THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Protocol Page

Phase II Study of CPX-351 (Cytarabine:Daunorubicin) Liposome Injection in Patients with Newly Diagnosed AML at High Risk for Induction Mortality
2014-0548

Core Protocol Information

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Which Committee will review this protocol?

(•) The Clinical Research Committee - (CRC)

Protocol Body

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PHASE II STUDY OF CPX-351 (CYTARABINE:DAUNORUBICIN) LIPOSOME INJECTION IN PATIENTS WITH NEWLY DIAGNOSED AML AT HIGH RISK FOR INDUCTION MORTALITY

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September 9, 2019

ABBREVIATIONS

7+3	Seven days of continuous infusion of cytarabine at 100 mg/m^2/day and three days of daunorubicin at 60 mg/m^2/day
5+2	Five days of continuous infusion of cytarabine at 100 mg/m^2/day and 2 days of daunorubicin at 60 mg/m^2/day
ADR	Adverse Drug Reaction
AE	Adverse Event
AHD	Antecedent Hematologic Disorders
ALL	Acute Lymphocytic Leukemia
ALT	Alanine Transaminase (SGPT)
AML	Acute Myeloid Leukemia
ANC	Absolute Neutrophil Count
Ara-U	Arabinosyluracil
ASCO	American Society of Clinical Oncology
AST	Aspartate Transaminase (SGOT)
ATPase	Adenosine triphosphatase
AUC	Area under the plasma concentration-time curve
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
C	Celsius
Cmax	Maximum plasma concentration
CL	Clearance
CNS	Central nervous system
CPX-351	CPX-351 (cytarabine:daunorubicin) Liposome Injection
CR	Complete Response
CRi	Complete Response with incomplete hematologic recovery
CRF	Case Report Form
CMMoL	Chronic Myelomonocytic Leukemia
CTCAE	Common Terminology Criteria for Adverse Events
DEHP	di(2-ethylhexyl)phthalate
dL	deciliter
DSMB	Data and Safety Monitoring Board
DSPG	Distearoylphosphatidylglycerol
DSPC	Distearoylphosphatidylcholine
EC	Ethics Committee
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EFS	Event-free Survival
ELN	European LeukemiaNet
EOI	End of Infusion
EP	European Pharmacopoeia
EU	European Union
FDA	Food and Drug Administration
g	grams
GCP	Good Clinical Practice
HCl	Hydrogen Chloride

HIPAA	Health Information Protection and Portability Act
HIV	Human Immunodeficiency Virus
HOVON	Hemato-Oncologie voor Volwassenen Nederland
HP	High Purity
HSCT	Hematopoietic Stem Cell Transplantation
ICF	Informed Consent Form
ICH	International Committee on Harmonization
ITT	Intent-to-treat
IRB/EC	Institutional Review Board/Ethics Committee
iv, IV	intravenous
K-M	Kaplan-Meier
L	liter
LDH	Lactate Dehydrogenase
LVEF	Left ventricular ejection fraction
m^2	square meters
MDR	Multi-drug Resistance
MDS	myelodysplastic syndrome
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram(s)
mL	milliliter(s)
MLL	Mixed Lineage Leukemia
MPN	Myeloproliferative neoplasm
MTD	Maximum Tolerated Dose
MUGA	Multiple Gated Acquisition scan
mw	molecular weight
N	Number, Population
NF	National Formulary
OS	Overall Survival
PD	Persistent Disease
PHI	Protected Health Information
PK	Pharmacokinetics
PS	Performance Status
q.s.	Quantum sufficiat
RBC	Red blood cells
SAE	Serious Adverse Event
SD	Standard deviation
sAML	Secondary AML
$T_{1/2}$	Half-life
t-AML	Therapy-related AML
Tmax	Time of occurrence of Cmax
u	Units
µL	Microliter
ULN	Upper Limits of Normal
USP	United States Pharmacopeia
WHO	World Health Organization

TABLE OF CONTENTS

1.0 General Information

This document is a protocol for a human research study. This study is to be conducted according to United States and international standards of Good Clinical Practice (FDA Title 21 parts 11, 50, 54, 56, 312, International Conference on Harmonization and the Declaration of Helsinki), applicable government regulations and Institutional research policies and procedures.

2.0 Background Information

2.1 Acute Myeloid Leukemia in the Elderly and its Treatment

Acute myeloid leukemia represents a group of clonal hematopoietic stem cell disorders in which both failure to differentiate and excessive proliferation in the stem cell compartment result in accumulation of non-functional cells termed myeloblasts.[1]

Untreated AML in all ages is rapidly fatal, with patients dying on average within a few months of diagnosis. Even with treatment, particular groups of AML patients continue to have a poor prognosis. AML in the elderly (age ≥60) is associated with increased risk of not responding to therapy and increased risk of dying from the treatment. Appelbaum, et al.[2] and Kantarjian, et al.[3] summarize the factors that contribute to poor outcomes in elderly patients with AML. Risk factors that decrease patient tolerance to therapy or sensitivity of the leukemia to therapy include increasing age, poor performance status, co-morbid medical conditions, accumulated chromosomal abnormalities, adverse mutations, and multi-drug resistance.

There is broad overlap of these risk factors with most elderly AML patients having one or more adverse features. The poor results of treatment in elderly AML lead to a reluctance to treat elderly patients with intensive regimens designed to induce aplasia and complete remission.

Clearing the marrow of leukemia has historically been the only means of obtaining prolonged survival in AML patients. This is usually accomplished by use of intensive cytotoxic/cytoreductive therapy.[4] The intensity of treatment needed to induce aplasia and complete remission is associated with early mortality rates of 10-20% in elderly patients considered fit for intensive therapy and is higher in patients with risk factors indicating less sensitive leukemia, co-morbidities, and poor performance status.[2, 3, 5-7]

A randomized study reported in 2009 showed that a double induction regimen with 90 mg/m^2 daunorubicin with cytarabine (first induction) followed by a second induction with intermediate dose cytarabine (1 g/m^2) could be safely administered with high rates of complete remission in older patients. The second induction was given even to patients already morphologically leukemia-free after first induction, a practice that is not routinely used in the U. S. and Canada. This publication from the HOVON group noted improvement in remission rate when compared to a traditional regimen of cytarabine plus daunorubicin (45 mg/m^2) but no improvement in disease-free survival or overall survival

was noted.(8) At the present time, the HOVON study has not been replicated in elderly patients by any other group.

Another study by Fernandez, et al., reported success for the same higher dose daunorubicin regimen (90 mg/m^2) versus 45 mg/m^2 of daunorubicin when used for first induction for younger patients.(9) For second inductions, the dose of daunorubicin was reduced to 45 mg/m^2 for all patients. Responding patients were taken to transplant. Neither this study nor the HOVON study used daunorubicin 90 mg/m^2 for second induction courses or in consolidation and neither study demonstrated that 90 mg/m^2 is more effective than 60 mg/m^2. Although of great interest, neither regimen is ready to be used as the control arm in a comparative study with CPX-351.

For patients at high risk of relapse (e.g. those with high-risk cytogenetics, underlying MDS, or therapy-related AML), allogeneic stem cell transplantation is usually recommended if the patient is able to tolerate a transplant and has a suitable donor.

For patients with older age or co-morbidities the risk of induction treatment remains high. Patients age 65 or older treated with standard 3+7-like chemotherapy have been reported to have an induction mortality rate of 20%.(8, 10) Those with adverse cytogenetic abnormalities have a significantly higher risk of early mortality.(8, 11) At MD Anderson, patients at relatively high (30-50%) risk for induction mortality (60-day) can be identified. Patients in this age group with adverse cytogenetic abnormalities have an early mortality rate of 35% compared to 22% for those in the same age group with diploid cytogenetics (Tambaro et al. ASH 2012). In addition, an analysis of 998 patients age 65 or older with AML or high-risk MDS analyzed the factors associated with increased risk for early mortality and, consequently, poor long-term outcome. By multivariate analysis, age 75 years or older, performance status ≥2, complex karyotype, treatment outside a laminar air-flow setting, antecedent hematologic disorder of at least 12 months duration prior to the diagnosis of AML, and a creatinine >1.3 mg/dL were identified as independent factors associated with increased risk of mortality by 8-weeks from the start of induction chemotherapy.(3) Patients with none of these risk factors have an 8-week mortality probability of 10%, with a median survival of 16 months and 1-year projected survival of 58% and are considered a low-risk group. In contrast, for those with 1 or 2 risk factors (i.e., the intermediate risk group) the 8-week mortality rate is 19-36% with median survival of 4-9 months and 1-year survival rates of 22-35%. Those with 3 or more risk factors (the high-risk group) have a 65% probability of early mortality, median survival of 1 month and 1 year projected survival of 8%.(3) For patients with intermediate or high risk the decision to treat with intensive chemotherapy is difficult because the benefits have been approximately equal to the risks. Studies using non-intensive treatment have been performed indicating reduced early risk with reduced benefit. The need for effective treatment with acceptable treatment safety remains urgent.

