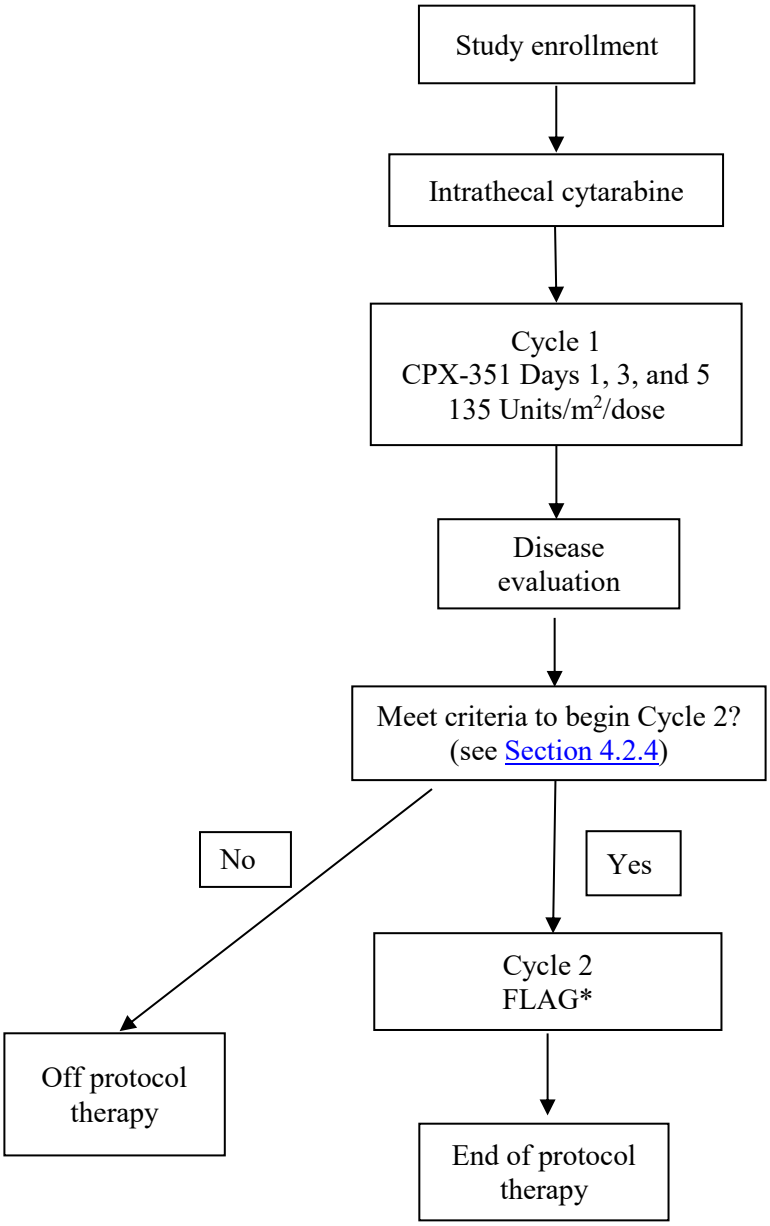
Anthracyclines are an essential element for the treatment of AML in children. Unfortunately, the use of anthracyclines is limited by cardiotoxicity that may result in devastating cardiomyopathy, a critical problem for some children who are AML survivors. Children's Oncology Group (COG) clinical trials for newly diagnosed AML currently deliver approximately 450 mg/m^2 cumulative daunorubicin equivalents for those who receive 4 courses of chemotherapy alone, or approximately 300 mg/m^2 to those who receive 3 courses followed by hematopoietic stem cell transplantation. Without better therapeutic options for children with AML, methods to minimize cardiac damage while maintaining anthracycline dose-intensity must be identified.

Liposomal delivery systems for anthracyclines are a promising strategy to mitigate cardiotoxicity while maintaining efficacy. DaunoXome (DNX), a liposomal anthracycline that has been studied extensively in adults and children, has shown superior re-induction rates in adults and children with AML when added to a fludarabine/cytarabine/G-CSF backbone. Unfortunately, there is presently not a plan to further develop DNX in the United States.

This clinical trial proposes to study CPX-351, a novel liposomal preparation of a fixed 5:1 ratio of cytarabine/daunorubicin. Preclinical studies and pharmacokinetics performed in adult trials have demonstrated that CPX-351 provides elevated and prolonged encapsulated drug concentrations when compared to DNX, and orders of magnitude higher than free drug. Phase 1 trials in adults with AML demonstrated safety and tolerability at 100 Units/m^2/dose given on Days 1, 3, 5. In addition, adult phase 2 studies demonstrated a statistically significant advantage in overall survival over standard regimens in newly diagnosed and relapsed AML, particularly those with adverse prognostic factors.

AAML1421 is a Phase 1/2 study of CPX-351 for children with AML in first relapse. The primary objectives of this study will be to determine the recommended Phase 2 Dose (RPD2) of CPX-351 in children with AML and to estimate the response rate (CR+CRp) by determining the best response after up to 2 cycles (Cycle 1 = CPX-351 followed by Cycle 2 consisting of FLAG). The phase 1 portion of the study has been completed as of 12/12/2016 and dose level #1 (135 Units/m^2/dose) was found to be the recommended phase 2 dose. This clinical trial is a critically important step in developing this novel liposomal anthracycline with the potential to reduce cardiotoxicity while maintaining efficacy for children with AML.

EXPERIMENTAL DESIGN SCHEMA



*FLAG: Fludarabine and cytarabine, Days 1-5, and filgrastim (G-CSF), Days 1-5 and Day 15 to count recovery.

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aims

1.1.1 To determine a recommended phase 2 dose (RP2D) and the toxicities associated with CPX-351 in pediatric and young adult patients with relapsed/refractory AML.

1.1.2 To estimate the response rate (complete remission (CR) plus complete remission with partial platelet recovery (CRp)) after CPX-351 (Cycle 1) followed by FLAG (Cycle 2) in children with AML in first relapse.

1.2 Secondary Aims

1.2.1 To estimate the response rate (CR + CRp + CRi) after one cycle of CPX-351.

1.2.2 To describe the pharmacokinetics of plasma cytarabine and daunorubicin after CPX-351 administration to pediatric and young adult patients with relapsed/refractory AML.

1.3 Exploratory aims

1.3.1 To describe the response in biomarkers of cardiac injury to a single cycle of CPX-351.

1.3.2 To explore the effect of CPX-351 on novel biochemical and imaging markers of cardiotoxicity, including plasma microRNAs (miRNA) and myocardial deformation.

1.3.3 To explore the role of rare coding variants as risk factors for anthracycline-induced cardiomyopathy.

2.0 BACKGROUND

2.1 Introduction/Rationale for Development

Anthracyclines are essential in the treatment of children with AML. However, it is clear that anthracycline-associated cardiotoxicity represents the major adverse sequela for survivors of childhood AML. CPX-351 demonstrates at least equivalent response rates to both 7+3 (cytarabine 100 mg/m^2/dose, Days 1-7; daunorubicin 60 mg/m^2/dose, Days 1, 3, 5) and other anthracycline-containing re-induction regimens in adults with AML. For adults with high-risk features, CPX-351 is statistically superior in terms of response rate and 1-year overall survival (OS). In children with AML, new therapies are needed to maintain or improve outcomes while abrogating the devastating consequences of cardiotoxicity for survivors. We propose to study CPX-351 in children with AML in first relapse.

Administration of a novel liposomal anthracycline-containing agent in children with AML in first relapse is warranted. The prognosis for children with AML in first relapse is poor, but historical data consistently demonstrates improved CR rates with anthracycline-containing re-induction regimens.[1,2] The International BFM (i-BFM) group reported in children with AML in first relapse, that those who received FLAG plus the liposomal

anthracycline DaunoXome (DNX) had superior CR rates compared to those who received FLAG alone. As a result, administration of DNX is standard for re-induction therapy in relapsed disease among the European consortia and DNX is currently being included in their clinical trials for children with de novo AML (Dirk Reinhardt, personal communication). Recent COG studies for relapsed AML have utilized anthracyclines such as idarubicin (AAML06P1, AAML07P1) resulting in a cumulative anthracycline exposure of approximately 600 mg/m^2 daunorubicin equivalents in combination with either high- or standard dosing cytarabine. The OS of patients who experienced bone marrow relapse following treatment on COG study AAML0531 revealed that those who relapsed within one year had a dismal prognosis, whether they received hematopoietic stem cell transplant (HSCT) or not (3 year OS 21% ± 17% with HSCT; 27% ±7% without HSCT p=0.524). Although those with a first complete response (CR1) > 1 year demonstrate better OS, they still fare poorly overall (3 year OS 51% ± 13% without HSCT; 2 year OS of 20% ± 31% with HSCT p=0.008; T. Alonzo, personal communication).

New therapies for children with AML are needed as often cure requires HSCT and comes at the expense of high cumulative anthracycline doses. Even with such aggressive therapies, OS is poor. CPX-351 offers the potential to maintain or improve survival with effective and tested agents, cytarabine and daunorubicin, while potentially reducing acute and late cardiac side effects through liposomal encapsulation. While the drugs are not novel, this may be the only liposomal anthracycline formulation available to children in the United States. With an acceptable toxicity profile, it is conceivable that CPX-351 could move quickly into a pediatric phase 3 study.

2.1.1 Anthracycline Use in Childhood AML

Cytarabine and anthracyclines have been the cornerstones of AML chemotherapy for decades. Anthracyclines are critical for improving survival, particularly through intensification of induction therapy.[3,4] Currently, the most common anthracyclines used for the treatment of de novo pediatric AML in the U.S. are daunorubicin and mitoxantrone. Idarubicin is an effective anthracycline but is more commonly used in the relapsed setting. Doxorubicin is more commonly used in acute lymphoblastic leukemia therapy and in patients with solid tumors. There is evidence that daunorubicin demonstrates 20-30% less cardiac toxicity per unit dose compared with doxorubicin.[5,6], and cumulative anthracycline calculations should be made accordingly. Higher cumulative doses with conventional preparations result in significant long term toxicity. Currently, COG utilizes Medical Research Council (MRC)-based chemotherapy, which has improved outcomes for children with AML with a 56% and 66% event free survival (EFS) and OS, respectively.[7-9] Children with newly diagnosed AML enrolled on COG trials (AAML0531, AAML1031) who do not receive HSCT receive a cumulative anthracycline dose of approximately 444 mg/m^2 (assuming a 1:3 conversion of mitoxantrone to daunorubicin equivalent), while those assigned to HSCT on AAML1031 (high risk cytogenetic/molecular abnormalities, + MRD) receive 294 mg/m^2. Randomized studies in adults have demonstrated that higher doses of anthracyclines lead to superior survival.[3,4] In fact, adult cooperative groups in the United States (SWOG, MDACC) are increasing cumulative anthracycline doses for adolescent/young adult high-risk AML patients in recognition that dose-intensification improves survival.

Anthracycline use is limited by dose-dependent cardiotoxicity. Anthracycline-associated cardiac myopathy remains a devastating problem for those children who are AML survivors.[10,11] Studies of long-term survivors utilizing cardiac MRI demonstrate reduced ejection fraction and reduced cardiac mass in patients with prior anthracycline exposure.[12,13] Emerging data also reveal a significant risk of cardiovascular deaths in pediatric AML survivors. Two studies from France and England reported a 43.7 standard mortality ratio from heart disease for children with AML, the highest among all pediatric cancers.[14-16] The literature clearly demonstrates that late cardiac effects, such as congestive heart failure, increases with higher cumulative doses of anthracyclines.[10] One analysis of anthracycline-induced clinical heart failure (A-CHF) in a cohort of 607 children who had been treated with anthracyclines concluded that up to 5% of patients will develop A-CHF 15 years after treatment, and patients treated with cumulative doses of more than 300 mg/m^2 are at highest risk. Those receiving cumulative anthracycline doses of 600 mg/m^2 had a 17.8% risk of developing A-CHF (95% CI, 5.5% to 30.1%).[17] It is important to note that cumulative doses of anthracyclines in AML patients are among the highest still administered to children and adults with cancer. Without better therapeutic options for children with AML, we must identify methods to minimize cardiac damage while maintaining anthracycline dose-intensity.

2.1.2 Strategies to Address Cardiotoxicity in Childhood AML

Rigorous testing of new agents that may mitigate the risk of cardiac toxicity is critically important to improving outcomes of children with AML. Previous strategies have included alternative derivatives of anthracyclines (mitoxantrone, idarubicin) and the use of pre-therapy cardioprotective agents such as dexrazoxane (DRZ).[15,18] The cardioprotective effect of DRZ was initially attributed to its iron chelating effect by ADR-925, an EDTA product of the enzymatic DRZ hydrolysis. This theory has been challenged, with studies demonstrating that the inhibition of the enzymatic conversion of DRZ to ADR-925 does not abolish the cardioprotective effect.[19] Another theory to explain the protective effect of DZR on cardiac tissue is that its antioxidant properties protect the heart from oxidative stress.[20-23] However, the use of other antioxidants has not demonstrated protection of cardiac tissue from oxidative stress. Most recently, DZR was found to prevent the accumulation of anthracycline-induced DNA double strand breaks via its effect on the depletion of topoisomerase II α and topoisomerase β. This mechanism is also a possible explanation for the reported DZR interference with anthracycline pharmacokinetics and efficacy.[24,25]

Liposomal delivery systems for anthracyclines are a promising strategy to mitigate cardiotoxicity while maintaining efficacy.[26-28] While data is limited in pediatric patients, adult studies have been published that demonstrate evidence of decreased cardiotoxicity with liposomal anthracyclines.[29,30] DNX is a liposomal anthracycline that has been studied extensively in adults and children with newly diagnosed and relapsed acute leukemia.[2,27,28,31-33] The i-BFM group recently published their results of a randomized trial comparing FLAG and FLAG + DNX in children with relapsed AML. In this study, DNX was given at a dose of 60 mg/m^2/day on Days 1, 3, and 5 in combination with fludarabine 30 mg/m^2/day x 5 days followed by cytarabine 2000 mg/m^2/day x 5 days. There were no

statistically significant differences in acute toxicities reported on the 2 arms. Of note, Grade 3 to 4 skin toxicity was reported in 4% of patients with FLAG/DNX compared with 1% of those treated with FLAG and acute cardiotoxicity ≥ Grade 3/4 was reported in a total of 5 patients with FLAG/DNX and 1 patient treated with FLAG. The study did not report cumulative anthracycline dose prior to enrollment. Notably, all cardiotoxicities occurred in the setting of infection. Overall, the CR rate (ANC > 1000, platelets > 50,000) after 2 courses was 69% with FLAG/DNX, and 59% with FLAG (p=0.07).[2] Most recently, DNX was compared to idarubicin during induction for children with newly diagnosed AML. In that study, DNX had antileukemic activity comparable to idarubicin, demonstrated superior EFS in patients with t(8;21), and caused less treatment related toxicity.[33] The potential advantages of liposomal preparations include: prolonged time in the circulation[34] due to protection of the drug from enzymatic inactivation, altered biodistribution of the liposome formulation and potential sparing of normal tissue with a resultant reduction in toxicity;[35] circumvention of drug efflux transporters responsible for drug resistance.[36,37] Data presented below suggests that CPX-351 has marked pharmacokinetic advantages when compared to DNX. More importantly, there is no plan to further develop DNX in the United States, and there are no data on the long term benefit of liposomal anthracyclines in children with AML. Developing CPX-351 in children with AML is an important step in studying the role of liposomal anthracyclines as a cardioprotectant strategy.

2.2 Preclinical Studies and Pharmacokinetics

CPX-351 is a liposomal formulation of a fixed combination of cytarabine and daunorubicin contained within the liposome at a 5:1 molar ratio. Each unit of CPX-351 contains 1.0 mg of cytarabine and 0.44 mg of daunorubicin. Prior studies have demonstrated the importance of ratio-dependent synergy and antagonism for combinations of cytarabine and daunorubicin. Specifically, chemotherapy combinations *in vitro* can be synergistic, additive, or antagonistic depending on the molar ratio between the two agents.[38] Maintaining a fixed ratio is difficult when using combinations of free drugs given the different distribution, metabolism, and elimination properties of the individual drugs. Controlling the exposure of drug ratios using liposomal preparations can lead to prolonged maintenance of the optimal drug ratio and result in improved efficacy *in vivo*. *In vivo* delivery of fixed cytarabine:daunorubicin ratios (5:1 molar ratio) shown to be synergistic in cell culture results in an increase in antitumor activity in tumor-bearing mice. In preclinical models, CPX-351 provided elevated and prolonged drug concentrations that were orders of magnitude higher than free drug and also higher daunorubicin concentrations compared to Daunoxome (DNX) based on literature reports of the latter. These pharmacokinetic advantages have held true in clinical studies of CPX-351 as well. When CPX-351 was given Days 1, 3, and 5 in the adult phase 1 study, the clearance of cytarabine and daunorubicin was less than 120 mL/h/m^2 across all dose levels. This is markedly less than the clearance of unencapsulated daunorubicin (38,600 mL/h/m^2) and cytarabine (134,000 mL/h/m^2).[39,40] This results in striking differences in plasma elimination half-life when comparing CPX-351 with free drug or with DNX. For example, the plasma elimination half-life of the daunorubicin component of CPX-351 is approximately 24 hours compared to 5-8 hours in DNX. At 48 hours, the plasma concentration of the daunorubicin component of CPX-351 is about 15 times greater than that of DNX. The plasma half-life of the cytarabine component in CPX-351 at the adult MTD is approximately 42.5 hours, significantly longer than unencapsulated cytarabine. Of

interest, there is almost no distribution phase of CPX-351 suggesting that virtually all detectable CPX-351 in the plasma exists in the encapsulated form. In addition, these concentrations are maintained in the circulation at the synergistic 5:1 ratio.[41-43] Therefore, CPX-351 accumulates and persists in the bone marrow at drug concentrations many fold higher than free drugs and significantly higher than DNX. Further, liposome encapsulation may result in the drug being selectively taken up by the leukemia cells with subsequent release of cytarabine and daunorubicin from intracytoplasmic liposomes.[43]

2.3 Adult Studies

2.3.1 Phase 1 Studies of CPX-351 in Adults

The first phase 1 study included 48 adult patients with relapsed/refractory leukemia (43 patients with AML) who received CPX-351 on Days 1, 3, and 5 of treatment. Multiple dose levels between 3 units/m^2/dose and 134 units/m^2/dose were tested. Pancytopenia was universal and was associated with fever, infection, and bleeding episodes. Dose-limiting toxicity (DLT) was seen at the 134 units/m^2/dose level and consisted of congestive heart failure (CHF; 1 patient), hypertensive crisis (1 patient), and persistent cytopenias beyond 56 days (1 patient). The patient with CHF had previously received 365 mg/m^2 cumulative anthracycline dose and sustained cardiac dysfunction during an episode of acute sepsis in which their LV ejection fraction dropped from 52% to 29% but subsequently increased to 47% after resolution of the septic episode. Responses were observed in 10 of 43 (21%) AML patients treated with CPX-351 including 9 CRs and 1 CRp. PK data from 13 patients showed a median elimination half-life of 31.1 hours for cytarabine and 21.9 hours for daunorubicin with the 5:1 ratio maintained for greater than 24 hours. Notably, encapsulated drug was detectable at least 7 days following the last dose.[44] Subsequent phase 2 studies have provided additional encouraging data regarding the tolerability and response rate to CPX-351.