2.2 CombiPlex® Technology

In vitro studies have shown that antitumor activity can be enhanced when cytotoxic drugs are used in combination. This has led to cytotoxic drug combinations as standard therapy

in many forms of cancer treatment. New anticancer drugs are typically first introduced in patients as single agents. After a maximum tolerated dose is determined for one agent, a second agent is added and the dose of one or both agents is adjusted on the basis of toxicity. The development of these combination regimens then is determined empirically on the basis of tolerability. However, *in vitro*, where the molar ratio of drugs used in combination can be controlled, it has been demonstrated that drug combinations providing synergy at one ratio may be simply additive or even antagonistic at other ratios.(12) When individual free drugs are administered, each agent is handled differently by the body, resulting in differences in the distribution and elimination of the individual drugs to tumor sites which must result in drug ratios that are sometimes suboptimal or ineffective. The manufacturer's technology is based on findings that *in vitro* synergistic activity of antineoplastic drugs depends on specific drug ratios and that the in vivo activity of a combination depends on maintaining the synergistic ratio. In this way, the development of a particular chemotherapeutic regimen can be based on the most efficacious ratio rather than empirically based on toxicity.

The development of CPX-351 (cytarabine:daunorubicin) Liposome Injection was based on 1) defining a synergistic ratio of the two active moieties, cytarabine and daunorubicin, using cell-based screening assays and 2) designing a liposomal drug carrier to maintain this ratio after intravenous administration. This ratio was not based on the empirically-derived, toxicity-guided regimens currently used for cytarabine and anthracyclines.

2.3 Physical, Chemical and Pharmaceutical Information

CPX-351 is a liposomal formulation of a fixed combination of the antineoplastic drugs cytarabine and daunorubicin. The two drugs are present inside the liposome in a 5:1 molar ratio. The liposome membrane is composed of distearoylphosphatidylcholine, distearoylphosphatidylglycerol and cholesterol in a 7:2:1 molar ratio. These liposomes have a nominal diameter of approximately 100nm and are suspended in sucrose. Sterilization is achieved by filtration through a 0.22 μm filter.

CPX-351 is provided as a sterile, pyrogen-free lyophilized formulation in 50 ml glass, single-use vials. Each vial contains 100 units of CPX-351 where each unit contains 1.0 mg cytarabine and 0.44 mg daunorubicin base in liposomes. The lyophilized cake is reconstituted with water for injection to obtain a homogeneous dispersion at 5 units/mL. The composition of the formulation after reconstitution is listed in Table 1 below.

Table 1: Quantitative Composition

Component	mw	Amount per Vial	Amount per unit
Cytarabine, USP/EP	243	100 mg	1.0 mg
Daunorubicin HCl USP/EP(reported as the free base)	528	44 mg	0.44 mg
Distearoylphosphatidylcholine	790	454 mg	4.5 mg
Distearoylphosphatidylglycerol	801	132 mg	1.3 mg
Cholesterol, HP	387	32 mg	0.3 mg
Copper gluconate, USP	454	92 mg	0.9 mg
Triethanolamine, NF	149	7 mg	0.07 mg

Sucrose, NF	342	2054 mg	20.54 mg

2.4 Product Label

OLD LABEL DELETED

NEW LABEL ADDED

For Clinical Trial Use Only

Protocol: [xxxx]

CPX-351 (daunorubicin and cytarabine) liposome for injection, for intravenous use only

Contents: 44 mg daunorubicin and 100 mg cytarabine. Single-dose vial for reconstitution.

Directions: Use as directed in Protocol [xxxx]

Store unreconstituted CPX-351 vials in a refrigerator at 2°C to 8°C (36°F to 46°F) in an upright position. Store in original carton to protect from light.

Reconstituted product can be stored up to 4 hours at 2-8°C.

Caution: New Drug – Limited by Federal (or United States) law to investigational use.

Lot No.: [ ]

Expiry Date: [MM/YYYY]

Manufactured for: Jazz Pharmaceuticals, 3180 Porter Drive, Palo Alto, CA 94304, USA, 1-650-496-3777

IST-6312-A

2.5 Pre-clinical Pharmacology & Toxicology

The pre-clinical pharmacology and toxicology is summarized in the Investigator's Brochure for CPX-351.

2.6 Brief Summary of Prior Clinical Studies

Three clinical studies have been conducted with CPX-351. One Phase I study, CLTR0305-101 and two Phase II studies: CLTR0308-204 and CLTR0308-205. Below is a summary of these studies.

2.6.1 Phase I Study of CPX-351: CLTR0305-101

The primary goal for this study was to establish the MTD for CPX-351 and recommend a dose for further study in a Phase II setting. Pharmacokinetics was assessed at every dose level and patients were monitored for signs of antileukemic activity.

The dosage regimen was designed to mimic the 7-day drug exposure provided by conventional 7+3 treatment. A single induction course administers doses on Days 1, 3, and 5, by 90 minute infusion. Patients with AML (multiply relapsed, refractory, or with first CR duration of 6 months or less), ALL, and high risk MDS were eligible.

Dose limiting toxicities were observed at the 10th dose level: 134 u/m^2 (134 mg/m^2 cytarabine + 59 mg/m^2 daunorubicin). One patient had significant reduction in post treatment LVEF and as a result both Phase II studies included a cap (500 mg/m^2) on cumulative anthracycline dose after one induction course of CPX-351 and patients with significant pre-existing cardiac disease were excluded. Other dose-limiting toxicities included hypertensive crisis and prolonged (>56 days) cytopenias.

The Phase I study of CPX-351 assessed the concentrations of cytarabine, daunorubicin, uracil arabinoside, and daunorubicinol at multiple dose levels and found that they exhibited mono-exponential, first order elimination with minimal early phase distribution. (Figure 1)

Figure 1: CPX-351 Day 1 and 5 Pharmacokinetics from Protocol CLTR0305-101

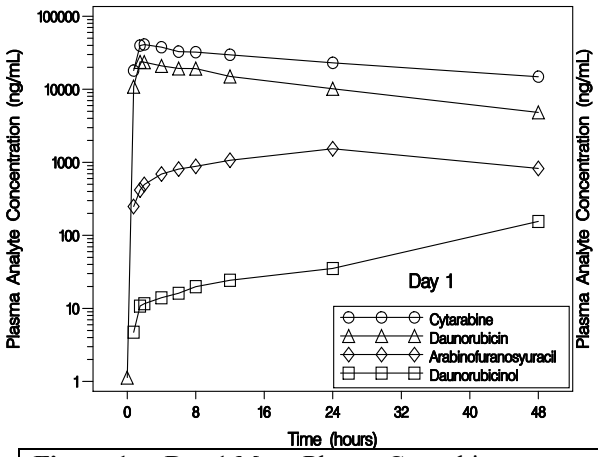


Figure 1a: Day 1 Mean Plasma Cytarabine, Daunorubicin, Ara-U and Daunorubicinol concentrations, Time 0-48hr (101u/m^2, 90 min infusion)

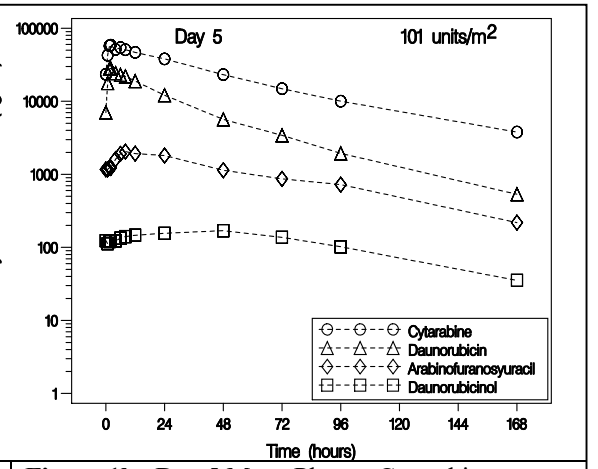


Figure 1b. Day 5 Mean Plasma Cytarabine, Daunorubicin, Ara-U and Daunorubicinol concentrations, Time 0-168hr (101u/m^2, 90 min infusion)

The day 1 (single dose) and day 5 (multiple dose) C_{max} and $AUC_{(0-\tau)}$ were linear and the 5:1 molar ratio of cytarabine to daunorubicin was maintained for up to 24 hours at all dose levels on days 1 and 5.

The pharmacokinetic analysis confirmed higher absolute plasma concentrations of both drugs compared to that expected with the conventional 7+3 regimen with a markedly prolonged mean half-life for both cytarabine and daunorubicin and greater drug exposure (AUC). Measurable drug levels were present seven days after the last infusion of CPX-351 (Study Day 12). Compared to infusions of the conventional drugs, CPX-351 administration was associated with substantially greater AUC and half-lives for plasma cytarabine and daunorubicin.(13) (Table 2)

Table 2: Cytarabine and Daunorubicin Pharmacokinetics following CPX-351 (101 u/m^2, Day 5)

Variable	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{(0-\tau)}$ (ng*hr/mL)	$T_{1/2}$ (hr)	CL (mL/hr/m^2)
Cytarabine					
N	13	13	13	13	13
Median	55,800	2	1,487,638	31.1	67.9
Mean	64,608	3.02	1,851,089	36.9	67.3
SD	23,230	2.25	934,523	24.5	30.6
Daunorubicin					
N	13	13	13	13	13
Median	29,200	2	633,579	21.9	70.1
Mean	30,185	1.87	666,640	25.2	72.9
SD	6,198	0.74	209,198	11.6	23

Abbreviations: C_{max}, maximum serum concentration; T_{max}, time to maximum serum concentration; $AUC_{(0-\tau)}$, area under the serum concentration-time curve from time 0 to time τ (end of the dosing interval); $t_{1/2}$, terminal half-life; CL, clearance; SD, standard deviation.

Three of the 48 patients entering the study had ALL and a transient CR was achieved in one patient at the 43 unit/m^2 dose level. Two patients with high risk MDS were treated at lower dose levels and neither responded. The remaining 43 patients had AML; 20 patients after multiple relapses or multiple treatment regimens, 18 after relapse from a first CR, and 5 after primary induction failure. Complete response was observed in 10 of 43 AML patients, a notable observation because most patients had received cytarabine and daunorubicin in the past and because of the low frequency of non-hematological toxicities observed with CPX-351.

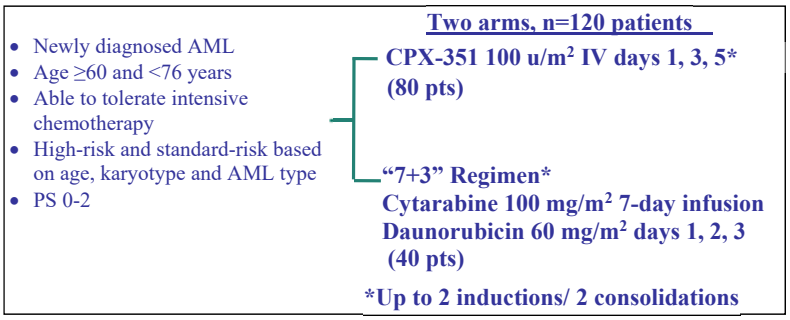
In conclusion, the Phase I study defined the maximally tolerated dose (101 u/m^2) for a day 1, 3 and 5 induction course of treatment and confirmed persistence of the 5:1 molar ratio for up to 24 hours in the plasma with markedly prolonged median half-life for both cytarabine and daunorubicin. Multiple responses in previously treated AML patients provided proof of antileukemic activity.

2.6.2 Phase II Study of CPX-351: CLTR0308-204

The 204 study was designed as a randomized study comparing CPX-351 head-to-head against 7+3, in newly diagnosed, older (age 60-75) patients with AML. The comparison of encapsulated cytarabine and daunorubicin (CPX-351) versus free cytarabine and daunorubicin (7+3) would be fully interpretable for relative efficacy and safety and would begin to provide evidence supporting or refuting the hypothesis that dosing based on molar ratios would lead to improved efficacy.

One hundred twenty-seven patients were randomized from 18 of 28 study sites. The study was amended to extend follow-up to 2 years when it became apparent that 1 year follow-up would be insufficient to capture median survival in both study groups.

A two to one randomization was used to maximize experience using CPX-351. Response rate (CR+CRi) was the primary endpoint and superior response with a one-sided p-value of <0.1 was deemed sufficient for moving forward in development. Secondary endpoints were overall survival, event-free survival, CR+CRi duration, rate of aplasia, safety and practicality of CPX-351 as consolidation therapy and the response rate of CPX-351 between de novo and secondary AML.



At entry patients were stratified by age (60-69 vs. 70-75), cytogenetics (< or ≥3 cytogenetic abnormalities), and type of AML (de novo vs. secondary). High risk patients were older (age 70-75) or had complex cytogenetics (≥3 cytogenetic abnormalities) or had secondary AML. Standard risk patients were younger (age 60-69), had non-complex cytogenetics (<3 abnormalities) and had de novo AML. After accrual was complete, Dr. Jeffrey Lancet (H.L. Moffitt Cancer Center, Tampa, FL) reviewed all of the cytogenetic reports and confirmed/corrected assignment of patients to <3 or ≥3 cytogenetic abnormalities. In addition he also assigned each patient to a specific cytogenetic risk group (adverse vs. intermediate vs. unknown) according to National Comprehensive Cancer Network guidelines (NCCN:www.nccn.org/professionals/physician_gls/PDF/aml.pdf). Dr. Lancet's assessment of cytogenetic risk is shown in Table 3.

Table 3 demonstrates balanced assignment of patients to both arms of the study indicating successful randomization and stratification.

Table 3: CLTR0308-204 Demographics

		CPX-351 n=85	7+3 n=41
Gender	Male	53 (62.4%)	25 (61.0%)
Race	Caucasian	76 (89.4%)	39 (95.1%)
Risk	Standard Risk	28 (32.9%)	14 (34.1%)
	High Risk	57 (67.1%)	27 (65.9%)
Age Group	≥70yrs	34 (40.0%)	15 (36.6%)
	Median (yrs)	68	68
AML Type	De novo AML	53 (62.4%)	22 (53.7%)
	Secondary AML	32 (37.6%)	19 (46.3%)
ECOG PS	0-1	70 (82.4%)	36 (87.8%)
	2	15 (17.6%)	5 (12.2%)
Cytogenetic Risk	Adverse	23 (27.1%)	13 (31.7%)
	Intermediate	54 (63.5%)	26 (63.4%)
	unknown	7 (8.2%)	2 (4.9%)

Table 4 demonstrates that CPX-351 produced superior rates of aplasia/hypoplasia and response with similar duration of remission. It was notable that the improvement in response occurred in the form of CRi (CR with incomplete hematologic recovery).

Table 4: CLTR0308-204 Response Rate and Duration of Remission

	CPX-351 n=84*	7+3 n=41	
Aplasia rate after 1st induction	64/81 (79.0%)	23/38 (60.5%)	
Aplasia rate after any induction	71/81 (87.7%)	27/38 (71.1%)	
CR+CRi	56 (66.7%)	21 (51.2%)	p=0.0712
CR	41 (48.8%)	20 (48.8%)	
CRi	15 (17.9%)	1 (2.4%)	
Median Duration of Remission (days)	271	262	p= ns

*Patient 12-003 was diagnosed with Philadelphia chromosome + AML and removed from study for treatment with imatinib

The study met the primary endpoint with a response rate of 66.7% compared to 51.2% (p= 0.0712). Further analysis of response according to the stratification factors demonstrated consistent benefit for CPX-351 in response rate across every subgroup.

Table 5: CLTR0308-204 Response Rate by Sub-group

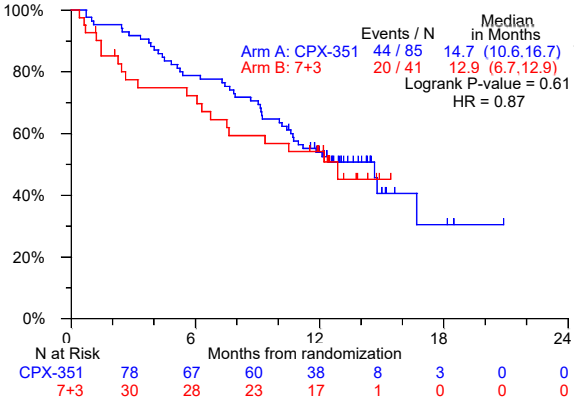
		CPX-351 n=84*	7+3 n=41
Risk	Standard Risk	22/28 (78.6%)	8/14 (57.1%)
	High Risk	34/56 (60.7%)	13/27 (48.1%)
Cytogenetic Risk	Intermediate	33/54 (61.1%)	15/26 (57.7%)
	Adverse	17/23 (73.9%)	5/13 (38.5%)
Age Group	60-69 yrs	34/50 (68.0%)	14/26 (53.8%)
	≥70yrs	22/34 (64.7%)	7/15 (46.7%)
AML Type	De novo AML	38/52 (73.1%)	15/22 (68.2%)
	Secondary AML	18/32 (56.3%)	6/19 (31.6%)

*Patient 12-003 was diagnosed with Philadelphia chromosome + AML and removed from study for treatment with imatinib

Kaplan-Meier (K-M) analysis after a minimum follow up of 1-year for Event Free Survival (EFS, based on documentation of persistent leukemia, relapse after CR, or death) and Overall Survival (OS) demonstrated non-significant improvements for CPX-351 in the overall study population and the high risk population. K-M analysis of the secondary AML population (including treatment-related AML and AML occurring in the setting of antecedent hematologic disorders, e.g. myelodysplasia, myeloproliferative disease, and chronic myelomonocytic leukemia) showed a significant improvement in overall survival (p-value=0.01) with a median survival improvement of 6 months (12.1 vs. 6.1 months). The overall survival and event-free survival curves shown in Figure 2 through Figure 4 include crossover patients in the Control Arm based on their original treatment assignment. This confounds the interpretation of OS, but as the patients are considered as having an event at crossover, this confounding is not present in the EFS curves. Given the number of EFS events, the EFS curves are relatively mature. The OS curves, with only about 50% of the events, are less mature.