2.3.2 Phase 2 Trials of CPX-351 in Adults

Trial 204 involving elderly patients with *de novo* AML consisted of a 2:1 randomization between standard 7+3 treatment and up to 2 courses of induction and 2 courses of consolidation therapy with CPX-351 given on Days 1, 3, 5. The CR + CRi (complete remission with incomplete blood count recovery) rate was 67% (N=56) for the patients treated with CPX-351 and 51% (N=21) for those treated with 7+3 (P=0.07). Ten patients unresponsive to 1 or 2 courses of 7+3 induction treatment were crossed over CPX-351 treatment. Of those, 4 achieved CR and survived > 12 months. Among those on study with an antecedent hematologic disorder and high-risk features, CPX-351 demonstrated significant superiority over 7+3 therapy [survival HR=0.40 (p=0.01), EFS HR=0.51, (p=0.04), and CR + CRi rate (57.6% vs. 31.6%), p=0.013].[45]

Trial 205 was a phase 2 study performed in patients 18-65 years of age and in first relapse. Patients were randomized 2:1 to receive either CPX-351 or to treatment selected by their physician, which was most often MEC (mitoxantrone/etoposide/cytarabine). Those with prior HSCT were eligible (22 patients in CPX-351 arm; 7 patients in the other arm). Overall CR+ CRi rates were 49.4% in CPX-351 arm and 40.9% in control arm (p=0.5). The bone marrow aplasia rate on Days 14-21 was superior in the CPX-351 arm (76% vs. 51%). For

this study, unfavorable risk leukemia was defined using the European Prognostic Index based on length of CR1, cytogenetics, age at relapse, and prior history of HSCT. For these patients, the CR+CRi rate was 39.3% in the CPX-351 arm compared to 27.6% (p=0.34) in those who received physician-choice chemotherapy. The 60-day mortality was approximately equal between the 2 study arms (14.8% vs 15.9%). However, the 1-year OS in unfavorable/poor risk patients was significantly better in the CPX-351 group with a hazard ratio of 0.55, p=0.02.[46]

As a result of the improved outcomes for high-risk patients in these phase 2 studies, Celator (A Jazz Pharmaceuticals Company) is sponsoring a multi-institution, randomized phase 3 study enrolling adults aged 60 to 75 with newly diagnosed, high-risk patients with AML. Results of this study were presented at the American Society of Clinical Oncology meeting in June of 2016. Adults with de novo, high-risk AML/MDS were randomized 1:1 to receive standard therapy with cytarabine/daunorubicin (7+3) or CPX-351. CR rates were superior in the CPX-351 arm 37.3% vs 25.6% in 7+3 arm (p=0.04). Sixty day mortality rates were superior in the CPX-351 arm (21.2% 7+3 vs. 13.7% CPX-351). This was largely due to decreased incidence of death due to disease in the CPX-351 arm (3.3% CPX-351 vs 13.3% in 7+3 arm). The study met its primary objective, with CPX-351 demonstrating superior OS compared to 7+3 (median survival 9.56 months vs 5.95 months, HR=0.69, p=0.005).[47]

The objectives of this study are to evaluate the safety and efficacy of CPX-351 in pediatric AML patients. While in pediatrics, ADE is commonly used for induction treatment, the standard-of-care in adult patients is 7+3 (cytarabine plus daunorubicin). Given that the adult phase 3 study demonstrates improved efficacy and an acceptable toxicity profile, it is likely to become a new standard of care in AML. In addition, the use of liposomal encapsulation offers the potential of reduced long-term cardiac toxicity. It is important to note that these adult studies have not demonstrated a difference between CPX-351 and intensive salvage regimens including anthracyclines on cardiac function in patients with prior anthracycline exposure. Further study is necessary to fully characterize the advantages of liposomal anthracyclines compared to conventional anthracycline therapy.

2.4 Pediatric Studies

2.4.1 Phase 1 Trial of CPX-351 in Children

Cincinnati Children's Hospital is conducting a phase 1 study of CPX-351 in children and adolescents (≤ 21 years of age). This is a single institution phase 1 pilot study that aims to assess the pharmacokinetics, toxicity and tolerability of a single course of CPX-351 in pediatric and young adults with relapsed/refractory hematologic malignancies. The trial utilizes a rolling six design, with dose level 1 (DL1) starting at the adult MTD of 100 units/m^2/dose once daily on Days 1, 3, and 5. Dose level 2 will escalate past the adult MTD to 134 units/m^2/dose once daily on Days 1, 3, 5. The trial was activated 09/09/13 and has accrued 8 patients (personal communication, Mike Absalon, MD, study PI). Of the 4 patients enrolled on DL1, 1 had AML that relapsed within 6 months of initial diagnosis. This patient with AML was taken off study with marrow aplasia on Day 37 to receive HSCT. Toxicities on DL-1 have been consistent with intensive chemotherapy reinduction

regimens for relapsed acute leukemia and there were no DLTs. To date, there have been 4 patients enrolled on to DL-2. Two of the 4 patients enrolled at that dose level have experienced pain that qualify as DLT per their protocol definition. Description of those toxicities are outlined here.

The first patient was a 2 year-old boy with relapsed AML after therapy per COG study AAML0531 (arm A) and persistent leukemia following a retrieval attempt with a clofarabine based regimen. He had a history of bilateral leg pain for which he received gabapentin 100 mg three times daily and oxycodone 1.5 mg once daily average during the week prior to initiation of CPX-351. CPX-351 was initiated at 135 Units/m^2/dose (Days 1, 3, 5). On Day 5 of protocol therapy he complained of leg pain that was not improved with oxycodone. Morphine 0.5 mg IV was administered with temporary relief. On Day 6 he appeared to have abdominal pain, leg pain, and a severe headache (holding his head with both hands and crying). There was neither trauma nor fall witnessed. He received morphine 0.5 mg scheduled every 4 hours with inadequate relief. A CT scan and an MRI/MRV of the head were obtained and were non-revealing. On Day 7 he developed fever to 39.5 and broad spectrum IV antibiotics were initiated. On Day 8 he continued to have headache that was refractory to scheduled morphine. Morphine continuous infusion was initiated. Due to poor pain control, dexamethasone (approximately 6 mg/m^2/day) and fentanyl by PCA were initiated on Day 9. His headache pain appeared to be well controlled by Day 10, and the dexamethasone was weaned over the following 3 days. Fentanyl by PCA was discontinued on Day 12 when he no longer appeared to be in pain.

A second patient who experienced a DLT was a 16 year-old girl with relapsed AML after therapy with COG study AAML1031 (arm A) and persistent leukemia following a retrieval attempt with FLAG. She had a history of a generalized pain syndrome during chemotherapy and from osteomyelitis isolated to a great toe. Pain medications prior to initiation of therapy with CPX-351 were fentanyl patch (25 mcg/h), gabapentin 300 mg three times daily and hydromorphone 2 mg p.o. prn break through pain (usual need 0-1 dose/day). CPX-351 was initiated at 135 Units/m^2/dose (Days 1, 3, 5). On Day 3 of protocol therapy she complained of headache with temperature of 38.4. The headache improved with acetaminophen and dexamethasone (initiated 4 mg q day). She became afebrile and remained afebrile until Day 20. On Day 5 of protocol therapy she complained of severe generalized aches stating 10 out of 10 pain that was worse than any she had previously experienced. Hydromorphone by patient controlled analgesia (PCA) was initiated with initially poor response and titrated upward over the following 5 days. Methocarbamol was initiated empirically on Day 8 for possible muscle-related pain. Dexamethasone was discontinued on Day 9. Her pain was thought to be very well controlled with hydromorphone PCA, methaocarbamol, fentanyl patch, and gabapentin by Day 12 of protocol therapy. The pain was reported as being significantly improved by Day 14 and a wean of the hydromorphone by PCA initiated. On Day 16 she developed pain related to a localized infection that resulted in continuation of narcotic therapy. The infection-related pain was qualitatively distinct from the pain syndrome she experienced previously during the course.

The plan in the Cincinnati Phase 1 trial of CPX-351 is to follow their dose finding guidelines and de-escalate to 100 Units/m^2/dose.

Preliminary evaluation of the data from the adult clinical trials demonstrated no events of ≥ Grade 3 pain related to CPX-351. There are no known mechanisms for pain related to CPX-351 that are published. In each case of the Cincinnati Phase 1 trial, the pain occurred in patients with pre-existing pain complaints, and was managed appropriately by the treating physician. The pain that was determined as possibly related to CPX-351 resolved 10 days from the time of the 3rd dose (Day 5) of study drug. Given the importance of testing CPX-351 at a dose level comparable to both conventional and liposomal preparations of anthracyclines (See Section 2.5), this study will incorporate specific supportive care guidelines and DLT criteria to address this particular toxicity. In AAML1421, there were no pain-related DLTs observed among the 6 patients treated at the 135 mg/m^2 dose level in the dose-finding phase.

2.4.2 Phase 1 portion of AAML1421

Six patients were enrolled onto the phase 1 portion of the AAML1421 at DL1 (135 units/m^2/dose on days 1, 3, 5). Each of the patients enrolled has experienced expected toxicities commonly seen with intensive reinduction regimens. Four of 6 patients experienced a documented bacterial infection with isolates identified as E. coli, streptococcus mitis, staphylococcus epidermidis, and coagulase negative staphylococcus. One of 6 patients experienced a DLT of grade 3 decrease of ejection fraction. This patient had a history of decreased cardiac function after induction therapy for *de novo* AML (baseline echo EF 64%, SF 44%; end of induction 1 EF 51%, SF 26%). At that time patient was started on Lisinopril and after HSCT had EF of 55%. At relapse, the patient had a cardiac MRI and EF was 58%. Echocardiograms performed as a baseline study for AAML1421 demonstrated EF 58% and SF 32%. The patient was no longer on Lisinopril. After reinduction with CPX-351 on AAML 1421, an echocardiogram performed on Day 38 showed EF of 58% and SF 32%. The start of cycle 2 was delayed due to count recovery and echocardiogram 17 days later showed EF of 36% and SF of 25%. Repeat echo 3 days later confirmed Grade 3 decrease in EF (40%). The patient was otherwise not ill, no evidence of sepsis. Lisinopril was started and echo 14 days later demonstrated resolution with EF 56% and SF 31.5%.

Therefore, 1/6 patients experienced a DLT and per protocol guidelines the Phase II dose for this study is 135 units/m^2/dose on days 1, 3, 5. There was one death on study of a 21 year old female who passed away approximately 7 weeks after initiation of therapy from progressive disease manifested as CNS myeloid sarcoma demonstrated on MRI. The patient was CNS negative at the onset of therapy. The toxicities and death were not attributed to CPX-351 by the treating physician.

2.4.3 Options for Development of CPX-351 in Children

Liposomal anthracycline-based strategies are moving forward in childhood AML. Based on available adult and pediatric data, our European colleagues in the MRC, i-BFM, and NOPHO are utilizing DNX in their pediatric phase 3 studies (personal communication). Given the superior pharmacokinetic properties and favorable

clinical studies of CPX-351, there is strong rationale to support its development in COG.

Results of a randomized Phase III study were presented at the American Society of Clinical Oncology meeting in June of 2016.[48] Adults with de novo, high-risk AML/MDS were randomized 1:1 to receive standard therapy with cytarabine/daunorubicin (7+3) or CPX-351. CR rates were superior in the CPX-351 arm 37.3% vs 25.6% in 7+3 arm (p=0.04). Sixty day mortality rates were superior in the CPX-351 arm (21.2% 7+3 vs. 13.7% CPX-351). This was largely due to decreased incidence of death due to disease in the CPX-351 arm (3.3% CPX-351 vs 13.3% in 7+3 arm). The study met its primary objective, with CPX-351 demonstrating superior OS compared to 7+3 (median survival 9.56 months vs 5.95 months, HR=0.69, p=0.005).[48] If CPX-351 demonstrates safety and efficacy in our proposed study, the COG Myeloid Disease Committee will have the opportunity to incorporate it, along with other novel agents, into the successor phase 3 or pilot study for *de novo* AML. The sponsor has enthusiastically supported the development of this agent in the pediatric population. Given the adult efficacy and potential beneficial reduction in cardiac toxicity a future randomized comparison of CPX-351 to standard of care *de novo* AML is compelling for the following reasons:

1) Phase 1/2 and phase 3 adult studies have demonstrated statistically significant benefit for high risk patients.
2) In AAML1031, mitoxantrone/cytarabine is proving to be a treatment block that has longer periods of neutropenia and an increased incidence of infection (personal communication, Richard Aplenc, MD).
3) The use of CPX-351 may be of benefit in addressing cardiotoxicity. It is reasonable to hypothesize that novel liposomal delivery systems may at least maintain anthracycline efficacy while diminishing the risk for anthracycline-induced cardiomyopathy.
4) Eventually, this agent may be able to replace ADE and avoid etoposide exposure and the possibility of secondary malignancies.
5) The i-BFM efficacy data for DNX is compelling; however, DNX is not available for future study in the United States.

Thus, AAML1421 will be the first prospective study of short and long-term cardiotoxicity with liposomal anthracycline delivery in pediatric patients receiving high cumulative doses of anthracyclines

2.5 Dosing Rationale

As detailed in the background, the pediatric phase 1 trial of CPX-351 at Cincinnati Children's Hospital completed the initial dose level of 100 units/m^2/dose on Days 1, 3, 5 (the adult MTD). There were no DLTs. This finding is consistent with the testing of prior liposomal anthracyclines (eg, Doxil, DNX) that have demonstrated safety in children at the adult MTD. Enrollment on AAML1421 will begin at DL1, which will be 135 units/m^2/dose on Days 1, 3, 5.

The rationale for studying this dose level includes:

1) There were no DLTs at 100 units/m^2/dose on Days 1, 3, 5 in the aforementioned and ongoing pediatric phase 1 study.
2) The DLTs in the adult study at 134 units/m^2 were persistent cytopenia > 56 days (1 patient), CHF (1 patient), and hypertensive crisis (1 patient). The episode of CHF occurred during a period of sepsis and was transient. Hypertensive crises are very rare in the pediatric/adolescent and young adult population (AYA) population; therefore this toxicity may not be relevant to the eligible patients in this study. The FDA agreed to a starting dose of 134 units/m^2/dose (our proposal rounds the dose to 135 units/m^2) upon review of the phase 1 protocol conducted at Cincinnati Children's Hospital.
3) CPX-351 at a dose of 135 units/m^2/dose delivers a total daunorubicin dose of approximately 180 mg/m^2, which is comparable to the liposomal daunorubicin delivered in DNX in the Kaspers et al study.[2] Also, this dose is comparable to the 150 mg/m^2 cumulative dose of daunorubicin administered during Induction I of AAML0531 and AAML1031, thus providing a dose that is comparable to published literature and potentially translatable to a randomized phase 3 study.
4) If 135 units/m^2/dose is not tolerated, there will be a single dose de-escalation to 100 units/m^2/dose (DL0).

The phase 1 portion of AAML1421 was completed in 12/2016 and DL1 (135 units/m^2/dose) was found to be the RP2D. The dose that will be used in the phase 2 portion of the study will be 135 units/m^2/dose given on days 1, 3, and 5.

2.6 Correlative studies

2.6.1 Testing of Cardiac Function and Biomarkers of Cardiac Injury

Cardiac damage remains a significant dose-limiting toxicity as well as a cause of treatment related morbidity and mortality in childhood AML. Echocardiogram data are not predictive of those at greatest risk for cardiac damage. The exploratory objectives outlined below will evaluate the feasibility of stringent testing of cardiac function and biomarkers of cardiac injury to provide a more timely assessment of a patient's risk for developing anthracycline-induced cardiomyopathy. These studies are important as they have not been studied prospectively in any published pediatric studies using liposomal anthracyclines.

The current COG phase 3 AML study, AAML1031, is collecting the most comprehensive and complete data set of cardiac function available for children with AML. The AAML1031 data will include ejection fraction/shortening fraction (EF/SF) values entered into the remote data entry systems at the start of each course of chemotherapy for more than 1000 patients. These longitudinal data will not only serve as baseline data for patients enrolling on future AML studies and demonstrate the feasibility of acquiring serial echocardiogram measurements on patients enrolled on COG trials.

The development of clinically evident cardiomyopathy is a relatively rare event during the duration of most clinical trial monitoring, and may only become symptomatic decades later[10,14] or during periods of high stress such as sepsis or pregnancy. Biomarkers of cardiac injury may provide a more timely assessment of a patient's risk for development of anthracycline-induced cardiomyopathy. In this study we will evaluate the feasibility of incorporating conventional and advanced echocardiogram monitoring as well as measurement of potential biomarkers of

cardiotoxicity in routine assessment of cardiac function. These data will provide the foundation for studying, in the next phase 3 AML trial, those markers predictive of cardiac morbidity and mortality. Reliable biomarkers for cardiotoxicity may allow for the identification of patients most likely to benefit from early medical intervention, and permit future evaluations of possible cardioprotective strategies.

Potential biomarkers of cardiac injury to be evaluated in this study include conventional and advanced echocardiography, serum cardiac troponin-T, N-terminal probrain natriuretic peptide (NT-proBNP), and high sensitivity C-reactive protein (hs-CRP). As an exploratory objective, a peripheral blood microRNA profile predictive of myocyte damage will be evaluated following anthracycline administration. Additionally, we plan to incorporate whole exome sequencing to investigate genetic predisposition to anthracycline-induced cardiomyopathy.