Figure 2: Kaplan-Meier estimates of OS and EFS in the Overall Population

Overall Survival



Event-free Survival

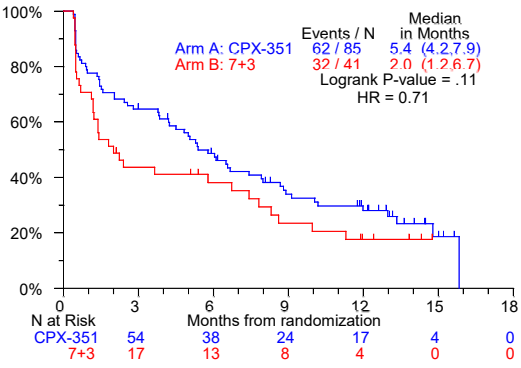
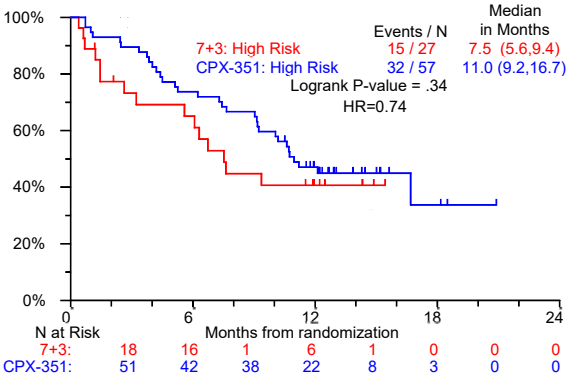


Figure 3: Kaplan-Meier estimates of OS and EFS in the High Risk Strata

Overall Survival



Event-free Survival

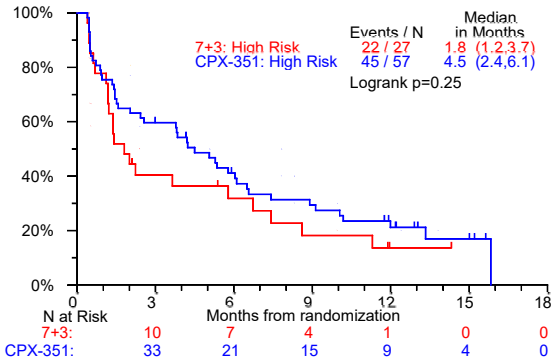
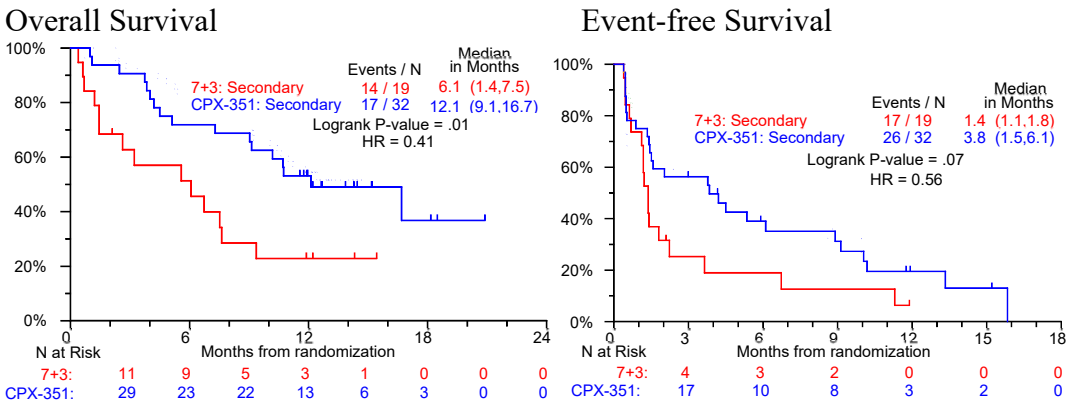


Figure 4: Kaplan-Meier estimates of OS and EFS in Patients with Secondary Leukemia



Induction mortality was assessed at Day 30 and 60. A lower rate of early mortality was observed for CPX-351 treated patients (See Table 6). This result is the best evidence that CPX-351 treatment is safe and suggests that rapid clearance of leukemia may assist in reducing the early death rate.

Table 6: CLTR0308-204 Induction Mortality (30 and 60 days)

	CPX-351 n=85	7+3 n=41	
30 Day Mortality	3 (3.5%)	3 (7.3%)	p=0.35
95% CI*	[0.01, 0.10]	[0.02, 0.20]	
60 Day Mortality	4 (4.7%)	6 (14.6%)	p=0.053
95% CI*	[0.01, 0.12]	[0.06, 0.29]	

*Two-sided confidence intervals (CIs) were calculated using the Fisher's exact method

Adverse events were qualitatively similar between both study arms. Adverse events with increased frequency in the CPX-351 arm were linked to greater myelosuppression with higher rates of febrile neutropenia (63.5% vs. 51.2%), bacteremia (42.4% vs. 22%), epistaxis (36.5% vs. 19.5%), and petechiae (32.9% vs. 12.2%). This appears to be due to more prolonged myelosuppression (median ANC recovery to ≥1000/μL: 36 vs. 29 days and median platelet recovery to ≥100,000/μL: 37 vs. 27 days). Serious fungal infections were more frequent on CPX-351 versus 7+3. These tended to occur during the period of nadir neutropenia and resolved at recovery. There were no grade 5 fungal events. A variety of non-hematologic adverse events appear to be more frequent after CPX-351, but most, including cough, rash, and headache were usually of grade 1-2 severity and were transient. The key safety findings were that CPX-351 had lower than expected 60-day mortality (4.7% vs. 14.6%), lower rates of adverse events with grade 5 outcome (11.8 vs. 17.1), and more prolonged myelosuppression with higher rates of febrile neutropenia, infections and bleeding events but no increase in deaths associated with infection when compared to 7+3. Non-myelosuppressive adverse events were similar in quality in both study arms and there was no evidence of excess cardiac events, in this group of anthracycline-naïve patients.

The 7+3 control arm performed as expected, based on the literature, with moderate induction mortality (60 day death rate=14.6%) and a 51.2% CR+CRi rate. However, a projected median overall survival of slightly greater than 12 months (12.9 months) was slightly better than expected.

A search for imbalances that could have biased study results in favor of CPX-351 was performed and no imbalance in post-induction bone marrow transplants, no major imbalance in the characteristics of secondary AML patients (including type of antecedent hematologic disorder), and no imbalance in prior treatment with hypomethylating agents was found. Fewer patients on the 7+3 arm received subsequent therapy after persistent or relapsed disease, but most of this imbalance was due to the high rate of early mortality among control arm patients, preventing subsequent therapies from being given to control arm patients.

2.6.3 Phase II Study of CPX-351: CLTR0308-205

This study compared CPX-351 (100u/m^2; Day 1, 3, 5) with salvage therapy in first relapse AML patients. This trial planned to accrue 120 patients with a 2:1 randomization. Investigator choice of control salvage regimen was expected to be between (1) high dose cytarabine with or without daunorubicin, (2) 7+3 or (3) mitoxantrone, etoposide and cytarabine. The allowance of investigator's choice reflects the fact that no single salvage therapy is superior and the necessity of individualizing treatment. Responding patients were expected to receive allogeneic stem cell transplant for consolidation if donors were available.

The European Prognostic Index was used to stratify patients. For this trial, the primary endpoint was survival at one year, which for the control arm was expected to be ≤30% based on previous clinical trials and the literature. Secondary endpoints were CR+CRi rate, remission duration, event-free survival and 30/60/90 day mortality. The 1 year survival rate was selected as the primary endpoint rather than response rate because of the substantial variability of the CR rate for different salvage regimens reported in the literature. Accrual was completed in November 2010 with 81 patients randomized to CPX-351 and 45 randomized to salvage therapy.

Patient demographics were well balanced between the two arms for durations of first complete remission, cytogenetics, and age. There was a slight imbalance in history of prior stem cell transplants with more patients in the CPX-351 arm having a history of transplant. Study stratification was successful with approximately 2/3's of patients in both study arms in the unfavorable EPI risk group.

205 Study Patient Demographics

		EPI Points	CPX-351 n=81 n (%)	Control n=44 n (%)
Risk Group	Favorable	1-6	9 (11.1)	6 (13.6)
	Intermediate	7-9	16 (19.8)	9 (20.5)

	Unfavorable	10-14	56 (69.1)	29 (65.9)
Relapse-Free Interval from CR1 (months)	> 18	0	11 (13.6)	8 (18.2)
	7-18	3	43 (53.1)	20 (45.5)
	≤ 6	5	27 (33.3)	16 (36.4)
Cytogenetics at Diagnosis	Inv(16) or t(16;16)	0	7 (8.6)	4 (9.1)
	t(8;21)	3	2 (2.5)	1 (2.3)
	Other	5	72 (88.9)	39 (88.6)
Age at First Relapse (years)	≤ 35	0	10 (12.3)	4 (9.1)
	36 - 45	1	14 (17.3)	7 (15.9)
	> 45	2	57 (70.4)	33 (75.0)
HSCT before Relapse	No	0	59 (72.8)	37 (84.1)
	Yes	2	22 (27.2)	7 (15.9)

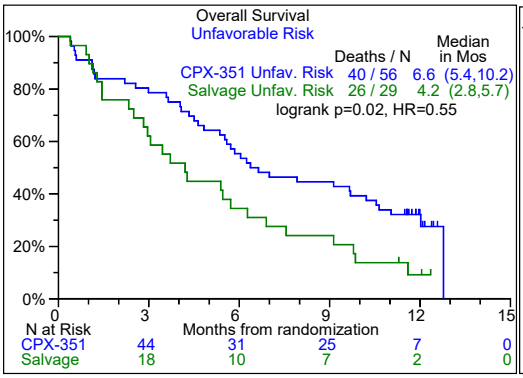
Consistent with results observed in Study 204, this study in patients with first relapse AML also demonstrated higher rates of treatment induced hypoplasia/aplasia and response with improvements in median EFS and OS.