2.6.2 Echocardiogram, Cardiac Troponin-T, N-terminal probrain natriuretic peptide (NT-proBNP), and hs-CRP

Biomarkers of cardiac injury may provide a more timely assessment of a patient's risk for development of anthracycline-induced cardiomyopathy. Serum levels of cardiac troponin-I (cTnI), troponin-T(cTnT), B-type natriuretic peptide (BNP), or the amino-terminal fragment of the BNP precursor (NT-proBNP) are associated with myocardial ischemia or strain injury and may be predictive of future subclinical or symptomatic cardiomyopathy (For reviews see: Dolci et al.[49] and Christenson et al.[50]). Lipsultz et al. have described elevations in cTnT and NT-proBNP among children with high risk ALL who were treated with moderate doses of doxorubicin during induction and in post-remission therapy.[51] Elevations of cTnT at any time during the investigation were seen in nearly half of the children that did not also receive dexrazoxane prior to doxorubicin, whereas elevations in cTnT were only seen in about 20% of the patients that received dexrazoxane. Increases in cTnT were associated with lower LV mass and LV end-diastolic posterior wall thickness by echocardiography 4 years later. Differentiating effects upon NT-proBNP were also see between the ALL cohorts. Elevations in NT-proBNP were associated with reduced LV thickness-to-dimension ratio by echocardiography. No significant association was noted between hs-CRP and changes by echocardiography. Longer follow up is necessary to determine when or to what extent these survivors of ALL develop symptomatic cardiac disease but this study illustrates how these markers may be used to compare different cardiac protection strategies for patients with leukemia in a significantly shorter time period.

Patients participating in this study of CPX-351 are likely to have previously received significant cumulative doses of anthracyclines and may harbor sub-clinical or asymptomatic heart disease even prior to treatment with CPX-351. Therefore changes in cTnT, NT-proBNP, and hs-CRP will be explored as potential biomarkers of cardiotoxicity prior to therapy and following therapy with CPX-351. Similarly, conventional echocardiogram parameters, including left ventricular ejection and shortening fractions, will be described with characterization of baseline abnormalities, as well as the decline from baseline post-CPX-351. Further, myocardial deformation, a novel measure of more subtle, regional

ventricular dysfunction will be explored (as described below). Together these data may allow indirect comparison of cardiotoxicity as similarly measured by echocardiogram and cardiac biomarkers following other anthracycline-containing regimens for hematologic malignancies. Further, it will provide critical preliminary data on which to base biomarker and imaging evaluations of cardiotoxicity during subsequent studies of CPX-351, such as a randomized phase 3 study comparing efficacy and cardiotoxicity of this drug with other anthracycline regimens used to treat AML. The optimal timing of cardiac biomarker acquisition such as cTnT following anthracycline therapy has not been fully elucidated. However, studies assessing cTnT or cTnI elevations following standard anthracycline formulations demonstrate troponin elevations as early as 4 hours after an anthracycline dose, but more often within several days after anthracycline exposure.[52] One adult study demonstrated the range of peak troponin concentrations being observed between days 6 and 35 of an anthracycline containing chemotherapy cycle (mean day 21).[53] Additionally, both pediatric and adult studies report prolonged troponin release up to 1 month following anthracycline exposure.[54] Preliminary data from the Cincinnati pediatric phase I study of CPX-351 has not demonstrated any elevations in high sensitivity troponin at early (24-48 hours after dose 3 of CPX-351) or late (end of cycle) time points. [Dr. Michael Absalon, personal communication] While this might reflect the absence of significant cardiac injury associated with the liposomal encapsulation of daunorubicin, it could also indicate that the longer half-life and sustained plasma concentration of the anthracycline component of CPX-351 results in a later rise in troponin concentration than seen with standard anthracycline regimens. Thus, serial cardiac biomarker samples will be collected following CPX-351 exposure on days 8, 15, 22 and 28-30 of cycle 1.

2.6.3 Novel Biochemical and Imaging Biomarkers of Cardiotoxicity

MicroRNAs (miRNAs) are posttranscriptional regulators of gene expression with a critical role in modulating cardiac regeneration, repair, and pathologic remodeling in response to stress and disease.[55] Circulating miRNAs have emerged as highly sensitive and specific markers of cardiomyocyte injury and cardiovascular disease.[56] While the role of miRNAs in modulating anthracycline-induced cardiomyocyte injury has been demonstrated in animal models,[57,58] less is known about circulating miRNA profiles in humans receiving anthracyclines. Pilot data from a small cohort of children receiving anthracycline chemotherapy demonstrates that miR-29b and miR-499 are specifically upregulated by 2-3-fold 6, 12, and/or 24 hours following a cycle of anthracycline (n=24), while both were unchanged in children receiving non-cardiotoxic chemotherapy (n=9). Further, plasma expression of these miRNAs was significantly higher 6 and/ or 24 hours post-anthracycline in patients with troponin evidence of cardiac injury compared to those with no troponin increase.[59] These data indicate that these miRNAs reflect anthracycline-induced cardiomyocyte injury and warrant further investigation.

To further explore the possibility of using peripheral blood miRNA as a prognostic indicator or anthracycline-induced heart injury in children, plasma will be collected at baseline, 6 hours following the 3rd dose of CPX-351, and weekly through one month after initiation of CPX-351. As above, the longer half-life and sustained plasma concentrations of the liposomal daunorubicin component of

CPX-351 warrant longer monitoring of miRNA as the timing of anthracycline-induced miRNA elevation may be delayed. The miRNA studies will be conducted and funded at Seattle Children's Hospital/Fred Hutchinson Cancer Research Center.

In addition to collecting conventional echocardiogram parameters of LV function (SF and EF) we will be requesting DICOM echo images to be sent for central analysis of myocardial strain. Myocardial strain is a dimensionless index reflecting the total deformation of ventricular myocardium during a cardiac cycle as a percentage of its original length and can be readily measured during routine echocardiography.[60] In contrast to SF and EF, which reflect global left ventricular function, myocardial strain, as assessed by speckle tracking echocardiography, is able to detect regional defects in myocardial contractility.[60] Anthracycline-induced reductions in myocardial strain have been demonstrated in children, both during and years following anthracycline therapy.[60,61] Increasing evidence demonstrates that early anthracycline-induced alterations in myocardial deformation precede reductions in left ventricular SF or EF.[60] Further, reduced peak systolic global longitudinal strain (GLS) has been shown to predict subsequent cardiotoxicity in adults receiving anthracycline.[60] The feasibility of central GLS analysis in the setting of this COG trial will be assessed with the goal of evaluating GLS as a functional predictor of myocardial damage in the setting of the next COG phase 3 study in AML. Additionally, change in GLS will provide a functional correlate against which to analyze both conventional cardiac biochemical biomarkers and plasma miRNAs.

Taken together cardiac biomarkers and advanced cardiac imaging may provide valuable information regarding the degree of anthracycline-induced cardiac injury, which upon further study may allow risk stratification and facilitate cardioprotective intervention in those at high risk for cardiac morbidity.

2.6.4 Whole Exome Sequencing to Evaluate the Role of Rare Coding Variants as Risk Factors For Anthracycline-Induced Cardiomyopathy

Current research indicates that anthracyclines directly damage cardiac myocytes and may cause a relative sarcopenia.[62,63] Moreover, anthracyclines suppress multiple cardiac transcription factors, and anthracycline uptake into cardiac myocytes may be mediated by neuregulin via erbB2 receptors,[64] thus varying cardiac myocyte anthracycline exposure and subsequent cardiac myocyte damage. Early studies demonstrated that increasing anthracycline dose,[65] cardiac irradiation,[66] young age,[66] and female gender,[67] increase the risk of anthracycline associated LV shortening dysfunction. These risk factors have been confirmed in more recent studies, which have sought to define genetic risk factors and to develop predictive models for the development of anthracycline-associated LV shortening dysfunction.[68-70] Additional work is needed to define genetic variation that may play a role in the development of short term, anthracycline associated cardiac toxicity.

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.

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*.

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.

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 (ie, 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.

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 on a Phase II study prior to study enrollment will be considered ineligible.** The only exception to this is for intrathecal cytarabine, which can be given within 1 week prior to administration of CPX-351. If IT cytarabine is given prior to enrollment, a separate institutional consent must be obtained.

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.

3.1.5.1 Cytogenetics

Specimens for cytogenetic analysis are required, and must be obtained prior to therapy initiation. It is strongly recommended that these specimens be sent to a COG-approved institutional cytogenetics laboratory (see Section 13.0). A listing of these laboratories may be found on the COG website, as well as methods of attaining COG approval for local cytogenetics laboratories without COG approval. For patient with refractory disease, the diagnostic specimen may be used.

The documentation of the chromosome analysis results and the reports/Forms should be sent to the appropriate reviewer (coordinating cytogeneticist) within 2 weeks (14 days) after enrollment (see Appendix II). Note: If the cytogenetics findings are available for the initial time of diagnosis, please submit the karyotype and/or Final Chromosome Report. If the information is not available, please indicate that information in the submission form.

Results of cytogenetics are not required to be completed prior to enrollment, but samples must be collected prior to therapy initiation and submitted to a cytogenetics laboratory.

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. Exception: the bone marrow aspirate may be performed within 14 days prior to enrollment. 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).

3.2.1 Age

Patients must be ≥ 1 year and ≤ 21 years of age at the time of enrollment.

3.2.2 Diagnosis

3.2.2.1 Patients must have had histologic verification of AML at original diagnosis.

3.2.2.2 Patient must have one of the following:

a) Recurrent disease with ≥ 5% blasts in the bone marrow (M2/M3 bone marrow), with or without extramedullary disease.

b) Recurrent disease with an absolute blast count greater than 1,000 per microliter in the peripheral blood with or without extramedullary disease.

3.2.2.3 To be eligible for the Dose-Finding Phase: **(The Dose-Finding Phase Completed in 12/2016)**

Relapse patients

- Patients must be in first relapse, and
- Patients must not have received prior re-induction therapy.

Refractory patients

- Patients must not have received more than one attempt at remission induction, which may consist of up to two different therapy courses. COG AAML1031 *de novo* therapy including Induction I and Induction II is an example.

Treatment-related AML (t-AML)

- Patients must be previously untreated for secondary AML.

3.2.2.4 To be eligible for the Phase 2 Efficacy Phase:

Relapse patients:

- Patients must be in first marrow relapse, and
- Patients must not have received prior re-induction therapy. Donor lymphocyte infusion (DLI) is considered a re-induction attempt.

3.2.2.5 CNS Disease

Patients must have the status of CNS1 or CNS2 only, and no clinical signs or neurologic symptoms suggestive of CNS leukemia, such as cranial palsy. See Section 3.3.

3.2.3 Performance Level

Patients must have a performance status corresponding to an ECOG scores of 0, 1 or 2. Use Karnofsky for patients > 16 years of age and Lansky for patients ≤ 16 years of age. See https://www.cogmembers.org/_files/protocol/Standard/PerformanceStatusScalesScoring.pdf under Standard Sections for Protocols. Note: Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

3.2.4 Prior Therapy

Patients must have recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, stem cell transplant or radiotherapy prior to entering this study. All prior treatment-related toxicities must have resolved to ≤ Grade 2 prior to enrollment.

a. Myelosuppressive chemotherapy: Must not have received myelosuppressive chemotherapy within 3 weeks of entry onto this study (excluding hydroxyurea).
   - Cyto-reduction with hydroxyurea can be initiated and continued for up to 24 hours prior to the start of CPX-351.

b. Biologic (anti-neoplastic agent): At least 7 days since the completion of therapy with a biologic agent such as steroids, retinoids.
   Note: For agents that have known adverse events occurring beyond 7 days after administration (i.e. monoclonal antibodies), this period must be extended beyond the time during which acute adverse events are known to occur.

c. Radiation therapy (RT): ≥ 2 weeks for local palliative RT (small port); ≥ 6 months must have elapsed if prior craniospinal RT or if ≥ 50% radiation of pelvis; ≥ 6 weeks must have elapsed if other substantial BM radiation.
   Note: Patients must have received ≤ than 13.6 Gy prior radiation to the mediastinum.

d. Stem Cell Transplant (SCT): No evidence of active graft vs. host disease for at least 4 weeks. For allogeneic SCT patients, ≥ 3 months must have elapsed since transplant.
   - Must have received no more than 1 prior autologous or allogeneic stem cell transplant.
   - Patients must be off all systemic immunosuppressive therapy for at least 2 weeks, excluding hydrocortisone for physiologic cortisol replacement.

e. Intrathecal cytotoxic therapy:
   - No waiting period is required for patients having received intrathecal cytarabine, methotrexate, and/or hydrocortisone.
   - At least 14 days must have elapsed since receiving liposomal cytarabine (DepoCyte) by intrathecal injection.

f. Growth factors:
   - Patients must not have received growth factors for 7 days prior to CPX-351.
   - Patients must not have received pegfilgrastim for 14 days prior to CPX-351.

3.2.5 Concomitant Medications Restrictions

Please see Section 4.1 for the concomitant therapy restrictions for patients during treatment.

3.2.6 Organ Function Requirements

3.2.6.1 Adequate Renal Function Defined As:

- Creatinine clearance or radioisotope GFR ≥ 70 mL/min/1.73 m^2 or
- A serum creatinine based on age/gender as follows:

Age	Maximum Serum Creatinine (mg/dL)	
	Male	Female
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR[71] utilizing child length and stature data published by the CDC.

3.2.6.2 Adequate Liver Function Defined As:

- Direct bilirubin <1.5x upper limit of normal (ULN) for age and institution , and
- SGPT (ALT) ≤ 3.0x ULN for age and institution (unless it is related to leukemic involvement)

3.2.6.3 Adequate Cardiac Function Defined As:

- Shortening fraction of ≥ 27% by echocardiogram, or
- Ejection fraction of ≥ 50% by radionuclide angiogram or echocardiogram.
- Corrected QT (QTcB) interval < 500 msecs.

3.2.6.4 Central Nervous System Function Defined As:

- Patients with seizure disorder may be enrolled if on anticonvulsants and if seizures are well controlled.
- CNS toxicity ≤ Grade 2.

3.2.7 HIV Disease

Patients with a known history of HIV are eligible, if they meet all of the following conditions:

- No history of HIV complications with the exception of CD4 count < 200 cells/mm^3
- No antiretroviral therapy with overlapping toxicity such as myelosuppression
- CD4 count > 500 cells/mm^3 prior to the diagnosis of relapsed AML
- HIV viral loads below the limit of detection
- No history of highly active antiretroviral therapy (HAART)-resistant HIV

3.2.8 Exclusion Criteria

3.2.8.1 Patients who have received > 450 mg/m^2 daunorubicin equivalents. This threshold was chosen because this is the approximate anthracycline exposure of patients who have received all 4 courses of chemotherapy according to AAML0531 or AAML1031 (300 mg/m^2 of daunorubicin and 48 mg/m^2 of mitoxantrone, using a 3-fold cardiotoxicity multiplier for mitoxantrone). Thus, patients who relapse after receiving AAML0531/AAML1031 therapy will be eligible for this study, provided they have not received any additional anthracyclines.

NOTE: For the purposes of determining eligibility for this protocol, the following cardiotoxicity multipliers will be used to determine daunorubicin equivalents:

- Doxorubicin: 1
- Mitoxantrone: 3
- Idarubicin: 3
- Epirubicin: 0.5

3.2.8.2 Patients who are currently receiving another investigational drug.

3.2.8.3 Patients receiving medications for treatment of left ventricular systolic dysfunction.

3.2.8.4 Patients with any of the following diagnoses:

- Acute promyelocytic leukemia (APL)
- Down syndrome
- Fanconi anemia, Kostmann syndrome, Shwachman syndrome or any other known bone marrow failure syndrome
- Wilson's disease and any other disorder of copper metabolism
- Juvenile myelomonocytic leukemia (JMML)

3.2.8.5 Patients with documented active, uncontrolled infection at the time of study entry.

3.2.8.6 Patients with known active HBV and HCV infections.

3.2.8.7 Patients with prior allergy to daunorubicin and/or cytarabine.

3.2.8.8 Pregnancy and Breast Feeding

3.2.8.8.1 Female patients who are pregnant are ineligible due to risks of fetal and teratogenic adverse events as seen in animal/human studies.

3.2.8.8.2 Lactating females are not eligible.

3.2.8.8.3 Female patients of childbearing potential are not eligible unless a negative pregnancy test result has been obtained.

3.2.8.8.4 Sexually active patients of reproductive potential are not eligible unless they have agreed to use an effective

contraceptive method for the duration of their study participation and for 6 months after the last dose of chemotherapy.

3.2.9 Regulatory Requirements

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

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

3.3 Definitions

CNS disease

CNS status is defined as:

CNS 1: In cerebral spinal 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 ≥ 5/µL WBCs 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, presence of ≥ 5/µL WBCs and cytospin positive for blasts (in the absence of a traumatic lumbar puncture) and/or clinical signs of CNS leukemia.

STEINHERZ/BLEYER ALGORITHM FOR EVALUATING TRAUMATIC LUMBAR PUNCTURES:

If the patient has leukemic cells in the peripheral blood and the lumbar puncture is traumatic and the cytospin contains ≥ 5 WBC/µL with blasts, the following algorithm should be used to distinguish between CNS2 and CNS3 disease:

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

Therefore, a patient with CSF WBC ≥ 5/µL blasts, whose CSF WBC/RBC is 2X greater than the blood WBC/RBC ratio, has CNS disease at diagnosis.

For example, the following patient would be classified as CNS3: CSF WBC = 60/µL; CSF RBC = 1500/µL; blood WBC = 46000/µL; blood RBC = 3.0 X 10^6/µL:

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

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

This is a two part study comprised of a Dose-Finding phase to determine the RP2D of CPX-351 followed by an Efficacy phase. In both phases of the trial, CPX-351 will be administered intravenously over 90 minutes on Days 1, 3, and 5 in Cycle 1 and FLAG will be administered in Cycle 2. A diagnostic lumbar puncture will be performed and intrathecal chemotherapy will be given prior to each cycle of systemic therapy. Patients without evidence of CNS disease (CNS1) do not require additional CNS-directed therapy during Cycle 1. Patients with CNS2 disease will receive further intrathecal chemotherapy according to the guidelines set in Section 4.2.3. Patients treated on the phase 2, Dose-Efficacy phase will receive CPX-351 at a dose of 135 units/m^2/dose. Pharmacokinetic (PK) sampling during the first cycle of treatment will be optional for the patients enrolled in the Efficacy phase of this study (see Section 14).

The use of dexrazoxane will not be allowed on this trial.

Post-treatment therapy: Please note that AAML1421 includes only 2 cycles of therapy. Patients in remission following cycle 2 should proceed to transplant or other therapy without delay and according to institutional practice.

The phase 1 Dose-Finding portion of the study was completed in 12/2016. The recommended Phase 2 dose was found to be 135 Units/m^2/ dose.
DLT assessment for CPX-351 will occur during Cycle 1 of the Dose-Finding phase. Patients will be assigned a CPX-351 dose level at the time of enrollment. The starting dose (DL1) will be 135 units/m^2/dose on Days 1, 3, 5. If 135 units/m^2/dose is not tolerated, there will be a single dose de-escalation to 100 units/m^2/dose (DL0).

CPX-351 Dose Level	CPX-351 Dose (1 Unit = 1 mg cytarabine and 0.44 mg daunorubicin)
0	100 Units/m^2/dose
1	135 Units/m^2/dose

Pharmacokinetic (PK) sampling during the first cycle of treatment will be required for the patients enrolled in the Dose Finding phase of this study (see Section 14).

Note: Central venous access in the form of a double lumen Broviac or Hickman, port-a cath, or peripherally inserted central catheter is highly recommended.

4.1.1 Concomitant Medications and Therapies

4.1.1.1 Other Anti-Cancer Therapy

No other cancer chemotherapy or immunomodulating agents may be given while the patient is on protocol.

4.1.1.2 Corticosteroid Therapy

Corticosteroids should not be used as anti-emetic therapy. Corticosteroid therapy is not permissible except for the following indications:

- As treatment or prophylaxis for anaphylactic reactions
- As a treatment for symptoms of Ara-C syndrome including fever, rash, or conjunctivitis (see Section 5.3.4)
- As treatment for rash possibly related to CPX-351 (see Section 5.3.1)
- Suspected or confirmed adrenal insufficiency
- Pain management per institutional guidelines. Note, it is not recommended to use prolonged courses (> 3 days) of corticosteroids for pain management. CPX-351 is a myelosuppressive chemotherapeutic agent and extended use of corticosteroids may result in increased susceptibility to infections.

4.1.1.3 Use of Filgrastim

During Cycle 1 (CPX-351), the use of filgrastim (G-CSF) or biosimilar may be used at the treating physician's discretion to enhance neutrophil recovery when clinically indicated. Routine use of filgrastim in clinically well patients awaiting count recovery is not recommended. If the treating physician determines that filgrastim use is indicated in Cycle 1, the pegylated formulation (pegfilgrastim) should not be used.

4.1.2 Supportive Care Guidelines

Necessary supportive measures for optimal medical care will be given throughout the study as indicated by the treating physician and the patient's medical need. For example, administration of antiemetics is strongly recommended. Monitoring for tumor lysis and preemptive or early intervention is recommended.

It is recommended that the patient's fluid status and hepatic and renal function be carefully monitored during the drug administration period. In this regard, patients should be well hydrated during the days of Cycle 1 and Cycle 2 chemotherapy drug administration, with the intent of maintaining urine output > 3 mL/kg/hr.

If copper toxicity is suspected, check serum copper levels. If abnormal, monitor until levels return to normal range. For patients receiving sources of copper such as in total parenteral nutrition (TPN), monitor for signs and symptoms of copper toxicity.

To prevent drug incompatibility, no other medications should be administered concurrently through the same IV line as study drugs.

For COG Supportive Care Guidelines see: https://childrensoncologygroup.org/index.php/cog-supportive-care-guidelines under Standard Sections for Protocols.

Antifungal prophylaxis

Antifungal agents per institutional guidelines should be administered when ANC falls below 200/µL and continued until count recovery.

Hospitalization/Hospital Environment

Hospitalization following each cycle of chemotherapy is strongly recommended until the absolute phagocyte count (sum of the neutrophils, bands and monocytes) rises for 2 successive days, and the patient is afebrile and clinically stable. An additional discharge criterion of an absolute neutrophil count (ANC) of at least 200/µL is also suggested.

It is recommended that patients should be assigned to rooms with special air filtration systems such as high efficiency particulate air filters (HEPA) or clean-air rooms with constant positive pressure airflow if at all possible.

Pain Management

A single-institution, Phase 1 study of CPX-351 was conducted at Cincinnati Children's Hospital that tested the doses prescribed in this protocol. As described in the background, 2 patients with pre-existing analgesic requirements experienced Grade 3 pain events at the 134 units/m^2 dose level that met protocol-derived DLT criteria and were considered possibly related to CPX-351. If pain develops in any patient after the initiation of CPX-351, it is recommended that the treating physician initiate institution-appropriate therapy with intravenous narcotics. Corticosteroids for pain are not recommended, but may be used with caution per institutional guidelines. For more information, please refer to Section 4.1.1.2.

4.2 Cycle 1

4.2.1 Therapy Delivery Map – Cycle 1 Cycle 1 lasts 28 days. This TDM is on 2 pages.	_____ Patient COG ID number _____ DOB

Criteria to start this cycle are found in the patient eligibility criteria found in Section 3.2.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
CNS 1 Patients Intrathecal Cytarabine (IT ARAC)	Intrathecal (IT)	Age (years) Dose 1-1.99 30 mg 2-2.99 50 mg ≥ 3 70 mg	**CNS1 Patients**: • Given at the time of diagnostic lumbar puncture **OR** Day 0* • Day 28-30$	*May be given up to 7 days prior to Day 1. $May be given when BM aspirate/biopsy performed at end of Cycle 1.
CNS 2 Patients# Intrathecal Cytarabine (IT ARAC)	Intrathecal (IT)	Age (years) Dose 1-1.99 30 mg 2-2.99 50 mg ≥ 3 70 mg	**CNS2 Patients#**: • Given at the time of diagnostic lumbar puncture **OR** Day 0* • Then give twice weekly until CNS is clear. • Day 28-30$	*May be given up to 7 days prior to Day 1. Twice weekly IT to begin a minimum of 48 hours following the 3rd dose of CPX-351. A minimum of 4 and a maximum of 6 (count includes day 0 IT) intrathecal treatments may be given. $May be given when BM aspirate/biopsy performed at end of Cycle 1. See Section 4.2.3 for full details
CPX-351 IND # 129443 Do not use commercial supply	IV (central venous catheter) over 90 minutes	135 Units/m²/dose	1, 3, 5	Round doses to nearest whole unit. Day 1 dosing should be at least 24 hours after IT therapy administration.

Ht _____cm Wt _____kg BSA _____m²

Date Due	Date Given	Day	IT ARAC _____mg	CPX-351 ____Units	Studies
			Enter calculated dose above and actual dose administered below		
		0	____ mg*		q
		1		____ Units	a-p, r, s, t
		3		____ Units	
		5		____ Units	r, t
		6			
		7			
		8	____ mg#		b, f, h, i, j, q, r, s, t
		11	____ mg#		q
		15	____ mg#		b, f, h, i, j, q, s, t
		18	____ mg#		q
		22	____ mg#		b, f, h, i, j, q, s, t
		28	____ mg$		l, m, n, p, q, s, t, u
		For criteria to begin Cycle 2, please see Section 4.2.4. See Section 7.1 for End of Therapy evaluations.			

Days IT therapy given for CNS2 patients may vary based on scheduling

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

Cycle 1

Cycle 1

4.2.2 Required Observations in Cycle 1

All baseline studies must be performed prior to starting protocol therapy unless otherwise indicated below. Observations a-l and n-q can be performed up to 7 days before the start of therapy. "m" can be performed within 2 weeks prior to the start of protocol therapy. If performed, the baseline studies need not be repeated on Day 1.

a. History
b. Physical exam (including VS): Day 1, and then weekly
c. Ht, Wt, BSA
d. Performance status
e. Pregnancy test: Female patients of childbearing potential require a negative pregnancy test prior to starting treatment; sexually active patients must use an acceptable method of birth control.
f. CBC, differential, platelets: twice a week until ANC > 1000/µL and PLT > 100,000/µL and then weekly.
g. Urinalysis
h. Electrolytes including Ca^{++}, Mg^{++}, PO_4: Day 1, then weekly
i. BUN and Creatinine: Day 1, and then weekly
j. Total and direct bilirubin, AST, ALT: Day 1, and then weekly
k. Total protein, albumin
l. Echocardiogram or MUGA, and EKG.
m. Imaging (CT or MRI) of chloroma (only required at baseline if clinically indicated, end of cycle only required if chloroma present at baseline)
n. BMA/clot section or biopsy (see Section 4.2.4).
o. BMA cytogenetics and FISH (It is recommended that cytogenetics/FISH be performed by a COG-approved laboratory (see Section 13.0).
p. Flow cytometry with minimal residual disease (MRD) determination, according to institutional standards. Perform with any subsequent bone marrow evaluation during the study.
q. LP-CSF for cell count, cytospin
r. Optional Pharmacokinetic study (see Section 14.1) on Day 1, Day 5, and Day 8, for patients enrolled in the Efficacy phase.
s. Echo and Blood Biomarkers Optional studies (see Section 14.2.1): **Echo:** Baseline (before CPX-351 infusion, Day 28 (±7 days) but before Cycle 2. **Blood:** Baseline (before CPX-351 infusion), Day 8, Day 15, Day 22 and once during Days 28-30.
t. Chemical and Imaging Biomarkers of Cardiotoxicity Optional study (see Section 14.2.2): Day 1 before CPX-351 infusion, Day 5, Day 8, Day 15, Day 22 and once during Days 28-30.
u. Blood Risk Factors for Cardiomyopathy Optional study (see Section 14.2.3): once during Day 28-30.

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)

4.2.3 Cycle 1 Treatment Details

Intrathecal cytarabine: IT

All patients will receive 2 doses of IT cytarabine using age-based dosing as follows:

1) At the time of diagnostic lumbar puncture, or on Day 0 of Cycle 1. Patients may be given intrathecal cytarabine up to 1 week prior to Day 1, but at least 24 hours prior to the start of CPX-351 on Day 1. If IT cytarabine is given prior to enrollment, a separate institutional consent must be obtained.

2) At the time the Day 28-30 bone marrow aspirate/biopsy is obtained, or up to one week prior to Day 1 of Cycle 2.

Age-based dosing:

Age (yrs)	Dose
1-1.99	30 mg
2-2.99	50 mg
≥ 3	70 mg

For CNS2 patients

The efficacy of CPX-351 in preventing and treating CNS AML is unknown, therefore patients with CNS2 leukemia are required to receive additional intrathecal chemotherapy as follows:

Patients with CNS2 involvement at screening will receive additional IT therapy. This additional therapy may not be given until at least 48 hours following the 3rd dose of CPX-351. The additional therapy will consist of IT cytarabine twice weekly until the CSF is clear. A minimum of 4 and a maximum of 6 intrathecal treatments may be given. This count includes the pre-study/Day 0 dose. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments and all other patients will receive 2 intrathecal treatments once the CSF is clear. Subjects whose CSF was cleared may then receive subsequent intrathecal cytarabine doses at the discretion of the treating physician. Subjects who have persistent CNS disease despite 6 doses of intrathecal cytarabine will be off study treatment (See Section 8.1). See Section 3.3 for definitions of CSF involvement.

For patients who do not have CNS disease at the time of enrollment but who are considered high risk for treatment failure in the CNS (examples include: a history of CNS leukemia, high WBC at relapse, monocytic leukemia, or KMT2A (MLL)-rearranged disease) additional intrathecal cytarabine may be given starting a minimum of 48 hours following the 3rd dose of CPX-351 at the investigators discretion.

Cycle 1

CPX-351: IV over 90 minutes
Days: 1, 3, and 5.
Dose: 135 Units/m^2/dose

CPX-351 should be administered by central venous catheter.
The dose of CPX-351 can be rounded to the nearest whole unit to avoid decimal dosing.

CPX-351 will be provided by Jazz Pharmaceuticals. **Do not use commercial supply.**

See Section 5.0 for Dose Modifications based on Toxicities.

4.2.4 Response Assessment

Criteria to Begin Cycle 2

- Patients will have a disease assessment on Day 28-30 of Cycle 1. If a bone marrow aspirate cannot be evaluated (dry tap, no spicules), a follow-up bone marrow aspirate and biopsy must be performed within 2 weeks to assess cellularity and response.
- If aspirate and/or biopsy results indicate a hypocellular marrow (< 10% cellularity on biopsy), then continue to repeat bone marrow aspirate and/or biopsy not less than once every 14 days until response can be assessed.
- If count recovery is noted within 14 days of an evaluable marrow, there is no need to repeat the marrow to confirm response. Once disease response assessment can be made after Cycle 1, and non-hematologic toxicities probably or definitely related to CPX-351 recover to ≤ Grade 2, then patients will receive Cycle 2 of FLAG.
- If the bone marrow aspirate and/or biopsy performed after Cycle 1 reveals ≥ 5% blasts and ≤ 25% blasts, then the patient should proceed to Cycle 2 of chemotherapy consisting of FLAG.
- If the patient meets criteria for CR, CRp, or CRi after Cycle 1, then the patient can proceed to Cycle 2. (See Section 10.2 for definitions of response.)

Note: The patient will be removed from protocol therapy if one of the following criteria is met:

- Patients with ≥ Grade 2 toxicities not related to CPX-351 lasting > 50 days from the start of Cycle 1 that are considered by the investigator to be medically significant.
- Patients who have > 25% blasts after Cycle 1 will be considered a treatment failure and will be removed from protocol therapy.
- In the Efficacy phase, if after Cycle 1 (CPX-351) a patient has a hypoplastic bone marrow for ≥ 60 days and failure to recover a peripheral ANC > 500/µL and a non-transfusion dependent platelet count > 20,000/µL not due to malignant infiltration or severe infection (defined as ≥ Grade 3 infection), they will be considered a treatment failure and will go off protocol therapy.
- In the Dose-Finding phase, patients meeting criteria for a hematologic DLT in Cycle 1. **The phase 1 Dose-Finding phase completed 12/2016.**

Cycle 1

4.3 Cycle 2

4.3.1 Therapy Delivery Map – Cycle 2 Cycle 2 lasts 28 days.	_____ Patient COG ID number	_____ DOB

Please refer to Section 4.2.4 for criteria to begin Cycle 2. This TDM is on 2 pages.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Filgrastim (G-CSF) or biosimilar	IV or Sub-Q	**5 micrograms/kg/dose**	Daily on Days 1-5; restart on Day 15 until ANC recovery.	Administer one hour prior to each dose of fludarabine. Continue until ANC>500/µL. Record below the last day G-CSF is administered. See Section 4.3.3 for administration details. The use of pegfilgrastim is not permitted.
Fludarabine (FLUD)	IV over 30 minutes	30 mg/m^2/dose	1-5	See Section 4.3.3 for administration details.
High Dose Cytarabine (HD ARAC)	IV over 1-3 hours	2000 mg/m^2/dose	1-5	Use eye drops as described in Section 4.3.3. To begin 4 hours after the start of the fludarabine infusion.

Ht _____cm Wt _____kg BSA _____m^2

Date Due	Date Given	Day	G-CSF ____ mcg	FLUD ____mg	HD ARAC _____mg	Studies	Comments
				Enter calculated dose above and actual dose administered below			
		1	____ mcg	____ mg	____ mg	a-k	
		2	____ mcg	____ mg	____ mg		
		3	____ mcg	____ mg	____ mg		
		4	____ mcg	____ mg	____ mg	c	
		5	____ mcg	____ mg	____ mg		
		8				a, c, e, f, g,	
		11				c	
		15	____ mcg			a, c, e, f, g,	
		18				c	
		22				a, c, e, f, g,	
		25				c	Record date of last dose of G-CSF. ___/___/___
		28				j, k, l See End-of Therapy observations in Sect. 7.1.	

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

Cycle 2

4.3.2 Required Observations in Cycle 2

a. Physical exam (including VS): Day 1, and then weekly
b. Ht, Wt, BSA
c. CBC, differential, platelets: twice a week until ANC > 1000/µL and PLT > 100,000/µL and then weekly.
d. Urinalysis
e. Electrolytes including Ca^{++}, Mg^{++}, PO_4: Day 1, then weekly
f. BUN and Creatinine: Day 1, and then weekly
g. Total and direct bilirubin, AST, ALT: Day 1, and then weekly
h. Total protein, albumin
i. LP-CSF for cell count, cytospin
j. Echocardiogram or MUGA, and EKG: Prior to Cycle 2 therapy (Echo done at the end of Cycle 1 can count as long as it was done within 2 weeks prior to the start of Cycle 2), Day 28 (± 7 days) but before any subsequent therapy.
k. BMA/clot section (biopsy if aspirate unsuccessful)
l. Flow cytometry with minimal residual disease (MRD) determination, according to institutional standards

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)

Cycle 2

4.3.3 Cycle 2 Treatment Details

Filgrastim (G-CSF) or biosimilar: IV or Sub-Q
Days: Daily, on Days 1 through 5, one hour prior to each dose of fludarabine. Restart on Day 15 and continue until the post-nadir ANC ≥ 500/µL
Dose: **5 micrograms/kg/dose**

Note: Pegfilgrastim cannot be utilized in place of filgrastim or biosimilar.

Fludarabine: IV over approximately 30 minutes
Days: 1-5.
Dose: 30 mg/m^2/dose

High Dose Cytarabine (HD ARAC): IV infusion over 1-3 hours once daily
Days: 1-5
Dose: 2000 mg/m^2/dose (begin 4 hours after start of fludarabine)

Please note: Following/during high-dose cytarabine, chemical conjunctivitis may occur. Administer steroid eye drops (0.1% dexamethasone or 1% prednisolone 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, artificial tears may be administered on an every 2-4 hour schedule.

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

See Section 7. 1 for End of Therapy evaluations.

See Section 5.0 for Dose Modifications based on Toxicities.

Cycle 2

5.0 DOSE MODIFICATIONS FOR TOXICITIES

5.1 Definition of Dose Limiting Toxicity (DLT)

The phase 1 Dose-Finding portion of the study was completed 12/12/2016.

DLT will be defined as any of the following events that occur after the first dose of CPX-351 during the first cycle of therapy. DLT assessment will occur during the Dose-Finding phase.

Non-Hematologic Dose-Limiting Toxicity will be defined as any ≥ Grade 3 non-hematologic toxicity that occurs after the first dose of CPX-351 that is possibly, probably or definitely related to CPX-351, and with the specific exclusion of:

- Alopecia
- Grade 3 fatigue, anorexia, nausea, vomiting, or diarrhea
- Grade 3 or 4 AST or ALT elevation that improves to ≤ Grade 2 within 14 days
- Grade 3 or 4 isolated electrolyte abnormalities that resolve, with or without intervention, to ≤ Grade 2 within 72 hours. Electrolyte supplementation is encouraged.
- Tumor lysis syndrome
- Grade 3 or 4 fever, febrile neutropenia, or infection with or without hospitalization
- Grade 3 rash
- Grade 3 mucositis that resolves (with or without supportive care) to ≤ Grade 2 elevation within 14 days
- Early nutritional intervention with TPN or enteral tube feeding for anorexia, nausea, or concern for poor nutritional status will not be considered a DLT
- Grade 3 troponin elevations without EKG or other evidence of myocardial ischemia
- Grade 3 or greater pain due to leukemia, mucositis, typhlitis, infection, or obvious injury.

Hematologic Dose-Limiting Toxicity will be defined as failure to recover a peripheral ANC > 500/μL and non-transfusion dependent platelet count > 20,000/μL due to documented bone marrow aplasia/hypoplasia (i.e., not due to malignant infiltration) for greater than or equal to 50 days from the start of the therapeutic cycle. This time period reflects approximately between 1 and 2 standard deviations above the approximate median time to ANC recovery for patients receiving mitoxantrone/cytarabine on COG study AAML0531. Failure to recover peripheral counts due to disease involvement of the bone marrow will not be considered dose-limiting.