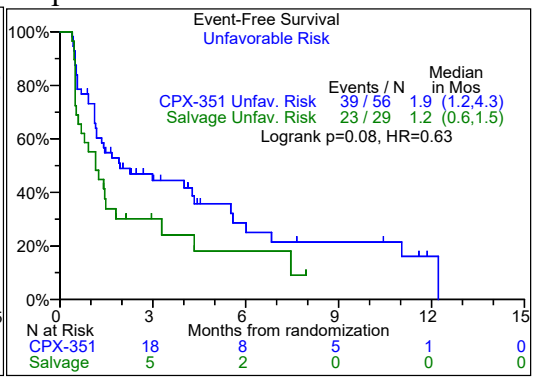
Aplasia, Response, and Early Mortality Results

	Overall (n=125)		**Unfavorable EPI Risk (n=85)**	
	CPX-351 (n=81)	**Salvage (n=44)**	**CPX-351 (n=56)**	**Salvage (n=29)**
Aplasia Rate	77.3%*	59.5%*	66.10%	44.80%
CR Rate	37.00%	31.80%	28.60%	20.70%
CRi Rate	12.40%	9.10%	10.70%	6.90%
Response Rate	49.40%	40.90%	39.30%	27.60%
60-Day Mortality	14.80%	15.90%	16.10%	24.10%
EFS (median)	4.0 months	1.4 months	2.0 months	1.2 months
OS (median)	8.5 months	6.3 months	6.6 months	4.2 months

The EFS and OS results did not demonstrate statistically significant improvements for the group as a whole, but significance was found for survival in the subset of relapsed patients with EPI Unfavorable disease. These results echo those found for the secondary AML population in Study 204, in that significant differences in patient outcomes were observed in patients with less sensitive AML because of a substantial fall off in efficacy for the control arm.

Overall Survival and Event-free survival in patients with EPI Unfavorable disease





A search for imbalances that could have biased study results in favor of CPX-351 was also performed and imbalances in post-induction bone marrow transplants were not found. The rate of post-induction HSCT was similar between the two study arms among patients with CR, CRi, and persistent AML, suggesting that transplants did not bias the study.

Safety data were consistent with those from Study 204. Patients with no history of prior stem cell transplant at study entry were in the majority (96/125, 76.8%) and were found to have slightly lower 60-day mortality (10.2% vs. 16.2%) and higher complete response rates (54.2% vs. 37.8%). Patients with prior stem cell transplants had poorer results after CPX-351 with somewhat higher 60-day mortality (27.3% vs. 14.3%) and lower response rate (35.4% vs. 57.1%). The prior stem cell transplant group was too small to reach definitive conclusions but it is possible that patient groups with extensive prior chemotherapy may be at higher risk following CPX-351 treatment.

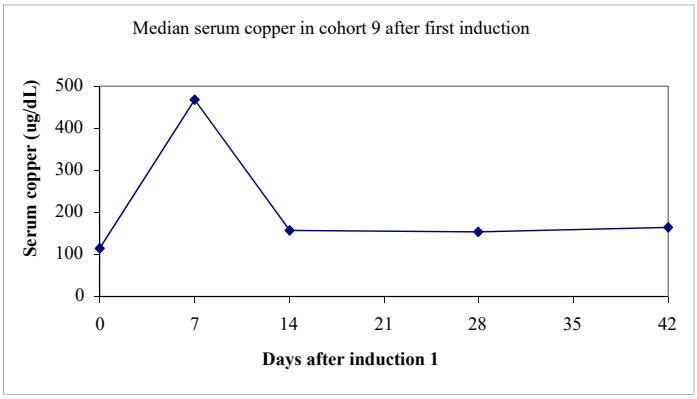
Overall, Study 205 confirmed and extended observations first made in Study 204. CPX-351 is highly active with improved clearance of leukemia and higher response rate, even in patients with prior exposure to cytarabine and anthracycline. CPX-351 was also shown to be safe with improved 60-day mortality in the majority of patients with no prior history of HSCT. These results suggest that CPX-351 will be an excellent candidate to test in newly diagnosed AML patients with high risk of treatment mortality.

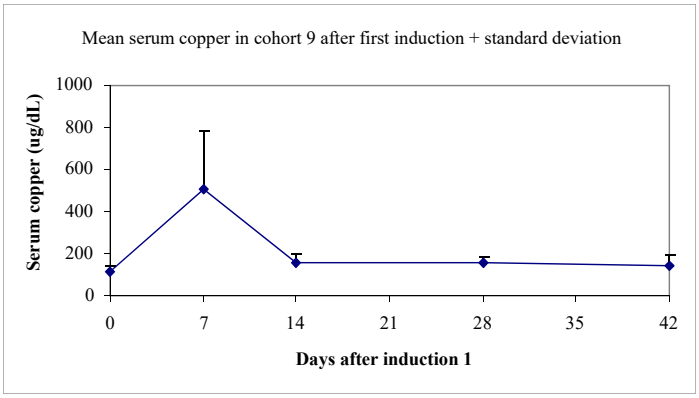
2.7 Copper Background

Copper is an essential element that is a component of a number of metalloenzymes acting as oxidases (e.g. diamine oxidase, monoamine oxidase, cytochrome c oxidase). The median absorption of copper from food (by an American adult) is 1.0 to 1.6 mg/day. The tolerable Upper Intake Level for adults is 10 mg/day. At a dose of CPX-351 of 100 u/m^2, a patient with a BSA of 2.0 m^2 would receive a maximum of 36 mg of elemental copper per dose. In the animal toxicology investigations for CPX-351, elevated copper levels were seen following dosing with levels generally returning to baseline 1 to 2 weeks after the last dose (see Figure 5). No toxicity seen in animal studies was attributable to copper.

The data from the clinical studies are consistent with the preclinical findings. Since CPX-351 contains copper (0.18 mg copper per unit, in the form of copper gluconate), serum total copper levels were monitored in the Phase 1 clinical study (Study CLTR0305-101). Figure 5 shows this data for cohort 9 (n=20, dose 101 units/m^2) graphically:

Figure 5: Median and Mean Serum Copper in Cohort 9 after the First Induction





As Figure 5 shows, both the median and mean copper levels in patients receiving 3 doses of CPX-351 at 101 units/m^2 was very high on day 7 (2 days after the last dose) but returned to normal levels in most patients by day 14. All patients had serum copper levels in the normal range by day 42 after the first induction. In summary, no safety problems are expected due to systemic copper exposure from CPX-351.

In this trial, serum copper levels will be monitored and elevated levels are assumed to be due to copper gluconate encapsulated within the CPX-351 liposome rather than free circulating copper. Clinical observation from a single patient treated with the identical copper-containing liposome encapsulating a different chemotherapy doublet (CPX-1, floxuridine and irinotecan) demonstrated sustained elevations of copper in a patient during a period of biliary obstruction, with return to baseline levels after stenting relieved the obstruction. This anecdote suggests that copper elimination after CPX-351 is likely via biliary excretion and elimination in the feces. Preclinical studies completed by Celator (acquired by Jazz Pharmaceuticals) demonstrated that virtually all of the copper administered in the form of CPX-351 is excreted via the feces within approximately 2 weeks of administration. It is expected that eligible patients with unobstructed biliary systems should be able to eliminate the copper administered within a few weeks. Serum copper levels will be monitored until they return to baseline levels (±20%) and patients will be monitored for hepatic dysfunction, a common manifestation of copper-related toxicity.

3.0 Study Objectives and Rationale

3.1 Primary Objectives

The primary objective of this study is to assess preliminary efficacy (as determined by the rate of CR or CRi) of two or three dose levels of CPX-351 in patients with newly diagnosed AML at high risk for induction mortality, defined as 30-50% predicted risk of death by Day 60, and to select the most promising dose level for further efficacy testing. Estimation of risk for induction mortality at patient screening will be based on factors associated with lower likelihood of AML response with or without factors that reduce tolerance to treatment-associated adverse events.

3.2 Secondary Objectives

To confirm the rate of dose limiting toxicities, including induction mortality (at day 60) for two different sub-MTD dose levels (50 and 75 U/m^2). If safety results suggest feasibility to test the MTD dose level of CPX-351 (100 U/m^2) in patients believed to be at high risk for induction mortality, the rate of dose limiting toxicities and induction mortality will be tested in this group as well.

3.3 Exploratory Objectives

To investigate the effect of CPX-351 on immune response, as determined by the effect on recovery of functional pathogen-specific and leukemia-specific immune responses and the recovery and function of NK cells.

To investigate the role of Troponin-T as an early marker for CPX-351-induced cardiotoxicity.

To investigate ex vivo the cytotoxicity of combination of Pim kinase inhibitor(s) and CPX-351 on circulating leukemia cells.

3.4 Study Rationale

The Phase I study identified the MTD in fit relapsed and refractory adult leukemia patients and confirmed that the intended 5:1 molar ratio was maintained for cytarabine:daunorubicin across multiple dose levels with markedly prolonged plasma half life of cytarabine and daunorubicin. At the MTD, CPX-351 was detectable in plasma at least 7 days after the last dose (Study Day 12). A substantial number of responses were observed among late stage patients already previously treated with cytarabine and daunorubicin.