5.2 Dose Modifications Due to Toxicity Related to CPX-351

5.2.1

Only one cycle consisting of three doses of CPX-351 will be administered to participants in this study. If ≥ Grade 4 non-hematologic dose limiting toxicity probably, or definitely related to CPX-351 occurs prior to completion of all planned doses, hold the remaining dose(s). These patients (in both the Dose-Finding and Efficacy phases) will be removed from protocol therapy.

5.3 Modifications and Supportive Care Recommendations for Toxicity Related to Protocol Therapy (Cycle 1 and Cycle 2)

5.3.1 Rash

A macular-papular rash occurring within one week of treatment initiation occurs in the majority of patients treated with CPX-351. Grade 3 rash occurred in 6% of adults participating in the phase 1 study. The rash typically responded to topical or systemic corticosteroids. Subjects experiencing a rash from CPX-351 should first receive topical corticosteroids. Pruritus may be treated with diphenhydramine. If extensive or refractory to topical therapy, a 3-5 day course of systemic prednisone or methylprednisolone may be used.

5.3.2 Hand-Foot Syndrome

Hand-foot syndrome has been reported in patients treated with other liposomal chemotherapy products and high-dose cytarabine regimens. Patients who develop hand-foot syndrome may receive topical emollients (such as Aquaphor) as well as topical or systemic steroids or antihistamines if appropriate. Oral administration of vitamin B6 (pyridoxine) can also be used for these patients - BSA <0.5 m^2: 50 mg per day; BSA 0.5-1.0 m^2: 100 mg per day; BSA 1.1- 1.5 m^2: 200 mg per day, and BSA >1.5 m^2: 300 mg per day.

5.3.3 Neurological Toxicity

The most common neurotoxicity related to fludarabine or cytarabine administration is an acute cerebellar syndrome that may manifest itself as ataxia, nystagmus, or dysarthria. However, seizures and encephalopathy have also occurred following therapy with high dose cytarabine. Patients experiencing Grade 3 or 4 cerebellar toxicity should be removed from protocol therapy.

5.3.4 Ara-C Syndrome including Fever, Rash, or Conjunctivitis

Do not withhold cytarabine for fever if it is likely to have been caused by the cytarabine. However, blood cultures should be obtained even if fever is thought to be likely due to cytarabine. Institution of antibiotics for fever associated with cytarabine infusion is at the discretion of the treating physician (see fever management recommendations in the COG Supportive Care Guidelines).

For rash or conjunctivitis, withhold cytarabine for Grade 3-4 toxicity until resolved to ≤ Grade 1. Make up missed doses and consider concurrent treatment with hydrocortisone or dexamethasone, and/or with dexamethasone ophthalmic drops for conjunctivitis only.

5.3.5 Renal Toxicity

Patients with nephrotoxicity secondary to antibiotics, or antifungals, may have prolonged excretion of cytarabine leading to more severe marrow and extramedullary toxicity.

- Patients with a serum creatinine > 2 mg/dL or > 2x normal for age should be hydrated orally or intravenously. Following hydration, the patient must have a creatinine clearance ≥ 60 mL/min/1.73m^2 as measured preferably by a nuclear GFR scan, timed urine collection for creatinine clearance, or calculated by the Schwartz formula before proceeding with cytarabine therapy.

- If the creatinine clearance is abnormal (< 60 mL/min/1.73m^2) then Cycle 2 high dose cytarabine should be reduced by 50% for each daily dose. With this approach, previous research has shown the prevention of subsequent neurotoxicity in recipients of high dose cytarabine in the face of renal insufficiency.[72]

6.0 DRUG INFORMATION

6.1 [redacted]

[redacted]

[redacted]

[redacted]

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6.2 CYTARABINE

(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 *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 D*ose: 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 prednisolone), 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.

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.

6.3 FILGRASTIM, TBO-FILGRASTIM, FILGRASTIM-SNDZ

(Granulocyte Colony-Stimulating Factor, r-metHuG-CSF, G-CSF, Neupogen®, Granix®, Zarxio®) NSC #614629 (11/15/16)

Source and Pharmacology:
Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. Filgrastim is a 175 amino acid protein with a molecular weight of 18,800 daltons manufactured by recombinant DNA technology utilizing E coli bacteria into which has been inserted the human granulocyte colony stimulating factor gene. It differs from the natural protein in that the N- amino acid is methionine and the protein is not glycosylated. G-CSF is a lineage specific colony-stimulating factor which regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens). Filgrastim exhibits nonlinear pharmacokinetics with clearance dependent on filgrastim concentration and neutrophil count. Filgrastim is cleared by the kidney. The elimination half-life is similar for subcutaneous and intravenous administration, approximately 3.5 hours. The time to peak concentration when administered subcutaneously is 2-8 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		Local irritation at the injection site, headache	Allergic reactions (more common with IV administration than subq):skin (rash, urticaria, facial edema), respiratory (wheezing, dyspnea) and cardiovascular (hypotension, tachycardia), low grade fever
Prompt: Within 2-3 weeks, prior to the next course	Mild to moderate medullary bone pain	Increased: alkaline phosphatase, lactate dehydrogenase and uric acid, thrombocytopenia	Splenomegaly, splenic rupture, rash or exacerbation of pre-existing skin rashes, sickle cell crises in patients with SCD, excessive leukocytosis, Sweet's syndrome (acute

			febrile neutrophilic dermatosis)
Delayed: Anytime later during therapy			Cutaneous vasculitis, ARDS
Late: Any time after completion of treatment			MDS or AML (confined to patients with severe chronic neutropenia and long term administration)
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of filgrastim in humans are unknown. Conflicting data exist in animal studies and filgrastim is known to pass the placental barrier. It is unknown whether the drug is excreted in breast milk.		

Formulation and Stability:
Neupogen® supplied as a clear solution of 300 mcg/mL in 1 mL or 1.6 mL vials. Neupogen® vials are preservative free single use vials. Discard unused portions of open vials.
Neupogen®, Granix®, and Zarxio® are also available as single use prefilled syringes containing 300 mcg/0.5 mL or 480 mcg/0.8 mL of filgrastim for subcutaneous administration.
Store refrigerated at 2º-8ºC (36º-46ºF). Protect from light. Do not shake. Prior to injection, filgrastim and filgrastim-sndz may be allowed to reach room temperature for a maximum of 24 hours (infusion must be completed within 24 hours of preparation). TBO-filgrastim may be removed from 2°C-8°C (36°F-46°F) storage for a single period of up to 5 days between 23°C to 27°C (73°F to 81°F). Avoid freezing and temperatures > 30ºC.

For IV use, dilute filgrastim (Neupogen®) and tbo-filgrastim (Granix®) in D5W only to concentrations >15 mcg/mL. Filgrastim-sndz (Zarxio®) may be diluted in D5W to concentrations between 5 mcg/mL and 15 mcg/mL. At concentrations below 15 mcg/mL, human serum albumin should be added to make a final albumin concentration of 0.2% (2 mg/mL) in order to minimize the adsorption of filgrastim to plastic infusion containers and equipment for all 3 products (communication on file from Teva Pharmaceuticals USA). Filgrastim or filgrastim-sndz dilutions of 5 mcg/mL or less are not recommended. TBO-filgrastim dilutions below 2 mcg/mL are not recommended. Diluted filgrastim biosimilar products should be stored at 2º-8ºC (36º-46ºF) and used within 24 hours. Do not shake.

Do not dilute with saline-containing solutions at any time; precipitation will occur.

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

Filgrastim biosimilar products should not be administered within 24 hours of (before AND after) chemotherapy.

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

6.4 FLUDARABINE

(Fludara®, fludarabine phosphate, 2-fluoro-ara-AMP) NSC# 312887 **(01/10/18)**

Source and Pharmacology:

Fludarabine phosphate is a synthetic purine nucleoside. It differs from the physiologic nucleosides, adenosine, in that the sugar moiety is arabinose instead of ribose, and by the addition of a fluorine atom to the purine base adenine. Fludarabine is also a fluorinated nucleotide analog the antiviral agent vidarabine, (ara-A). The addition of fluorine results in increased aqueous solubility and resistance to enzymatic degradation by adenosine deaminase. Fludarabine (2-fluoro-ara-A) is commercially available as the monophosphate salt (2-fluoro-ara-AMP). The monophosphorylation increases the drug's aqueous solubility while maintaining pharmacologic activity. The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2-fluoro-9-(5-0-phosphono β-D-arabino-furanosyl) (2-fluoro-ara-AMP) and the molecular weight is 365.2.

Fludarabine is a purine antagonist antimetabolite. *In vivo*, fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then it is phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

Phase 1 studies in humans have demonstrated that within several minutes after intravenous infusion, fludarabine phosphate is converted to the active metabolite, 2-fluoro-ara-A and becomes undetectable. Therefore, pharmacokinetics studies have focused on 2-fluoro-ara-A. Fludarabine phosphate 25 mg/m^2 infused intravenously over 30 minutes to adult cancer patients, showed a moderate accumulation of 2-fluoro-ara-A. During a 5-day treatment schedule, 2-fluoro-ara-A plasma trough levels increased by a factor of about 2.

Fludarabine is widely distributed. The volume of distribution at steady state (V_{ss}) reported after daily administration of 25 mg/m^2 for 5 days to adults averaged at 96-98 L/m^2. Tissue distribution studies in animals indicate that the highest concentrations of the drug are in liver, kidney, and spleen. Although the extent to which fludarabine and/or its metabolites distribute into the CNS in humans has not been determined to date, severe neurologic toxicity (eg,, blindness, coma) has been reported in patients receiving the drug, particularly in high dosages. There is evidence from animal studies that fludarabine distributes into the CNS and that a toxic metabolite (2-fluoroadenine, possibly formed by bacteria in the GI tract), can be absorbed systematically via enterohepatic circulation and distributed into CSF. According to *in vitro* data, about 19-29% of fludarabine is bound to plasma proteins.

Following IV administration, fludarabine phosphate is dephosphorylated rapidly to fludarabine. Plasma concentrations of fludarabine decline in a linear, dose-independent manner. The elimination profile of fludarabine also has been reported to be either biphasic or triphasic; however, reported terminal elimination half-lives have been similar. In adult cancer patients receiving fludarabine 25 mg/m^2 as a 30-minute IV infusion daily for 5 days, a terminal half-life of about 20 hours was reported. In a limited number of pediatric patients, the plasma concentration profile of fludarabine exhibited both monoexponential and biexponential decay, with a mean $t_{1/2}$ of 10.5 hours in patients with monoexponential elimination and a $t_{1/2}$ of 1.2-1.4 and 12.4-19 hours, respectively, in patients with biexponential elimination.

Renal clearance accounts for about 40% of the total body clearance of fludarabine. Renal elimination appears to become more important at high dosages of the drug. The dose of fludarabine needs to be adjusted in patients with moderate renal impairment.

The use of fludarabine in combination with pentostatin is not recommended due to the risk of severe pulmonary toxicity.

Toxicity:

	Common Happens to 21-100 subjects out of every 100	**Occasional** Happens to 5-20 subjects out of every 100	**Rare** Happens to < 5 subjects out of every 100
Immediate: Within 1-2 days of receiving drug	Fever, fatigue, weakness, pain, nausea, vomiting, anorexia, cough, dyspnea	Edema including peripheral edema, chills, rash, diarrhea, rhinitis, diaphoresis, malaise, abdominal pain, headache, back pain, myalgia, stomatitis, flu-like syndrome	Anaphylaxis, tumor lysis syndrome, dehydration*
Prompt: Within 2-3 weeks, prior to next course	Myelosuppression (anemia, neutropenia, thrombocytopenia), infection (urinary tract infection, herpes simplex infection, pneumonia, upper respiratory)	Weight loss, gastrointestinal bleeding, hemoptysis, paresthesia, allergic pneumonitis, bronchitis, pharyngitis, visual disturbance, hearing loss, hyperglycemia	Sinusitis, dysuria, opportunistic infections and reactivation of latent viral infections like Epstein-Barr virus (EBV), herpes zoster and John Cunningham (JC) virus (progressive multifocal leukoencephalopathy [PML])[L], EBV associated lymphoproliferative disorder, panycytopenia (can be prolonged), pulmonary hypersensitivity[a] (dyspnea, cough, hypoxia, interstitial pulmonary infiltrate), pulmonary toxicity (acute respiratory distress syndrome [ARDS], pulmonary fibrosis, pulmonary hemorrhage, respiratory distress, respiratory failure), pericardial effusion, skin toxicity (erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, pemphigus), liver failure, renal failure, hemorrhage, transfusion-associated graft-versus-host disease has occurred following transfusion of nonirradiated blood products, phlebitis*, sleep disorder*, cerebellar

			syndrome*, depression*, mentation impaired*, alopecia*, pruritus*, seborrhea*, esophagitis*, constipation*, mucositis*, dysphagia*, hesitancy*, cholelithiasis*, abnormal liver function tests *, osteoporosis*, arthralgia*, abnormal renal function test*, proteinuria*, epistaxis*, hemorrhagic cystitis*, eosinophilia*
Delayed: Any time later during therapy, excluding the above conditions			Neurotoxicity (increased with high doses): seizures, agitation, confusion, weakness, visual disturbances, optic neuritis, optic neuropathy, photophobia, blindness, paralysis, coma, death, peripheral neuropathy[a]); autoimmune phenomena: thrombocytopenia/thrombocyt openic purpura (ITP), Evans syndrome, hemolytic anemia, acquired hemophilia
Late: Any time after completion of treatment			Myelodysplastic syndrome/acute myeloid leukemia (mainly associated with prior or concomitant or subsequent treatment with other anticancer treatments), skin cancer (new onset or exacerbation)
Unknown Frequency and Timing:	**Pregnancy Category D** Based on its mechanism of action, fludarabine phosphate can cause fetal harm when administered to a pregnant woman. Fludarabine phosphate was embryolethal and teratogenic in both rats and rabbits.		

(L) Toxicity may also occur later.

* Reported in ≤ 3% of subjects. Since these are not considered life threatening they are not included in the consent.

[a] These effects were not reported in children.

Formulation and Stability:

Fludarabine phosphate injection is available as sterile lyophilized powder and in solution. Each single-dose vial of powder contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. After reconstitution, the pH range for the final product is 7.2-8.2. The single-dose solution vial contains 25 mg/mL, 2 mL of fludarabine phosphate. It may contain mannitol and is preservative-free.

Fludarabine phosphate vials should be stored refrigerated at 2-8ºC (36-46ºF).

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

Fludarabine phosphate powder should be reconstituted with 2 mL of Sterile Water for Injection. The solid cake should fully dissolve in 15 seconds or less. The resulting concentration is 25 mg/mL. When reconstituted to a final concentration of 25 mg/mL, the drug is stable for at least 16 days at room temperature and normal light conditions. The manufacturer recommends that the solution be used within 8 hours after reconstitution.

Prior to administration, fludarabine 25 mg/mL solution or the reconstituted 25 mg/mL solution should be further diluted in 100 mL or 125 mL of D5W or NS. Concentrations of 0.25 to 1 mg/mL have been used in clinical trials. When diluted to a final concentration of 1 mg/mL, fludarabine is stable for at least 16 days at room temperature and normal light conditions. The manufacturer recommends that the diluted solution be used within 8 hours after preparation. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

Supplier: Commercially available from various manufacturers. See package insert for further 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	Follow-Up	Relapse
Physical Exam	X		X
Ht, Wt, BSA	X		
Performance status	X		
CBC, differential, platelets	X		
Electrolytes (Ca^{++}, Mg^{++}, K^{+}), and creatinine	X		
LP-CSF for cell count, cytospin	X		X
Flow cytometry with minimal residual disease (MRD) determination[a]	X[b]		X
Echocardiogram or MUGA, and EKG	X	X[#]	X
BMA/clot section (biopsy if aspirate unsuccessful) for morphology[b]	X		X
BMA cytogenetics & FISH			X[@]

a Perform per institutional standard
b Need not be repeated if performed at the end of Cycle 2.
[#] Echocardiogram or MUGA should be repeated annually from the start of therapy; the EKG should be repeated if clinically indicated.
[@] See Section 13.2.6.
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 Research Studies for which Patient Participation is Optional

The following table lists samples to be obtained from patients who have provided consent for optional correlative studies.

Study	Baseline	During Cycle 1	Sample Type/Tube
Biomarkers of cardiac injury (Cardiac troponin-T, NT-proBNP, hs-CRP)^ See Section 14.2.1	X	• Baseline (before CPX-351 infusion) • Day 8* • Day 15* • Day 22* • Day 28-30 (one sample in this timeframe)	3-5 mL blood in a gold top tube (serum separator tube)
MicroRNA^ See Section 14.2.2	X	• Day 1, before CPX-351 infusion • Day 5, 6 hours (± 1 hr) after the end of the infusion of the 3rd dose of CPX-351 • Day 8* • Day 15* • Day 22* • Day 28-30 (one sample in this timeframe)	5-10 mL peripheral blood in a purple/lavender top (EDTA) tube
Whole exome sequencing^ See Section 14.2.3	-----	• Day 28-30 (one sample in this timeframe)	2-4 mL peripheral blood in a purple top (EDTA) tube
Pharmacokinetic sampling See Section 14.1	-----	• Day 1: 30 minutes after end of infusion; Day 5 prior to infusion, 30 minutes after end of infusion, 6 ± 2 hours from start of infusion; Day 8, 72 ± 24 hours from start of Day 5 infusion	2 mL blood in a tube provided in PK sampling kit (See protocol web page for PK manual)

*Samples for Biomarkers of cardiac injury and MicroRNA may be drawn up to 24 hours prior to the time point and up to 24 hours after the time point.
^ See Section 14.2.4 for shipping details.

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Removal from Protocol Therapy

a) Relapsed disease (see Section 10.2).
b) Treatment failure (see Section 10.2).
c) Unacceptable toxicity due to protocol therapy (see Section 5.0).
d) Refusal of further protocol therapy by patient/parent/guardian.
e) Completion of planned therapy.
f) Physician determines it is in patient's best interest.
g) Development of a second malignancy.
h) Pregnancy.
i) Repeat eligibility studies (if required) are outside the parameters required for eligibility (see Section 3.2).

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 (eg, 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

To complete this study, up to 50 evaluable patients (12 for the Dose Finding portion, 38 for the Efficacy portion) are required. Allowing for 10% of patients being inevaluable, an enrollment of up to 56 patients is required.

Based on the accrual to the Dose Finding portion of AAML0523 at a similarly restricted number of sites, it is estimated that 1.5 patients per month will be enrolled on the Dose Finding portion of this study. We estimate that it will require up to 12 months to complete safety and DLT assessments for Cycle 1 (CPX-351), allowing 2 months between the first dose of study drug and DLT assessment. Based on accrual to recent COG studies for patients with relapsed/refractory AML (2.2 patients per month on AAML0523 and 1.8 patients per month on AAML07P1 Arm B, run simultaneously), it is estimated that 5 patients overall will accrue to this study each month for the Efficacy phase. To evaluate the efficacy of CPX-351, up to 38 evaluable patients will be enrolled. Therefore, we expect accrual for the Efficacy portion study to take approximately 11 months allowing for 2 months between stages 1 and 2 to assess response for the last patient treated on stage 1. Allowing for DLT and efficacy assessment, we anticipate that 56 patients could be enrolled in approximately 23 months.

As described above, we anticipate that 38 patients will be enrolled on the Efficacy phase of the study. In order to more precisely estimate the PK parameters of CPX-351 in children, we will extend the PK collection of this study to continue during the Efficacy phase. In reviewing the experience on COG AALL07P1, where optional PK studies were collected in a similar manner in the Efficacy phase of that study, samples from 25 of 37 patients (68%) were successfully collected. Therefore, we would expect approximately 25 patients to participate in PK collection during the Efficacy phase of AAML1421. In parallel, Jazz Pharmaceuticals also has collected PK samples on 16 patients participating in the Cincinnati Phase I/II single-institution trial (11 at 100 units/m^2/dose, 5 at 134 units/m^2/dose). They anticipate 5 more patients will accrue to that study and provide additional samples

A population PK analysis will be conducted by Jazz Pharmaceuticals using PK data from this study, the aforementioned pediatric study in Cincinnati, and future Phase 3 pediatric study to better estimate CPX-351 PK parameters in children. We will recruit children with all age groups, with an effort to enroll as many young children as possible. We anticipate the PK collected on this study will make significant strides in fulfilling their requirements to more precisely estimate the PK parameters of CPX-351 in children.

9.2 Study Design

9.2.1 Dose-Finding Phase

The phase 1 Dose-Finding portion of the study was completed in 12/2016.

The Dose-Finding Phase will be conducted using a modified rolling 6 design. Two to six patients can be concurrently enrolled onto a dose level, dependent upon (1) the number of patients enrolled at the current dose level, (2) the number of patients who have experienced DLT at the current dose level, and (3) the number of patients entered but with tolerability data pending at the current dose level. Accrual is suspended when a cohort of six has enrolled or when the study endpoints have been met. Dose level assignment is based on the number of participants currently enrolled in the cohort, the number of DLTs observed, and the number of participants at risk for developing a DLT (ie, participants enrolled but who are not yet assessable for toxicity). For example, when three participants are enrolled onto a dose cohort, if toxicity data is available for all three and there are no DLTs, the recommended dose has been reached. If data is not yet available for one or more of the first three participants, or if one DLT has been observed, the new participant is entered at the same dose level. Lastly, if two or more DLTs have been observed, the dose level is de-escalated. This process is repeated for participants five and six. In place of suspending accrual after every three participants, accrual is only suspended when a cohort of six is filled. When participants are inevaluable for toxicity, they are replaced with the next available participant if escalation or de-escalation rules have not been fulfilled at the time the next available participant is enrolled onto the study.

The following table provides the decision rules for enrolling a patient at (i) the current dose level (ii) at an escalated dose level, (iii) at a de-escalated dose level, or whether the study is suspended to accrual:

# Pts Enrolled	# Pts with DLT	# Pts without DLT	# Pts with Data Pending	*Decision*
2	0 or 1	0, 1 or 2	0, 1 or 2	Same dose level
2	2	0	0	De-escalate*
3	0	0, 1 or 2	1, 2 or 3	Same dose level
3	1	0, 1 or 2	0, 1 or 2	Same dose level
3	0	3	0	Escalate**
3	2	0 or 1	0 or 1	De-escalate*
4	0	0, 1, 2 or 3	1, 2, 3 or 4	Same dose level
4	1	0, 1, 2 or 3	0, 1, 2 or 3	Same dose level
4	0	4	0	Escalate**
4	2	0, 1 or 2	0, 1 or 2	De-escalate*
5	0	0, 1, 2, 3 or 4	1, 2, 3, 4 or 5	Same dose level
5	1	0, 1, 2, 3 or 4	0, 1, 2, 3 or 4	Same dose level
5	0	5	0	Escalate**
5	2	0, 1, 2 or 3	0, 1, 2 or 3	De-escalate*
6	0	0, 1, 2, 3, or 4	2, 3, 4, 5 or 6	Suspend
6	1	0, 1, 2, 3 or 4	0, 1, 2, 3 or 4	Suspend
6	0 or 1	5 or 6	0 or 1	Escalate**
6	2	0, 1, 2, 3 or 4	0, 1, 2, 3 or 4	De-escalate*

* If six patients already entered at next lower dose level, the MTD has been defined.
** If final dose level has been reached, the recommended dose has been reached.

9.2.2 Efficacy Phase

The Efficacy Phase is a single arm two-stage design. The following optimal Simon two-stage design will be used to test the null hypothesis that the overall response rate is ≤ 40% versus the alternative hypothesis that the response rate is ≥ 60%.

	Cumulative Number of Responses	Decision
Stage 1: Enter 12 patients	5 or fewer	Terminate the trial because the therapy is ineffective
	6-12	Inconclusive result, continue trial (proceed to Stage 2)
Stage 2: Enter 26 additional patients	18 or fewer	Conclude therapy is ineffective
	19-38	Conclude therapy is effective

If the therapy is associated with a 40% response rate (CR+CRp) after up to 2 cycles of therapy, the therapy will be identified as effective with probability 0.10. If the therapy is associated with a 60% response rate, the therapy will be identified as effective with probability 0.80.

Results from the CCG 2951 study showed that a combination of mitoxantrone/cytarabine (MA) used for induction in pediatric patients with refractory/relapsed AML achieved a marrow remission rate of 76% and an overall response (CR + CRp) rate (criteria similar to

those used in this study) of 58%.[73] Response was assessed after one cycle of MA, however, these children received significantly less intensive treatment for their *de novo* AML than those who will relapse after receiving current *de novo* regimens. In addition to fewer high dose cytarabine consolidation cycles, they received a lower cumulative anthracycline exposure compared with patients who will relapse following AAML0531 and AAML1031 (≈ 290 mg/m^2 vs. ≈ 440 mg/m^2). Thus, a response rate lower than 58% would be expected after one cycle for those who now receive anthracycline-containing reinduction regimens. However, this response was measured after one cycle of protocol therapy.

More recently, COG AAML0523 tested the combination of clofarabine and cytarabine in children with AML in first relapse. In this trial, response was defined as best response (CR + CRp) after up to 2 cycles. Of 48 patients, the overall response rate was 48%.[74] Of 23 responders, 11 (48%) were SD after Cycle 1, then achieved CR or CRp after Cycle 2. This supports the importance of receiving two cycles of reinduction therapy in order to determine the optimal response to therapy. Importantly, only 1/11 (9%) of patients who had > 20% blasts after Cycle 1 achieved CR or CRp in Cycle 2. This is consistent with data from the FLAG vs. FLAG/DNX study published by Kaspers et al (Appendix Fig A1, online only) demonstrating dismal outcomes and low second remission rates in patients with > 20% blasts after Cycle 1.[2] However, Partial Remission is (PR) is a standard response definition for COG leukemia studies and includes blasts percentages between 5 and 25%. Therefore, patients who have > 25% blasts after Cycle 1 will be considered non-responders and removed from protocol therapy in order to be able to pursue other options.

Kaspers et al. randomized patients to receive either FLAG or FLAG/DNX in children with AML in first relapse.[2,75] In this trial, the primary endpoint was Day 28 bone marrow status. That is, patients who had < 20% blasts at Day 28 were determined to have a good early response to therapy. There was not a determination of CR rates after the first cycle of FLAG or FLAG/DNX. The secondary endpoint of the study was CR rate after two cycles of chemotherapy (FLAG/DNX then FLAG vs. FLAG then FLAG). As mentioned previously, the CR rate (ANC > 1000, platelets > 50K) after two cycles was 69% with FLAG/DNX, and 59% with FLAG (p=0•07).[29] However, their CR definition was different in that study (ANC > 1000, platelets > 50K), than what is used for COG relapsed AML studies (ANC > 1000, platelets > 100K).

9.3 Methods of Analysis

The response rate (CR+CRp), best response after up to two cycles, will be determined using the approach of Jung and Kim.[76] The corresponding 90% confidence interval will be calculated using the approach of Koyama and Chen.[77] The response rate will be calculated as the ratio of the number of patients who demonstrate response after up to two cycles of therapy divided by the number of patients evaluable for response. Descriptive statistics will be used to summarize the proportion of patients experiencing ≥ Grade 3 non-hematologic toxicities, cardiac toxicities, and infections, summarize length of hospitalization time, and estimate time to bone marrow and peripheral blood cell count recovery. The impact of the timing of relapse on outcomes will be investigated in this study, but these analyses will be exploratory.

Pharmacokinetic properties of CPX-351

Steady state pharmacokinetics of CPX-351 will be obtained in all patients treated in the Dose Finding phase of the study. Descriptive statistics will be used to summarize the systemic exposure of CPX-351 (Clearance (CL), Volume of Distribution (Vd), Tmax½,

Area Under the Curve (AUC)). These parameters will be summarized with summary statistics, including means, medians, ranges, and standard deviations (if numbers and distribution permit).

PK samples will also be collected during the efficacy portion of the study following the sparse sampling scheme outlined in Section14.1. Participation in these PK samples will be optional for patients treated on the efficacy phase. Descriptive statistics will be used to summarize plasma concentrations of total cytarabine and daunorubicin at each time point.

Monitoring of Toxic Death

Dose modification or termination will be considered if 3 or more of the first 20 patients in the Efficacy phase experience a toxic mortality (TM), defined as death within 30 days of the last dose of CPX-351 and not associated with disease progression, or if 5 or more of the 38 patients in the Efficacy phase experience a TM. Dose modification or termination will be considered with probability 0.05 if the true TM rate is 5% and with probability 0.79 if the true TM rate is 20%.

Monitoring of Cardiac Toxicity

Dose modification or termination will be considered if 3 or more of the first 20 patients in the Efficacy phase experience a severe cardiac toxicity, defined as Grade 4 or higher ejection fraction probably or definitely related to CPX-351 during the first cycle of chemotherapy, or if 5 or more of the 38 patients in the Efficacy phase experience a severe cardiac toxicity. Note: Grade 4 or higher ejection fraction related to sepsis will not be considered a severe cardiac toxicity for this monitoring rule. Dose modification or termination will be considered with probability 0.05 if the true rate is 5% and with probability 0.79 if the true rate is 20%.

9.4 Evaluability for Response

A patient will be considered evaluable for response if: (1) the patient meets the eligibility criteria for the Efficacy Phase; (2) the patient receives at least one dose of CPX-351 at the RP2D; and (3) the patient is under follow-up for a sufficient period to evaluate the disease at the end of the Cycle 1 or meets the definition of Treatment Failure in Section 10.2. Patients who demonstrate a CR or CRp as delineated in Section 10.2 will be considered to have experienced a response. All other evaluable patients will be considered non-responders. A patient who dies as a result of toxicity during Cycle 1 after receiving all or part of protocol therapy will be considered a nonresponder. In addition, the proportion of patients with CRi will be estimated.

9.5 Evaluability for Toxicity

Any patient who receives at least one dose of study drug will be considered evaluable for toxicity.

9.6 Correlative Biology Studies

9.6.1 Analytic Plan for Study Objective 1.3.1: Response in Biomarkers of Cardiac Injury to a Single Cycle of CPX-351

The incidence, timing and magnitude of elevations in troponin, NT-BNP, hsCRP will be described. Spearman correlation coefficients will be used to correlate the

post-treatment values of troponin, NT-BNP, and hsCRP with previous cumulative anthracycline dose prior to CPX-351, change in ejection fraction between pre- and post-CPX-351 baseline assessed by echocardiogram before and after Cycle 1, and change in global longitudinal strain (GLS). A sample size of 30 patients will provide 80% power (α=0.05) to detect a minimum correlation of 0.49. Additionally, two-sample t-tests will be used to compare mean post-treatment values of troponin, NT-BNP, and hsCRP for those with an ejection fraction decrease of less than 10% from baseline versus those 10% or greater from baseline. These descriptive analyses will provide critical preliminary data on which to base the timing and power calculations for subsequent phases of CPX-351 investigations in order to allow more definitive assessment of its cardiotoxic effects relative to other anthracycline regimens.

9.6.2 Analytic Plan for Study Objective 1.3.2: Effect of CPX-351 on Novel Biochemical and Imaging Markers of Cardiotoxicity, Including Plasma miRNAs and Myocardial Deformation

Quantification of change in GLS from baseline

A paired t-test will be used to compare the mean global longitudinal strain (GLS) between the pre-treatment (at relapse) baseline and post-Cycle 1 echocardiogram. A sample size of 30 patients will provide 80% power (with 2 sided α=0.05) to detect a 1.6% reduction in GLS, assuming a GLS standard deviation of 3%.[78]

Quantification of miRNA change from baseline

Candidate plasma miRNAs will be quantified at baseline (prior to relapse therapy) and Day 5 (6 hours from the 3rd dose of CPX-351), Day 6, and once Day 28-30 post-CPX-351 using TaqMan microRNA Assays. Plasma miR-29b and -499 fold change from pre-treatment baseline will be determined at each post-treatment time point using the DDCt method. A sample size of 30 patients will provide 80% power (α=0.05) to detect a Ct difference from baseline of 1.7 for miR-29b and 1.6 for miR-499, assuming ΔCt variability of 3.2 and 3 standard deviations, respectively, for miR-29b and miR-499 (based on K. Leger pilot data).

Correlation of GLS and miRNA

The relationship between 6-hour miR-29b and -499 expression and change in LV GLS between the pre-cycle and end of Cycle 1 echocardiograms will be determined by calculating a Spearman correlation coefficient. The a priori focus on the 6-hour time point as the primary independent variable is based on preliminary data revealing that differences in miRNA expression were greatest 6 hours following anthracycline therapy. However, a linear mixed effects model incorporating the additional time points will be used to explore the longitudinal relationship with each time point as a predictor variable for the measures of early cardiotoxicity. A sample size of 30 patients will provide 80% power (α=0.05) to detect a minimum correlation of 0.49. Power calculations were generated in PASS13.

9.6.3 Analytic Plan for Study Objective 1.3.3: The Role of Rare Coding Variants as Risk Factors for Anthracycline-Induced Cardiomyopathy

Our computational approach can be summarized as a two-step process with alignment of the sequence data using a commercial aligner followed by subsequent statistical analyses from post-alignment data. Sequence analysis will follow GATK's best

practices in which duplicate variants from the sequencing process are removed (https://www.broadinstitute.org/gatk/guide/best-practices?bpm=DNAseq). In addition, local realignment is performed on mismatching bases around indels, a known artifact often produced by the initial mapping algorithm. Quality scores on each individual mapped base are also recalibrated to account for expected errors emitted by the sequencing machine. Finally, variations on genomic sites are identified relative to a reference genome and scores are assigned to each discovered variant to minimize both false positives and negatives. Custom Amazon Machine Image with all necessary tools and reference datasets in the Docker format will be developed for deployment in the Amazon cloud infrastructure. StarCluster will be used to manage the creation of all Amazon EC2 regular instances. Requested data as well as all intermediaries and final processed files are stored in Amazon S3 buckets with protected access. Finally, processed results will be downloaded to internal servers at the Children's Hospital of Philadelphia (CHOP) for visualization.

9.7 Gender and Minority Accrual Estimates

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

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	1	4	0	0	**5**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	7	1	0	0	**8**
White	23	15	1	4	**43**
More Than One Race	0	0	0	0	**0**
Total	**31**	**20**	**1**	**4**	**56**

This distribution was derived from AAML07P1.

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 (ie, v4.02 and all subsequent iterations prior to version 5.0).

10.2 Response Criteria

Response criteria will employ revised AML International Working Group Criteria,[79] and include:

Overall Response (OR): OR rate is defined as the sum of the number of patients with complete remission (CR) plus those with complete remission in the absence of total platelet recovery (CRp) divided by the total number of evaluable enrolled patients.

Complete Remission (CR): Attainment of an M1 bone marrow (< 5% blasts) with no evidence of circulating blasts or extramedullary disease and with recovery of peripheral blood counts (ANC ≥ 1000/μL and platelet count ≥ 100,000/μL). Flow cytometry may also be useful to distinguish between leukemia and a regenerating bone marrow. There is no requirement for bone marrow cellularity.

CR With Partial Recovery of Platelet Count (CRp): Attainment of an M1 bone marrow (< 5% blasts) and no evidence of circulating blasts or extramedullary disease and with recovery of ANC ≥ 1000/μL and platelet transfusion independence (defined as: no platelet transfusions x 1 week).

Complete Remission with Incomplete Blood Count Recovery (CRi): Attainment of an M1 bone marrow (< 5% blasts) and no evidence of circulating blasts or extramedullary disease and with ANC < 1000/μL or platelet count < 100,000/μL without platelet transfusion independence (defined as: no platelet transfusions x 1 week).

Partial Response (PR): M2 bone marrow (5% to 25% blasts) and at least a 50% decrease in bone marrow blast percent from baseline. Bone marrow must have adequate cellularity (eg, ≥ 10%, if a biopsy is performed) to determine response. PR status will not be included in calculation of response to the regimen. A repeat bone marrow aspiration within 14 days may be required to distinguish between a PR and increased blasts caused by bone marrow regeneration, and is left at the discretion of the investigator.

Treatment Failure (TF):
In the Efficacy phase, if after Cycle 1 (CPX-351) a patient has a hypoplastic bone marrow for ≥ 60 days and failure to recover a peripheral ANC > 500/μL and a non-transfusion dependent platelet count > 20,000/μL not due to malignant infiltration or severe infection

(defined as ≥ Grade 3), they will be considered a treatment failure and will go off protocol therapy.

The definition of treatment failure includes:

a. An increase in the extent of bone marrow infiltration by leukemic cells (absolute increase of ≥ 20% blasts) OR
b. Development of extramedullary disease (EMD) OR
c. M2 marrow that does not qualify for PR status OR
d. An M1 marrow with circulating blasts OR
e. > 25% blasts in the bone marrow after Cycle 1 of therapy.