The randomized Phase II results with CPX-351 suggest that CPX-351 is likely better tolerated than conventional cytarabine and daunorubicin-based chemotherapy with reduced 60-day mortality (4.6%) observed among newly diagnosed patients. Moreover, preclinical observations that CPX-351 concentrates in bone marrow with evidence for relatively selective internalization of liposomes within the cytoplasm of leukemia cells

suggests that CPX-351 action will be potent and that non-hematopoietic toxicities will be modest. Clinical observations from the Phase I study of complete response occurring at dose levels as low as one third to one half the MTD (32 to 43 units/m^2), strongly suggest that 25% and 50% dose reductions to 75 and 50 units/m^2 will still be effective and should be better tolerated. By using reduced dose CPX-351 as induction therapy it may be possible to improve the outcome of patients with AML considered at high risk for 60-day mortality, patients not ordinarily offered induction therapy for AML at all.

Data from the Phase I and both Phase II studies also demonstrated a relatively shallow dose/toxicity curve, with relatively little increase in non-hematopoietic toxicities with increasing doses up to the MTD. If sub-MTD dose levels of CPX-351 are well tolerated in patients at higher risk of early mortality, it may be possible to evaluate treatment at the MTD for patients at high risk for induction mortality. To the extent that higher dose levels maximize treatment efficacy, demonstration of treatment safety and feasibility at the MTD may permit treatment with the most efficacious dose level in patients at high risk for induction mortality.

4.0 Study Design

One cycle equals 28 days.

This study is an open-label, two-arm or possibly 3-arm, phase II trial. CPX-351 is administered as approximately a 90-minute infusion on days 1, 3, and 5 with one study arm receiving 50 units/m^2 and the other receiving 75 units/m^2. Both the 50 and the 75 unit/m^2 dose levels are considered to have acceptable safety based on observations from a pair of randomized Phase II studies conducted in adult patients, where the observed 60-day mortality results in patients considered fit for intensive chemotherapy and with no prior history of stem cell transplant, was observed to be reduced compared to control.

Patients with newly diagnosed AML who are predicted to be at high risk (30-50%) for induction mortality (death by day 60) are eligible. Every patient entered must have at least one AML-related factor (adverse cytogenetics, secondary AML, MDR phenotype, etc.) that contributes to elevated risk of induction mortality. Patients may or may not have patient-related factors (poor performance status, co-morbidities, poor organ function, etc.) that also contribute to elevated risk of induction mortality. 30 patients will be randomized with a 1:1 ratio to the two dose arms with 15 patients per arm to assess for safety and efficacy. If safety is demonstrated for both of the sub-MTD dose levels, and escalation is deemed feasible above 75 units/m^2, a cohort of 15 patients will be studied at 100 units/m^2, the MTD dose level. The latter dose (i.e., 100 units/m^2) was found safe and an additional 25 patients will be enrolled at this dose (for a total of 40 evaluable patients) to define efficacy at this standard dose.

At the end of the study a single dose level will be confirmed to have acceptable safety if the observed rate of dose limiting toxicity (DLT) is <u>≤5 DLT/15 patients.<33</u>%). Dose limiting toxicity is defined as induction mortality (death occurring during the first 60 days from start of study therapy), grade 3 or 4 non-hematologic toxicity, or dose limiting

hematologic toxicity at least possibly related to the study drug occurring during the first 60 days from the start of study therapy (i.e., during the first cycle of therapy).

For non-hematologic toxicity the following are counted as dose limiting toxicities only under the conditions noted:

- Liver toxicity: Grade 4, related to the study drug, not resolving to grade 2 within 7 days.
- Decrease in ejection fraction: Grade ≥3 (nadir LVEF <40-20%), related to study drug
- Creatinine elevation: Grade ≥3, related to study drug.
- Any other adverse event of Grade ≥3, related to study drug that in the opinion of the PI is inconsistent with the expected adverse events with standard chemotherapy.

Dose limiting hematologic toxicity is defined by bone marrow and peripheral blood examination at Day ≥56 showing:

- Absence of recurrent or persistent leukemia AND
- Persistently hypocellular marrow (<20% cellularity) AND
- Peripheral blood:
    - ANC< 500 and/or
    - Platelet count <10,000

If more than one dose level is considered acceptably safe, the decision about which one to use for future clinical study will be based on efficacy. Efficacy success for an individual patient is defined as achievement of CR or CRi. Observation of ≤3 CR or CRi among 15 patients (≤20%) defines a non-efficacious study arm. In situations where both study arms are considered equally safe, the study arm with the higher rate of response will be recommended for future study, provided it equals or exceeds 20%.
When the dose recommended for future study is defined, 10 additional newly diagnosed patients will be treated at that dose level (not to exceed a total of 25 patients at that dose level) to obtain additional evidence of safety and efficacy among previously untreated patients with high risk of induction mortality.

4.1 Patient Recruitment

All patients will be screened by a principal investigator or sub-investigator prior to entry on the study. An explanation of the study and discussion of the expected risks and benefits will be fully discussed with patients prior to the screening process in order for the patient to provide a voluntary written informed consent. Only eligible and consenting patients will be entered into the study.

4.2 Registration Procedures

Patients will be registered through CORe.

4.3 Patient Sample Size

A maximum of 80 patients will be treated.

A minimum of 15 patients will be treated at each dose level, unless conditions for early stopping are met based on excessive toxicity or insufficient safety (see section 10.0).

4.4 Toxicity Assessment

Toxicities will be evaluated and graded according to the NCI Common Terminology Criteria for Adverse Events, version 4 (CTCAE).

If a patient is discontinued due to progressive disease or intercurrent illness unrelated to toxicity attributable to CPX-351 and before safety can be assessed to rule out DLT, an additional patient will be recruited, to ensure that the minimum number of patients are evaluable for safety at each dose level.

4.5 Second Inductions

A second induction may be given if:

- The first induction does not produce a day 28 bone marrow demonstrating morphologic leukemia free state (≤5% blasts)
- Second inductions are given at the discretion of the investigator but are recommended in any patient with documentation of reduced leukemic burden, especially those with evidence of partial remission (≥50% reduction in % blasts count to 5-25% blasts).
- Second induction may be started on Day 28 from 1st cycle. In patients with rapidly proliferating disease second induction may be started earlier (but not earlier than Day 21) provided there is no residual study drug-related grade 2 non-hematologic toxicity after discussion with the principal investigator.

The CPX-351 dose for second induction is the same as for initial induction except that doses are given on Days 1 and 3 only. Patients unable to achieve a response (CR+CRi) after two inductions are discontinued from further treatment and are followed for survival only.

4.6 Consolidation Therapy

Only patients with documented response (CR or CRi) are eligible for consolidation. Consolidation with stem cell transplant (HSCT) is permitted either in place of chemotherapy consolidation or after chemotherapy consolidation. Prior to beginning every other chemotherapy consolidation after the cumulative DNR dose exceeds 300 mg/m^2,or prior to every course of treatment after the cumulative DNR dose exceeds 500 mg/m^2, patient LVEF must be documented to be ≥50% and patient PS must be 0-2.

Patients in CR with full recovery of peripheral blood counts within 56 days may be consolidated starting within one week of documentation of response. Patients with CRi may start consolidation no earlier than 42 days after the start of the last induction course

and only if ANC is >500/μL and Platelets are >50,000/μL. Consolidation courses consist of 2 doses administered at 65 units/m^2 on days 1 and 3.
A maximum of 4 courses of consolidation will be administered. It is understood that variations in timing of second induction and consolidations may be necessary and will be decided on a case by case basis by the PI. The rationale for variations in schedule will be documented by the PI. If consolidation is well tolerated and hematopoietic recovery is not delayed beyond 56 days, additional courses of consolidation may be offered immediately at time of recovery. In cases of poor patient tolerance or delayed hematopoietic recovery, the principal investigator will document his decision to either continue or terminate consolidation treatment.

Consolidation dosing:

First Consolidation	
Induction Outcome	**Consolidation Dose**
CR after induction ≤56 days	May start within one week of documentation of CR
CR after >56 days	Delay start of consolidation until day 70
CRi with at least ANC to ≥500/μL and platelets to ≥50,000/μL	Starts after day 42.
CRi with ANC<500/ μL or platelets <50,000/ μL	No consolidation to be given
Second and Subsequent Consolidations	
Previous Consolidation Outcome	**Consolidation Dose**
Count recovery to pre-consolidation baseline by Day 56	Same as first consolidation
Partial count recovery ANC (≥500/μL and platelets to ≥50,000/μL by Day 56	Delay second induction until day 70
Failure to recover (ANC <500/μL or platelets to <50,000/μL) by Day 56	No consolidation to be given

Variations of the schedule mentioned above may be considered in individual patients if felt to be in the best interest of the patient after discussion with the principal investigator and with proper documentation of the rationale for the change.

4.7 Salvage Therapy

Patients who never achieve CR/CRi following initial induction and patients who achieve CR/CRi and later relapse may receive salvage therapy.

4.8 Patient Follow-up

Follow-up for CR duration, EFS and safety (if applicable) terminates at the time of relapse, start of salvage or non-protocol treatment for leukemia (for persistent disease or transplant conditioning chemotherapy).

4.9 Study Modification/Discontinuation

Any amendments to the study will be documented in a revised protocol with a new assigned version.

The Investigator and the IND Office may stop the trial early for the following reasons:

- Unacceptable toxicity
- Discontinuation of drug development
- Poor enrollment
- Request by a regulatory authority

In the case of study discontinuation, the principal investigator will notify and discontinue patients from the trial and inform the IRB.

5.0 Selection and Withdrawal of Patients

5.1 Study Population

5.1.1 Inclusion criteria

5.1.1.1 Ability to understand and voluntarily sign an informed consent form

5.1.1.2 Age >18 years at the time of diagnosis of AML

5.1.1.3 Pathological diagnosis of AML according to WHO criteria (with at least 20% blasts in the peripheral blood or bone marrow):

- Newly Diagnosed De novo AML; except for APL
- Newly Diagnosed Secondary AML, defined as having a history of an antecedent hematologic disorder (myelodysplastic syndromes [MDS], myeloproliferative disease [MPD] or history of cytotoxic treatment for non-hematologic malignancy) or apparent de novo AML with MDS-associated karyotype. (Appendix 9)

5.1.1.4 Eastern Cooperative Oncology Group (ECOG) performance status 0-3

5.1.1.5 Laboratory values fulfilling the following:

- Serum creatinine ≤ 2.0 mg/dL
- Serum total bilirubin ≤ 2.0 mg/dL
- Serum alanine aminotransferase < 3 times the ULN Note: If elevated liver enzymes are related to disease ALT should be <5 times ULN.

5.1.1.6 To be considered at high risk for induction mortality patients must have 1 or 2 of the following risk factors (patients ≥60 must have at least 1 risk factor, patients <60 must have at least 2 risk factors) present. At least one risk factor in every patient must be an AML-related factor:

AML-related factors include:

- AHD (MDS, CMML, or MPD) or history of exposure to cytotoxic chemotherapy (t-AML)), or WHO-defined AML with MDS-related changes or apparent de novo AML with MDS-associated karyotype.
- Unfavorable cytogenetics as defined by the European Leukemia Net (~~Appendix F)~~ Appendix 11

Patient-related factors:
- Age ≥70
- ECOG PS ≥2
- Co-morbidities: Serum creatinine >1.3 g/dL

5.1.1.7 Cardiac ejection fraction ≥ 50% by echocardiography or MUGA (when LVEF expressed as a range, at least the upper limit should include 50%).

5.1.1.8 Able to adhere to the study visit schedule and other protocol requirements

5.1.1.9 All men and women must agree to practice effective contraception during the study period if not otherwise documented to be infertile.

5.1.2 Exclusion Criteria

5.1.2.1 Patients with history of second malignancy are eligible if they have documentation of disease stability, off therapy, based on CT scan or other measures for the 6 months prior to entry in core.

5.1.2.2 Any serious medical condition or psychiatric illness that would prevent the patient from providing informed consent.

5.1.2.3 Chemotherapy or other investigational anticancer therapeutic drugs in the two weeks prior to study entry; in the event of rapidly proliferative disease, however, the use of hydroxyurea is permitted up to 24 hours before study entry in core

5.1.2.4 Evidence of active CNS leukemia

5.1.2.5 Pregnant or lactating women

5.1.2.6 Uncontrolled infection; to be eligible, patients receiving treatment for an infection (antibiotic, antifungal or antiviral treatment) must be afebrile (<38.3ºC) and without hemodynamic instability or dyspnea from pneumonia for >48 hrs prior to the start of induction therapy.

5.1.2.7 Hypersensitivity to cytarabine, daunorubicin or liposomal products

5.1.2.8 History of Wilson's disease or other copper-metabolism disorder

5.2 Withdrawal of Patients

Patients will be discontinued from treatment and begin follow-up for survival under the following circumstances:

- Persistent disease: lack of a response to treatment after two cycles of induction therapy
- Relapsed disease: re-appearance of disease following CR or CRi
- Unacceptable toxicity
- Patient non-compliance with protocol
- Administration of non-protocol chemotherapy
- Intercurrent illness which, in the judgment of the investigator, affects assessment of clinical status to a significant degree, and requires discontinuation of protocol therapy.

During any phase of the study, if a patient requests termination of treatment and/or follow-up, the patient will be discontinued and no further information collected. The patient will be classified as withdrawal of consent.

6.0 Treatment of Patients

See APPENDIX 1: Patient Evaluation Flow Sheet

Copper Levels: MDACC will draw the serum copper, send it out for processing and will report the results in clinic station in approximately 4 days. This will be included in the budget for the cost incurred by the MDACC lab. MDACC will be reimbursed by the Supporter of this study (Jazz Pharmaceuticals) for this test.

6.1 Pre-Treatment Evaluations

The date of the first test or exam will be considered as date of the screening visit.

Procedure	Evaluation	Timing
Informed Consent	It should be personally signed and dated by the patient. The investigator must also personally sign and date the document. A copy of the Informed Consent must be given to the patient. The patient's study screening must be conspicuously noted in the source documentation.	Informed consent should be obtained prior to initiation of screening procedures. If the period between ICF signature date and screening visit is ≥30 days the patient must sign another ICF.
Demography	Date of birth, sex Basis for high risk of induction mortality	Within 14 days prior to registration in core
Medical History	Complete medical history	Within 14 days prior to registration in core
Physical Exam	Objective review of body systems ECOG Performance Status	Within 3 days prior to registration in core
Vital Signs	Heart rate Blood pressure Temperature Respiratory rate	Within 3 days prior to registration in core
Hematology	Hemoglobin White Blood Count Platelets Differential Count (may be omitted if WBC ≤0.5 x10^9/L)	Within 1 day prior to registration in core
Biochemistry	BUN Creatinine Electrolytes (Sodium, Potassium, Chloride) Bilirubin AST or ALT	Within 1 day prior to registration in core
Bone Marrow Aspiration and/or Biopsy	Morphology	Within 14 days prior to registration in core
Copper levels	Serum copper	Within 14 days prior to registration in core

Procedure	Evaluation	Timing
Diagnostic Imaging	Chest X-ray Echocardiography or MUGA scan	Within 28 days prior to registration in core
ECG		Within 14 days prior to registration in core
Cytogenetics	Cytogenetics (performed locally) Molecular abnormalities: Evaluation of CEBPA, FLT3, and NPM1	Within 3 months prior to registration in core: patients may be registered and treated prior to the cytogenetic test results; however, every attempt should be made to have the results prior to registration in core
Correlative studies (optional)*	Immune reconstitution	Before the start of induction therapy
Correlative studies (optional)*	BNP, Troponin I, high-sensitivity troponin (I or T)	Before the start of induction chemotherapy
Correlative studies (optional)*	Pharmacodynamics	Before the start of induction chemotherapy
Pregnancy Test	Urine or serum pregnancy test	Within 7 days prior to registration in core

* Failure to collect samples for optional studies will not constitute a protocol deviation.

6.2 Evaluation during Treatment

Inductions and consolidations are administered as courses. A course consists of the administration of therapy with scheduled assessments to evaluate the response to treatment. The first induction may end before the completion of all evaluations if a second induction is necessary, (see Section 4.5). Induction is completed when a patient has

- A confirmed CR (see section 8.4)
- A CRi (see section 8.4) and is to begin consolidation treatment before documentation of hematologic count recovery
- Persistent/recurrent disease (PD/relapse)
- Response evaluation cannot be performed because of the patient's condition and no further study treatment can or will be administered.

Patients with a CR or CRi may receive consolidation treatments. Evaluations on Days 1-10 must be performed on the day indicated (±2 day); all other evaluations are to be performed on the Study Day indicated ±3 days, except when specifically indicated. Each course requires the following evaluations:

Procedure	Evaluation	Timing
Physical Exam	Objective review of body systems	Days 14 and 42
Vital Signs	Heart rate Blood pressure Temperature Respiratory rate	Days 14 and 42

Procedure	Evaluation	Timing
Hematology	Hemoglobin White Blood Count Platelets Differential Count (may be omitted if WBC $\leq$0.5 x10^9/L)	Days 1, 3, 5, 7, 10±2, 14±3, then weekly (±3days) until Day 42 or peripheral blood count recovery
Biochemistry	BUN Creatinine Electrolytes (Sodium, Potassium, Chloride) Bilirubin AST or ALT	Days 1, 3, 5, 7, 10±2, 14±3, then weekly (±3days) until Day 42
Copper levels	Serum Copper	Perform prior to next treatment course
Bone Marrow Aspiration and/or Biopsy	Morphology	Day 28 (±7 days) after every induction.
Cytogenetics	Cytogenetics (performed locally) Molecular abnormalities: Evaluation of CEBPA, FLT3, and NPM1	Required in patients with a CR or CRi with positive baseline findings (perform at the time of bone marrow assessment for CR or CRi). Optional in patient with normal findings at baseline
Diagnostic Imaging	Echocardiography or MUGA scan to assess LVEF	Prior to every other course of treatment after the cumulative DNR dose exceeds 300 mg/m^2, prior to every course of treatment after the cumulative DNR dose exceeds 500 mg/m^2, or 30 days after the last course.
Response Assessment		See Section 8.4.1
Adverse Events/Toxicity	CTCAE v.4 assessment	Beginning at the start of the first infusion, continually assess during Treatment Period and record and report any new serious adverse events (up to 30 days after completion of Treatment Period)
Concomitant Medications		Continually assess during Treatment Period
Correlative studies (optional)*	Immune effects	Before induction and at start of 1st and 2nd consolidation
Correlative studies (optional)*	BNP, Troponin I, high-sensitivity troponin (I or T)	Before the start of each cycle, and approximately 24 hrs from end of infusion on day 1 and day 5 of each cycle, and approximately on day 14 (±4 days) of each cycle, end of treatment.
Correlative studies (optional)*	Pharmacodynamics	Pre-dose and 24-hrs (±4 hrs) after first dose.