Relapse: Morphologic relapse after CR/CRp/CRi is defined as a reappearance of leukemic blasts in the peripheral blood or ≥ 5% blasts in the bone marrow not attributable to any other cause (eg, bone marrow regeneration after therapy). In the setting of recent treatment, if there are no circulating blasts and the bone marrow contains 5% to 20% blasts, a repeat bone marrow performed at least a week later is necessary to distinguish relapse from bone marrow regeneration. Should flow cytometric or molecular analyses suggest relapse (by the reappearance of a similar immunophenotype or mutation to the original leukemia) in the presence of < 5% blasts, or ≥ 5% blasts in a regenerating marrow, a repeat bone marrow(s) performed at least a week later is necessary to confirm relapse by morphologic methods. In such instances the date of recurrence is defined as the first date that more than 5% blasts were observed in the marrow. The reappearance or development of cytologically proven extramedullary disease also indicates relapse. Molecular and/or genetic relapse is characterized by reappearance of a cytogenetics or molecular abnormality.

Unevaluable: Aplastic or severely hypocellular marrow. In this instance, marrow evaluation should be repeated weekly until response determination can be made through at least Day 60.

Bone Marrow Classification:

M1 is < 5% blasts
M2 is 5 to 25% blasts
M3 is > 25% blasts

11.0 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Purpose

Adverse event 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 which 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, 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 Specific Examples 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, COG, or industry sponsor 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: A disorder characterized by cessation of life during the first 28 days of life.
- 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.

All secondary malignancies that occur following treatment need to 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.

11.4.6 Pregnancy, Pregnancy Loss, and Death Neonatal

NOTE: When submitting CTEP-AERS reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the Pregnancy Information Form, available at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportForm.pdf, needs to be completed and faxed along with any additional medical information to 301-230-0159. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the CTEP-AERS report.

11.4.6.1 **Pregnancy**

Patients who become pregnant on study risk intrauterine exposure of the fetus to agents that may be teratogenic. For this reason, pregnancy needs to be reported in an expedited manner via CTEP-AERS as **Grade 3 "*Pregnancy, puerperium and perinatal conditions - Other (pregnancy)*"** under the ***Pregnancy, puerperium and perinatal conditions*** SOC.

Pregnancy needs to be followed **until the outcome is known**. If the baby is born with a birth defect or anomaly, then a second CTEP-AERS report is required.

11.4.6.2 **Pregnancy Loss (Fetal Death)**

Pregnancy loss is defined in CTCAE as "*Death in utero*". Any pregnancy loss needs to be reported expeditiously, as **Grade 4 "*Pregnancy loss, puerperium and perinatal conditions*" SOC.** Do NOT report a pregnancy loss as a Grade 5 event since CTEP-AERS recognizes any Grade 5 event as a patient death.

11.4.6.3 **Death Neonatal**

Neonatal death, defined in CTCAE as *"Newborn death occurring during the first 28 days after birth*" should be reported expeditiously, as **Grade**

4 "*Death neonatal*" under the "*General disorders and administration*" SOC when the death is the result of a patient pregnancy or pregnancy in partners of men on study. Do NOT report a neonatal death resulting from a patient pregnancy or pregnancy in partners of men on study as a Grade 5 event since CTEP-AERS recognizes any Grade 5 event as a patient death.

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 (eg, 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

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 reporting methods described below are specific for clinical trials evaluating agents for which the IND is held by COG, an investigator, or a pharmaceutical company. It is important to note that these procedures differ slightly from those used for reporting AEs for clinical trials for which CTEP holds the IND.

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 beginning April 1, 2018. 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 must be submitted electronically via CTEP-AERS at: https://eapps-ctep.nci.nih.gov/ctepaers

- 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 requires expedited reporting **within 24 hours** via e-mail to the COG CTEP-AERS Coordinator and Study Chair.
- Any death occurring greater than 30 days of the last dose with an attribution of possible, probable, or definite to an agent/intervention requires expedited reporting **within 24 hours** via e-mail to the COG CTEP-AERS Coordinator and Study Chair.

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.

Fax or email supporting documentation **for AEs related to investigational agents** to COG: 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 (COG) **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.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">7 Calendar Days</td><td rowspan="2">24-Hour Notification
5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not Required</td><td>7 Calendar Days</td></tr>
</table>

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 CTEP-AERS reportable events and Grade 3 and higher non-hematologic Adverse Events and all grades of the following cardiac events: prolonged QTc interval, ventricular arrhythmia, and left ventricular systolic dysfunction.

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). Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31. This 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 (eg, 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.

13.0 CYTOGENETIC ANALYSIS GUIDELINES AND REQUIREMENTS

13.1 Cytogenetic Analysis Overview

Specimens for cytogenetic analysis are required, and must be obtained prior to therapy initiation. It is strongly recommended that all patients enrolled on AAML1421 have a cytogenetics study performed by a COG-approved laboratory at the time of diagnosis and subsequent relapse, should it occur.

The institutional CRA must inform the cytogenetics laboratory that the patient has been enrolled in a COG Myeloid study and that the cytogenetics/FISH data must be submitted

within 2 weeks after enrollment on the AAML1421 protocol. See Appendix II for cytogenetics procedures and for the Sample Authorization Form for reflexive FISH testing.

13.2 Specimen Collection and Submission

13.2.1
It is strongly recommended that specimens for cytogenetic analysis be sent to a COG-approved institutional cytogenetics laboratory. If a COG institution does not have a COG-approved laboratory, the institution may send the samples for karyotyping/FISH studies to any COG-approved cytogenetics laboratory on a fee-for-service basis. Prior arrangements for performing cytogenetics and/or FISH studies should be made with the laboratory. An authorization form for the reflexive FISH testing signed and dated (by the physician or designees just as with any order) sent to the cytogenetics laboratory with the sample will simplify and enhance the ability to obtain FISH testing and results.

13.2.2
A minimum of 2 mL (optimal volume, 3 mL) of fresh whole bone marrow aspirated through a needle into a syringe containing sodium heparin (preservative-free is preferable) is recommended in all cases. A first or second draw, or a draw from a repositioned needle, is best to ensure a sufficient number of leukemic cells in the aspirate. The specimens should be kept at **room temperature** and transported to the institutional cytogenetics laboratory as quickly as possible (**always within 24 hours of collection**). If shipping is done by overnight courier to an approved laboratory, that laboratory should be contacted to obtain instructions on transport. Some laboratories request specimens to be transferred in a 15-mL conical tube filled with RPMI-1640 and 15% heat-inactivated fetal calf serum. Specimens should be kept at ambient temperature.

13.2.3
If bone marrow cannot be aspirated, a bone marrow core biopsy should be submitted.

13.2.4
Peripheral blood (3-5 mL) collected in sodium heparin should be submitted as a back-up to the bone marrow when the marrow sample is suboptimal or unobtainable, if more than 20% circulating blasts are identified, or when a constitutional abnormality (eg, trisomy 21) is a possibility. For documenting a constitutional abnormality, a PHA stimulated blood study should be performed.

For patients with myeloid sarcoma:
In addition to the requirements described above, if touch preps are able to be prepared from the tumor, the institutional pathologist should distribute/triage order(s) for FISH to be performed accordingly (eg, MLL, RUNX1T1-RUNX1, CBFB).

13.2.5
Results of these studies should be submitted electronically to the corresponding coordinator of the Myeloid COG cytogenetics committee for central review (see below).

13.2.6
Requirements for Data Submission
The following are required for each case: a completed generic COG Cytogenetics/FISH Reporting Form (found on the COG Member website) and 2 original karyotypes (different

cells), with corresponding full-size metaphase spreads of each abnormal clone or 2 karyotypes of normal cells with corresponding full-size metaphase spreads in the case of normal cytogenetic analysis. This information must be sent to the appropriate coordinating cytogeneticist **within 2 weeks after enrollment on the AAML1421 protocol and within 1 month of subsequent relapse, should it occur** (see Appendix II). Reports must be sent electronically (preferred as PowerPoint presentation). If the laboratory is unable to send an electronic file with the documentation, please contact the COG cytogenetics coordinator for your area for advice. Reporting forms must be filled out for all cases, whether or not the cytogenetic analyses were successful. A separate form is required for each specimen (ie, bone marrow and blood) analyzed in each case. Published data on FISH screening report an overall incidence of occult aberrations in AML with normal karyotypes between 3% to 10%.[80-82] Thus, for all cases with only normal karyotypes FISH must be performed to evaluate for inv(16)/t(16;16), t(8;21), t(15;17) and 11q23. However, if the sample did not yield sufficient quantity of metaphases for analysis, -7 and -5/5q- should also be performed in addition to the inv(16)/t(16;16), t(8;21), t(15;17) and 11q23. Published data indicate that 10%-25% of patients who have one of these recurring translocations may have a concomitant deletion encompassing a region adjacent to the breakpoint on one of the involved derivative chromosomes. Such deletions may portend a less favorable prognosis.[83-85] Thus, for all cases in which a recurring chromosomal abnormality [t(8;21), inv(16)/t(16;16), 11q23] is identified, it is recommended that FISH be performed with probes specific to that rearrangement, to evaluate any variant pattern that might include a deletion associated with a gene fusion. FISH forms should also be completed and sent to the coordinating cytogeneticist with images documenting the abnormal or normal FISH patterns. If the case has a suspected 12p it is recommended to perform FISH using ETV6 probe. If the laboratory is not able to perform FISH, contact the COG cytogenetic coordinators for advice. Any discrepancies in interpretation between cytogenetics/FISH results from the laboratory and the coordinator will be discussed between the coordinators, with consult of additional cytogenetics committee members as necessary. The COG cytogenetics committee decision will prevail.

Note: Please provide karyotype or Final Chromosome Report findings at initial time of diagnosis if available.

Cytogenetic Coordinators

Please send above materials by e-mail (preferably as a PowerPoint file) to the following COG Cytogenetics Laboratories.

WEST OF MISSISSIPPI RIVER
(INCLUDE MINNESOTA AND WISCONSIN), AUSTRALIA, NEW ZEALAND, WESTERN CANADA
SEND TO:
Betsy Hirsch, Ph.D.
Telephone: (612) 273-4952/3171
E-mail: hirsc003@umn.edu

EAST OF MISSISSIPPI RIVER
(EXCLUDE MINNESOTA AND WISCONSIN), EUROPE, EAST CANADA
SEND TO:
Susana C. Raimondi, Ph.D.
Telephone: (901) 595-3537/3536
E-mail: susana.raimondi@stjude.org

Recommendations for Case Analysis by Institutional, COG-Approved Cytogenetics Laboratories (see Appendix II.)

14.0 SPECIAL STUDIES SPECIMEN REQUIREMENTS

14.1 CPX-351 Pharmacokinetics

The patients participating in the Dose-Finding Phase of the trial are required to provide serial blood samples for the assessment of CPX-351 serum concentrations. Patients participating in the Efficacy Portion of the protocol may participate in the pharmacokinetics study as an optional correlative biology study. Blood samples for the Efficacy portion can be collected during Cycle 1 with a sparse sampling scheme as described below in Section 14.1.1.

All material necessary for collection and shipping of PK specimens will be provided in a kit sent by Celator Pharmaceuticals Inc. (A Jazz Pharmaceuticals Company). Once you have a potential patient identified, please order a PK kit. Please refer to the PK manual on the protocol page for kit ordering instructions.

[redacted]

14.1.1 Timing of Pharmacokinetic Sampling for the Efficacy Phase

Blood will be drawn during Cycle 1 only. A blood sample will be collected in a tube provided in the kit. Timing of pharmacokinetic sampling will be as follows:

Day	Time	Note
1	• 30 minutes after end of infusion	On Day 1 & 5, samples should be obtained through a central venous catheter lumen or peripheral IV that is NOT used for the CPX-351 infusion.
5	• Prior to infusion • 30 minutes after end of infusion • 6 ± 2 hours from start of infusion	
8	• 72 ± 24 hours from start of Day 5 infusion	

14.1.2 Sample Collection and Processing

- Collect approximately 2 mL of blood in a tube provided in the kit and immediately invert the tube gently 180° and back, 5 times.
- Place on crushed ice/water immediately after inverting the tube. Record the calendar date and exact time of the blood draw on the AAML1421 PK Specimen Shipping Form.
- Separate the plasma from the cells by centrifuging under refrigeration (2-8°C) for 10 minutes at 1500 x g.
- Using a disposable pipette, remove plasma (from the top) without disturbing the blood cells.
- Label two 3 mL cryovials provided in the kit as detailed below in Section 14.1.3. Pipette plasma aliquots into each cryovial. Leave approximately 20% of dead space in the cryovials to avoid cracking upon freezing.
- Place cryovials onto crushed ice/water until placed in the freezer or freeze immediately. Freeze at -70°C ± 10°C within 60 minutes of collection.
- Plasma will be kept at -70ºC ± 10°C until shipment to the bioanalytical laboratory on dry-ice.

14.1.3 Sample Labeling and Shipping

Each tube should have a freezer-safe label with the following information:

- AAML1421
- COG patient ID number
- COG NCI Site Number
- PK time point
- Date and start and stop time of CPX-351 infusion.
- Sampling date and time

NOTE: Samples should be batched and shipped once all specimens for a patient have been collected.

All shipments must be accompanied by a completed AAML1421 PK Specimen Shipping Form.

Package the shipping tubes to prevent breakage and contamination in Styrofoam boxes provided in the kit containing a generous supply of dry ice. If samples cannot be shipped overnight, use a carrier that is capable of replenishing the dry ice in the polyfoam box.

Upon shipment of samples, please email Sample.Receipt@inventivhealth.com, lavanya.arivelu@inventivhealth.com and Danielle.Morgen@inventivhealth.com or fax at 609-951-0080 notifying us that the samples have been shipped and provide the number of boxes in shipment and carrier tracking number if available. Advise if any special handling is required. Also, please attach associated electronic sample files to the email, if any.

Samples should be shipped on dry ice, via courier Priority Overnight to:

inVentiv Health Clinical Lab, Inc
Attn: Sample Receipt
301A College Road East
Princeton, NJ 08540
Ph: (609) 951-0005
Fx: (609) 951-0080

Note: The laboratory facilities are closed on Sundays and on certain holidays. To ensure a timely delivery, please schedule your shipment no later than 3 days before any holiday.

14.2 Exploratory studies

The exploratory studies detailed in this section are for research purposes only, and results will not be used to make clinical treatment decisions. Thus, results will not be obtained in real-time (but rather batched for retrospective review) and results will not be returned to the treating physician. The optional studies described below require patient consent.

Please note, if there is an inadequate amount of blood for all optional studies, the studies should be prioritized in the following order:

1. Biomarkers of cardiac injury (see Section 14.2.1)
2. MicroRNA study (see Section 14.2.2)
3. Whole exome sequencing (see Section 14.2.3)

Additionally, if patient/parent/responsible party consents, any specimens left over on this study after required tests are performed will be banked for future research studies at the COG Leukemia Biospecimen Bank.

14.2.1 Echocardiogram, Cardiac Troponin-T, N-terminal Probrain Natriuretic Peptide (NT-proBNP), and hs-CRP

The study of biomarkers for cardiotoxicity will be conducted under the oversight of Dr. Michael Absalon and Cincinnati Children's Medical Center.

For enquiries regarding this study, please contact:
Michael Absalon, MD, PhD,
Phone: (513) 636-2061
Email: michael.absalon@cchmc.org

14.2.1.1 Echocardiogram

Study participants will undergo standard 2D-echocardiograms prior to CPX-351 therapy, Day 28 (± 7 days) of Cycle 1 and must be before Cycle 2 begins, Day 28 (± 7 days) of Cycle 2 and must be before any subsequent therapy, then yearly from the start of therapy. Echocardiographic data will include LV systolic and diastolic function, LV shape and size. The echocardiograms are considered as standard of care and data will be collected via study case report forms.

14.2.1.2 Blood biomarkers

Sample collection and processing

Samples will be obtained from consenting patients at the following time points:

- Baseline (before CPX-351 infusion begins)
- Day 8*
- Day 15*
- Day 22*
- Day 28-30 of Cycle 1 (one sample in this timeframe)

*Samples for Biomarkers of cardiac injury and MicroRNA may be drawn up to 24 hours prior to the time point and up to 24 hours after the time point.

At each protocol specified time point, dispense 3 to 5 mL of blood into a labeled Serum Separator drawing tube (SST, gold top tube).

Allow the tube to clot for 30-60 minutes in a vertical position. Centrifuge at 1300-1800 g for 10-15 minutes at room temperature. Do not use brake to stop centrifuge.

Label each cryovial with:

- Study number (AAML1421)
- COG patient ID number
- BPC number
- Day of cycle and treatment cycle
- Sampling date and time
- Type of specimen (serum)

Using a pipette, remove serum from the top of the tube without disturbing the blood cells and transfer into the labeled cryovial(s).

Immediately place the cryovial(s) containing the serum sample in a -70 ± 10^0C freezer.

NOTE: Frozen samples should be batched and shipped once all specimens for a patient have been collected.

Shipping

See Section 14.2.4 for shipping details.

NOTE: Each specimen should be sent with a completed AAML1421 Specimen Transmittal form.

14.2.1.3 Leukemia Bank Sample Processing

The COG Leukemia Biospecimen Bank will forward specimens received for the cardiac troponin-T, N-terminal probrain natriuretic peptide (NT-proBNP), and hs-CRP studies to Dr. Absalon in Cincinnati, OH.

14.2.2 Novel Biochemical and Imaging Biomarkers of Cardiotoxicity

For this study, blood samples and echocardiograms images and reports are requested as detailed below.

14.2.2.1 Peripheral blood miRNA study

To explore the possibility of using peripheral blood miRNA as a prognostic indicator or anthracycline-induced heart injury in children, plasma will be collected at baseline and at several time points following CPX-351 administration. The miRNA studies will be conducted under the oversight of Dr. Kasey Leger and Seattle Children's Hospital.

For enquiries regarding this study, please contact:
Kasey Leger, MD
Seattle Children's Hospital
Email: kasey.leger@seattlechildrens.org

14.2.2.1.1 Specimen Time points

Samples from consenting patients, will be collected at the following time points in Cycle 1:

- Day 1, prior to CPX-351 infusion
- Day 5, 6 (± 1 hr) hours following the end of the CPX-351 infusion
- Day 8*
- Day 15*
- Day 22*
- Day 28-30 (one sample in this timeframe)

*Samples for Biomarkers of cardiac injury and MicroRNA may be drawn up to 24 hours prior to the time point and up to 24 hours after the time point.

14.2.2.1.2 Sample collection and processing

Peripheral blood (5-10 mL) must be obtained in a purple/ lavender top tube (EDTA tube). Process within 2 hours of collection:

- Spin in refrigerated (4°C) centrifuge at 2500g for 10 minutes
- Using sterile pipettes, transfer plasma into cryovials in 0.5 mL aliquots.
- Samples can be stored at -20°C for up to 3 days, or indefinitely at -80°C until shipped.

14.2.2.1.3 Labeling

Label the tubes with:

- Study number (AAML1421)
- COG patient ID number
- BPC number
- Date and time of collection
- Time point (include treatment cycle and day of cycle)

- Specimen type (plasma)

14.2.2.1.4 Shipping

Samples should be sent frozen, on dry ice, using overnight delivery.

See Section 14.2.4 for shipping details.

14.2.2.1.5 Leukemia Bank Sample Processing

The COG Leukemia Biospecimen Bank will forward specimens received for the peripheral blood miRNA study to Dr. Leger in Seattle, WA.

14.2.2.2 Myocardial deformation (strain)

Study participants will undergo a standard 2-dimensional, M-mode, and Doppler echocardiogram at baseline and at the end of Cycle 1 and Cycle 2, then yearly. Please request that both the ejection fraction and shortening fraction be calculated for the exam. Echocardiograms will be performed per AHA/ACC task force practice guidelines at participating institutions.[86] All tracings will be sent to Seattle Children's for central review for myocardial deformation and other echocardiographic parameters. Myocardial deformation will be measured using Tomtec software program, which allows post-hoc strain analysis on standard echocardiogram images across vendor platforms.

In addition to the standard echocardiogram report generated by the cardiologist at the performing institution, participating institutions are requested to send a digital copy of the original tracings from echocardiograms to be reviewed for study purposes.

In addition to collecting conventional echocardiogram parameters of LV function (SF and EF) we will be requesting DICOM echo images to be sent for analysis of myocardial strain.

Following ascertainment of end of Cycle 2 echocardiogram, ship:

- de-identified echocardiogram reports and
- DICOM echocardiogram images including the baseline echocardiogram (prior to protocol therapy), the beginning of Cycle 2 and end of therapy echocardiograms.

Note: if a patient goes off protocol therapy at any point during the trial, please send the baseline and end of therapy echocardiogram report and images.

These files can be burned to a CD and mailed to the address below. Multiple studies for the same patient may be submitted on one CD; however, please submit only one patient per CD. These should be mailed after Cycle 2 and preferably within 30 days from the end of Cycle 2.

Ship to:
Kasey Leger, MD
Seattle Children's Hospital
4800 Sand Point Way NE
M/S MB.8.501
Seattle, WA 98105

14.2.3 Evaluation of the Role of Rare Coding Variants as Risk Factors For Anthracycline-Induced Cardiomyopathy

For enquiries regarding this study, please contact:
Richard Aplenc, MD PhD
Office phone: (267) 426-7252
Lab phone: (267) 426-2288
Email: Aplenc@email.chop.edu

14.2.3.1 Specimens

2 mL to 4 mL of blood will be collected from consenting patients on Day 28-30 of Cycle 1 (one sample in this timeframe) for germline whole-exome sequencing.

14.2.3.2 Sample collection and Processing

Blood must be obtained in purple top tube (EDTA tube). The samples can be kept at 4ºC for storage and shipment.

14.2.3.3 Labeling

Label the tubes with:

- study number (AAML1421)
- COG patient ID number
- BPC number
- date and time of collection
- time point (include treatment cycle and day of cycle)
- source of material (blood)

14.2.3.4 Shipping

See Section 14.2.4 for shipping details.

14.2.3.5 Leukemia Bank Sample Processing

The COG Leukemia Biospecimen Bank will forward specimens received for the study on evaluation of the role of rare coding variants as risk factors for anthracycline-induced cardiomyopathy to Dr. Aplenc in Philadelphia, PA.

14.2.4 Instructions for Shipping to the COG Leukemia Biospecimen Bank

Samples must be shipped with a AAML1421 Specimen Transmittal form.

Ambient blood in EDTA tube should be placed inside a leak proof biohazard envelope with absorbent material and then a pressure resistance Tyvek envelope and then shipped at room temperature in a shipping container labeled with an Exempt Human Specimen label. Ambient blood may be shipped on Monday through Friday for a Tuesday through Saturday delivery.

Serum and plasma must be batch shipped frozen on dry ice. Specimens must be placed inside a leak proof biohazard envelope with absorbent material and then a pressure resistance Tyvek envelope. Place packaged specimens in an insulated shipping container with at least 5 lbs. of dry ice. Complete a dry ice label (UN 1845) and place the dry ice label and an Exempt Human Specimen label on the side of the shipping container. Place a FedEx shipping label on the top of the shipping container. Frozen samples may only be shipped on Monday through Thursday for a Tuesday through Friday delivery.

NOTE: Specimen collection and shipping supplies are not provided by the Leukemia Biospecimen Bank (or the Biopathology Center). Institutions are responsible for providing their own collection and shipping supplies.

Samples should be mailed by FedEx Priority Overnight. COG sites may use the COG Federal Express account number available at:
https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf

Ship to the following address:
COG Leukemia Biospecimen Bank
Nationwide Children's Hospital
575 Children's Crossroads, Room WB2255
Columbus, OH 43215
Phone: (614)722-2866
Fax: (614)722-2887
Email: MGLab@nationwidechildrens.org

Call or email the Leukemia Bank before shipping an ambient blood sample that will be delivered on a Saturday.

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 individuals contributing to NCI-sponsored trials 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).

Downloading Site Registration Documents:

Site registration forms may be downloaded from the AAML1421 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 AAML1421
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided.

Requirements for AAML1421 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 via the Regulatory Submission Portal, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' section) → Regulatory Tab → Regulatory Submission

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.

When applicable, original documents should be mailed to:

CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103

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: SAMPLE SUBMISSION FOR CYTOGENETIC/FISH STUDIES

(A COPY OF THIS SECTION MUST BE SENT TO THE RECOMMENDED INSTITUTIONAL, COG-APPROVED CYTOGENETICS LABORATORY)

CHROMOSOME ANALYSIS

- Bone marrow should be studied by cytogenetics methods in all cases of acute myeloid leukemia.
- A back-up blood specimen should be studied when the bone marrow aspirate or bone marrow core biopsy is inadequate or unobtainable (in which case short-term unstimulated cultures are established to examine spontaneously dividing [presumably leukemic] cells) or when a constitutional chromosomal abnormality is a possibility (phytohemagglutinin-stimulated cultures should be established to examine presumably nonleukemic [constitutional] lymphocytes).
- Short-term (15 - 48 hours) unstimulated cultures are recommended for each bone marrow sample. Analysis of direct preparations is successful in some laboratories. Mitogen-stimulated cultures of bone marrow samples should not be initiated.
- All preparations must be G-banded. (Q- or R-banding will not be accepted as a stand-alone banding method.)
- Complete analysis of 20 G-banded metaphases is required for each case, except as noted below. Complete analysis is defined as follows: the chromosomes in each metaphase cell have been counted; each chromosome has been examined to determine whether the banding pattern is normal, and all abnormalities present in the cell have been defined. Analysis may be accomplished by examining metaphase spreads under the microscope or by imaging. Sometimes analysis of 20 metaphases is not possible because of poor *in vitro* growth or a very limited quantity of specimen. However, limited characterization of the abnormal clone can still be informative. Such informative cases will be considered acceptable. A minimum analysis of 20 metaphases is required for a normal case.
- Identification of clones will follow the criteria of the Second International Workshop on Chromosomes in Leukemia, as stated in the: General Report (Cancer Genet. Cytogenet 2:93-96, 1980): at least 2 metaphases with identical structural abnormalities or extra chromosomes, or at least three metaphases with identical missing chromosomes will constitute a clone. Nonclonal abnormalities (excluding random loss) should also be recorded.
- Karyotypes are to be designated according to the guidelines described in ISCN 2013, An International System for Human Cytogenetic Nomenclature (2013), LG Shaffer, J McGowan-Jordan and M Schmid (eds); S. Karger, Basel, 2013.

FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH)

- FISH using commercially available probes will be required for the following cases A.) Normal B) Insufficient Quantity. C) Abnormal case with an 11q23 or 12p (see below). The laboratory is expected to follow the standards and guidelines for FISH put forth by the American College of Medical Genetics. If the laboratory is unable to perform FISH tests, contact cytogenetics coordinators for advice.

A) For all cases for which G-banding reveals a normal karyotype, FISH with *RUNX1T1/RUNX1*, *CBFB*, *or CBFB-MYH11*, *PML-RARA* and *MLL* (probes must be performed to rule out a cryptic

t(8;21), inv(16) or t(16;16), t(15;17) and 11q23 abnormality. FISH testing should be performed on 1 day cultures (unstimulated sample).

B) For all cases with failed cytogenetics, FISH with *RUNX1T1/RUNX1*, *CBFB or CBFB-MYH11*, *PML-RARA*, *MLL,* 7 (LSI, D7Z1/D7S486), and 5 (LSI, *EGR1*/D5S23, D5S721). The threshold for a positive FISH finding for –7, -5/5q- will be >30%. If possible, morphologic and immunophenotypic data should be obtained as such data can be suggestive of a particular abnormality and thereby help the laboratory director to prioritize these studies.

C) When G-banding reveals a known recurring chromosomal rearrangement, specifically one involving an t(8;21), inv(16)/t(16;16), or abnormal 11q23, FISH is recommended with a probe set targeting the involved loci (ie, *RUNX1T1/RUNX1*,, *CBFB or CBFB-MYH11*, *MLL*) should be performed on metaphase or interphase cells to determine whether there is an associated deletion involving the regions 3' or 5' of the participating genes, and to establish the pattern that can be used to monitor this patient's disease. When G-banding reveals an abnormality of 12p, FISH with a probe that can assess *ETV6* status (*ETV6* breakapart or *ETV6-RUNX1*) in order to detect *ETV6* rearrangement or deletion.

CYTOGENETIC STUDY/*FISH* SUBMISSION
Steps to obtain the FORMS by the institution's CRA

1. **www.childrensoncologygroup.org**
2. **COG** members
3. Committees ►Clinical Research Associates ►Resources ►Data Management ►Generic Study Forms
4. Under COG ►Cytogenetics Reporting/FISH Forms

Alternatively, the cytogenetics reporting form can be found at : https://cogmembers.org/site/pages/modal.aspx?mid=9000000007. The COG Forms should be completed by the designated individual in the Cytogenetics Laboratory and signed by the Cytogenetics Director. It is highly recommended to scan the Forms and e-mail with the appropriate documentation (PowerPoint presentation preferred) to COG reviewers for the Myeloid Committee. If the laboratory is unable to send an electronic file with the documentation, please contact the COG cytogenetics coordinator for your area for advice

The case should be sent to the appropriate reviewer by Day 14.

Cytogenetic Coordinators
Please send above materials by e-mail (preferably as PowerPoint file) to the following COG Cytogenetics Laboratories:

WEST OF MISSISSIPPI RIVER
(INCLUDE MINNESOTA AND WISCONSIN), AUSTRALIA, NEW ZEALAND, WESTERN CANADA
SEND TO:
Betsy Hirsch, Ph.D.
Telephone: (612) 273-4952/3171
E-mail: hirsc003@umn.edu

EAST OF MISSISSIPPI RIVER
(EXCLUDE MINNESOTA AND WISCONSIN), EUROPE, EAST CANADA

SEND TO:
Susana C. Raimondi, Ph.D.
Telephone: (901) 595-3537/3536
E-mail: susana.raimondi@stjude.org

CYTOGENETICS REVIEW

The region's cytogenetics coordinator will review each case when it is submitted. She/he will determine whether each case is adequate in terms of the numbers of metaphase cells analyzed, quality of banding, and interpretation of the karyotypes. If the coordinator agrees with the submitting laboratory, the results of the study will be entered into the appropriate Rave form. If the coordinator does not agree and the results are significant for patient stratification, she/he will send the case to another member of the COG Cytogenetics Review Committee for rapid review. If the coordinator does not agree with the submitting laboratory but the results are not significant for patient stratification, the case will be taken to the next central review session to be reviewed there. If the case is determined as not adequate it will be registered as unknown cytogenetics.

(A SIGNED AND DATED COPY OF THIS AUTHORIZATION FORM FOR REFLEXIVE FISH TESTING MUST BE SENT TO THE CYTOGENETICS LABORATORY, TOGETHER WITH THE BONE MARROW SAMPLE)

AUTHORIZATION FORM FOR REFLEXIVE FISH TESTS

A). REQUIRED FISH if a case is cytogenetically normal or inadequate

t(8;21)	[*RUNX1T1/RUNX1*]
inv(16)/t(16;16)	[*CBFB or CBFB-MYH11*]
t(15;17)	[*PML-RARA*]
11q23	[*MLL*]

B). REQUIRED FISH if a case is cytogenetically inadequate

-7:	[LSI, D7Z1/D7S486]
-5/5q-:	[LSI, *EGR1*/D5S23, D5S721]
t(8;21)	[*RUNX1T1/RUNX1*]
inv(16)/t(16;16)	[*CBFB* or *CBFB-MYH11*]
t(15;17)	[*PML-RARA*]
11q23	[*MLL*]

C). RECOMMENDED FISH if a case has a cytogenetically detectable

11q23	[*MLL*]
Abnormality of 12p	[*ETV6* breakapart or *ETV6-RUNX1*]

It is authorized to perform reflexive FISH testing to rule-out cryptic aberrations and/or to evaluate molecular deletions.

Patient Registration # _____

_____
Print Name of Attending Physician

_____
Print Name of Institution and City, State

Signature of attending physician or designee: _____

Date: _____

APPENDIX III: YOUTH INFORMATION SHEETS

INFORMATION SHEET REGARDING RESEARCH STUDY
(for children from 7 through 12 years of age)

A treatment study of a new drug to treat children with leukemia that has not responded to treatment or has come back.

1. We have been talking with you about your illness, acute myeloid leukemia (AML). Leukemia is a type of cancer that grows in the bone marrow. Bone marrow is the spongy tissue inside the bones of your body that make blood cells. Relapse means that the cancer has come back after treatment. You have received treatment for AML before. We are asking you to take part in a research study because the AML has come back. A research study is when doctors work together to try out new ways to help people who are sick. In this study we are trying to learn more about how to treat AML that has come back.

2. Children who are part of this study will be given a new drug called CPX-351. Doctors want to see if this new treatment will make children with leukemia get better, with fewer heart problems later in life. You will also get other drugs used to treat AML. We do not know how well the new drug will work in children, teens and young adults. That is why we are doing this study.

3. Sometimes good things can happen to people when they are in a research study. These good things are called “benefits.” We hope that a benefit to you of being part of this study is your cancer will go away. We also hope that the study treatment will help keep more children from having heart problems later in life. However, we don’t know for sure if there is any benefit of being part of this study.

4. Sometimes bad things can happen to people when they are in a research study. These bad things are called “risks.” One risk to you is that the study treatment may not work as well as other treatments to get rid of the cancer for as long as possible. Another risk may be more problems, or side effects, from the study treatment. Other things may happen to you that we don’t yet know about.

5. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. There may be other treatments for your illness that your doctor can tell you about. Make sure to ask your doctors any questions that you have.

6. We are asking your permission to collect extra blood. Doctors want to learn more about how children’s bodies handle the new drug you will receive. Doctors also want to see if there are ways to tell how your heart is affected by the treatment. Most of these samples would be taken when other standard blood tests are being performed, but there may be a few extra needle sticks. You can still take part in this study even if you don't allow us to collect the extra blood samples for research.

INFORMATION SHEET REGARDING RESEARCH STUDY
(for teens from 13 through 17 years of age)

A treatment study of a new drug to treat children with leukemia that has not responded to treatment or has come back.

1. We have been talking with you about your illness, acute myeloid leukemia (AML). Leukemia is a type of cancer that grows in the bone marrow. Bone marrow is the spongy tissue inside the bones of your body that make blood cells. You have received treatment for AML before. Relapse means that the cancer has come back after treatment. After doing tests, we have found that the AML has come back.

2. We are asking you to take part in a research study because your AML has come back. A research study is when doctors work together to try out new ways to help people who are sick. In this study we are trying to learn more about how to treat AML that has come back. Children and teens who are part of this study will be given a new drug called CPX-351. Doctors want to see if this new treatment will make the leukemia get better, with fewer heart problems later in life. You will also get other standard drugs used to treat AML. We do not know how well the new drug will work in children, teens and young adults. That is why we are doing this study.

3. Sometimes good things can happen to people when they are in a research study. These good things are called “benefits.” We hope that a benefit to you of being part of this study is a better chance at getting rid of the cancer for as long as possible. We also hope that fewer late effects and heart problems will occur. However, we don’t know for sure if there is any benefit of being part of this study.

4. Sometimes bad things can happen to people when they are in a research study. These bad things are called “risks.” One risk to you is that the study treatment may not work as well as other treatments to get rid of the cancer for as long as possible. Another risk may be more problems, or side effects, from the study treatment. Other things may happen to you that we don’t yet know about.

5. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. There may be other treatments for your illness that your doctor can tell you about. Make sure to ask your doctors any questions that you have.

6. We are asking your permission to collect additional blood. Doctors want to learn more about how children’s and teens’ bodies handle the new drug you will receive. Doctors also want to see if there are ways to tell how your heart is affected by the treatment. Most of these samples would be taken when other standard blood tests are being performed, but there may be a few extra needle sticks. You can still be treated on this study even if you don't allow us to collect the extra blood samples for research.

APPENDIX IV: POSSIBLE DRUG INTERACTIONS

The list below does not include everything that may interact with your chemotherapy. Talk to your doctor before starting any new medications, over-the-counter medicines, or herbal supplements and before making a significant change in your diet.

To this date, there are no data on specific food or supplement interactions with CPX-351. The drug interactions observed with CPX-351 are the same as for each individual components (cytarabine and daunorubicin).

Some drugs, food, and supplements may interact with <u>cytarabine (by vein)</u>. Examples include:

Drugs that may interact with cytarabine*
• Clozapine, digoxin, flucytosine, leflunomide

Food and supplements that may interact with cytarabine**
• Echinacea

**Sometimes these drugs are used with cytarabine on purpose. Discuss all drugs with your doctor.*
***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 <u>daunorubicin</u>. Examples include:

Drugs that may interact with daunorubicin*
• Some antibiotics and antifungals (clarithromycin, erythromycin, itraconazole, ketoconazole)
• Some antiepileptics (carbamazepine, phenobarbital, phenytoin, fosphenytoin)
• Some antiretrovirals (darunavir, lopinavir; nelfinavir, ritonavir, saquinavir, telaprevir, tenofovir, tipranavir)
• Some heart medications (amiodarone, carvedilol, digoxin, dronedarone, nicardipine, propranolol, verapamil)
• Other agents, such as atorvastatin, clozapine, cyclosporine, dexamethasone, ivacaftor, leflunomide, natalizumab, nefazodone, progesterone, 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"

**Sometimes these drugs are used with daunorubicin on purpose. Discuss all drugs with your doctor.*
***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 fludarabine. Examples include:

Drugs that may interact with fludarabine*
• Clozapine, leflunomide, natalizumab, pentostatin, tofacitinib

Food and supplements that may interact with fludarabine**
• Echinacea

**Sometimes these drugs are used with fludarabine on purpose. Discuss all drugs with your doctor.*
***Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.*

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