* Failure to collect samples for optional studies will not constitute a protocol deviation.

6.3 Early Termination or End of Treatment

Any patient that completes or discontinues treatment must have the following evaluations performed within 30 days after termination and prior to the initiation of any salvage therapy, if not performed within the last 30 days:

Procedure	Evaluation	Timing
Physical exam	Objective review of body systems ECOG Performance Status	Within 30 days after discontinuation
Vital signs	Heart rate Blood pressure Temperature Respiratory rate	Within 30 days after discontinuation
Hematology	Hemoglobin White Blood Count Platelets Differential Count (may be omitted if WBC $\leq$0.5 x10^9/L)	Within 30 days after discontinuation
Biochemistry	BUN Creatinine Electrolytes (Sodium, Potassium, Chloride) Bilirubin AST or ALT	Within 30 days after discontinuation
Diagnostic Imaging	Echocardiography/MUGA	Within 30 days after discontinuation if a study has not been performed since last treatment but before the initiation of any non-protocol treatment
ECG		Within 30 days after discontinuation
Copper levels	Serum Copper	Within 30 days after discontinuation if a study has not been performed since last treatment
Adverse Events/Toxicity	CTCAE v.4 assessment	Assess Adverse events that were ongoing at the time of discontinuation and record and report any new serious adverse events (up to 30 days after discontinuation)
Correlative studies (optional)*	BNP, Troponin I, high-sensitivity troponin (I or T)	Within 30 days after discontinuation

* Failure to collect samples for optional studies will not constitute a protocol deviation.

6.4 Evaluation during Long-term Follow-up

The following evaluations are completed during the follow-up phase (i.e., after completion of all therapy to continue for one year except for survival and relapse status which will continue until death and relapse, respectively):

Procedure	Evaluation	Timing
Patient status report	Survival status	Until death
	Relapse status New anti-leukemic therapies	Monthly until relapse or the initiation of new therapy
Hematology	Hemoglobin Hematocrit Red Blood Count White Blood Count Platelets Differential Count (may be omitted if WBC $\leq$0.5 x10^9/L)	Every 2-3 months for one year until relapse or the initiation of new therapy, then as clinically indicated.
Bone Marrow Aspiration and/or Biopsy	Morphology	Every 3-4 months. For patients in CR, consider performing if peripheral blood counts fall below 1000/µL for ANC or 100,000/µL for platelets for >1 month with no obvious explanation for the drop or at any time there is suspicion of relapse; for patients in CRi perform if counts fall significantly below peak recovery. If the peripheral blood counts in a patient with a CRi recover to CR levels ($\geq$1000/µL for ANC or $\geq$100,000/µL for platelets), consider performing a bone marrow evaluation to confirm full CR. Following the first year of follow up, aspirations will be done as clinically indicated; record relapse information, including any bone marrow evaluations. Not required following the initiation of new therapy and or relapse.
Diagnostic Imaging	Echocardiography or MUGA	Perform every 3-4 months if last Treatment Phase LVEF was reduced >10% from baseline and is less than 50%. Continue assessments until LVEF returns to baseline or until 1 year from start of therapy.
Biochemistry	BUN Creatinine Bilirubin AST or ALT	Every 2-3 months for one year until relapse or the initiation of new therapy, then as clinically indicated.
Copper levels	Serum Copper	Perform monthly only if elevated copper (>20% above ULN) present at EOT. Perform every 1 month until abnormality returns to within 20% of ULN, or until 1 year, or the initiation of new therapy, or relapse (whichever is earliest)

7.0 Drug Administration

The responsibility for treatment of patients rests with the individual investigator.

7.1 Drug Preparation and Administration

7.1.1 Drug Preparation

The appropriate number of vials of CPX-351 (cytarabine:daunorubicin) Liposome Injection should be removed from the refrigerator prior to reconstitution. Reconstitute with 19 mL of water for injection using a 20 mL syringe. Do not heat CPX-351 (cytarabine:daunorubicin) Liposome Injection. After reconstitution, invert vials gently 3-4 times and let rest for 15 minutes prior to withdrawing drug for dilution. CPX-351 (cytarabine:daunorubicin) Liposome Injection should be diluted in approximately 500 mL of saline injection or dextrose injection.

The IV bags and infusion sets must be non-DEHP. Aseptic technique must be strictly observed throughout the handling of CPX-351 (cytarabine:daunorubicin) Liposome Injection, since no bacteriostatic agent or preservative is present. The infusion of CPX-351 (cytarabine:daunorubicin) Liposome Injection must be started within 4 hours of dilution. Vials are for single use. Unused material should be recorded as such and discarded according to institutional policies. Procedures for proper handling and disposal of anticancer drugs should be implemented.

7.1.2 Drug Administration

The infusion of CPX-351 (cytarabine:daunorubicin) Liposome Injection will be performed through a central venous catheter, using an infusion pump to ensure that the drug is infused over the specified time period. Non-DEHP containing administration sets should be used. **Do not use an in-line filter.** CPX-351 should never be given by the intramuscular or subcutaneous route. Administer CPX-351 over approximately 90 minutes via an infusion pump. Flush the line to ensure administration of the full dose.

The dosage (total units and u/m^2), start/stop time of the infusion, total volume infused, must be documented in the patient's chart.

7.2 Drug Accountability

The study pharmacist or designee must maintain records of the delivery of CPX-351 to the study site, the inventory at the site, the use by each patient, and the disposition of unused product. These records should include dates, quantities, lot numbers, expiration dates and patient identifications. The institution should maintain records that document adequately that patients were provided the doses specified by the protocol and reconcile all investigational product received from the manufacturer. Records of storage conditions (temperature logs) must be kept for the entire period that CPX-351 is maintained at the institution. Unused or expired drug will be disposed per MDACC policy.

7.3 Dose Reductions and Delays

Induction doses are not reduced. Consolidation doses are 65 units/m^2 on days 1 and 3. In instances with prolonged myelosuppression or other medical conditions where the investigator feels further dose reduction might be required, 50 units/m^2 on days 1 and 3 may be used after discussion with PI and sponsor and documentation of rationale. Patients with evidence of delayed recovery from cytopenias or acute toxicities following induction or consolidation (>56 days) may have consolidation doses delayed by up to 2 weeks to permit recovery. Any doses missed or delayed due to toxicity may be administered as soon as the patient has recovered from the toxicity. Toxicities will be graded using the CTCAE Version 4.0 (See APPENDIX 5: Common Terminology Criteria for Adverse Events V4.0 (CTCAE)).

7.4 Concomitant Therapy

7.4.1 Premedication

Nausea and vomiting:
Patients may receive prophylaxis or therapy for nausea and/or vomiting according to institutional standards.

Hypersensitivity/Infusion-related reactions:
Patients will not be routinely pre-medicated for hypersensitivity or infusion-related reactions initially during the first infusion of the first treatment course. If the patient develops a hypersensitivity reaction then he/she should be pre-medicated at all subsequent infusions.

7.4.2 Suggested guidelines for management of hypersensitivity reactions:

Mild symptoms (e.g., mild flushing, rash, pruritus):
Stop infusion and supervise at bedside with monitoring of vital signs
Reinitiate infusion slowly (halving the rate of infusion) +/- premedication

Moderate symptoms (e.g. moderate rash, flushing, mild dyspnea, chest discomfort):
Stop infusion and give IV diphenhydramine, 20-25 mg (or equivalent) and IV dexamethasone 10 mg.
Do not reinitiate infusion. Subsequent doses should be administered with premedication (e.g., IV diphenhydramine, 20-25 mg (or equivalent) and IV dexamethasone 10 mg) and may be given at the same dose and rate.

Severe/life-threatening symptoms (e.g. hypotension requiring vasopressor therapy, angioedema, respiratory distress requiring bronchodilation therapy, generalized urticaria):
Stop infusion. Administer IV diphenhydramine and dexamethasone as indicated above. Add epinephrine (adrenaline) or bronchodilators if indicated. Do not reinitiate infusion. Do not retreat. Report this event as a serious adverse event.

If hypersensitivity or infusion-related reactions become a clinically relevant toxicity, then premedication for hypersensitivity reactions will be instituted with drugs, doses and schedule according to each investigator's preference. Additionally, a decision may be made to prolong the infusion time to two hours or more.

7.4.3 Permitted therapy

Patients may receive ongoing supportive and palliative care (e.g. pain control) as clinically indicated throughout the study.

Infection Prophylaxis: Prophylactic use of anti-infectives is highly recommended during the period of profound neutropenia until ANC returns to 500/μL or greater. The choice of anti-infectives will be according to institutional protocol. Use of anti-infective agents as prophylaxis and treatment must be documented on the case report forms.

Growth Factor support: The use of growth factors will be according to institutional protocol and/or according to ASCO criteria.(14) Use of growth factors must be documented on the case report forms.

Transfusion support: The use of transfusion support (RBCs and platelets) will be according to institutional protocol. Use of transfusion support must be documented on the case report forms.

7.4.4 Therapy that is not permitted

Other anti-cancer treatment and other investigational therapy(ies) are not permitted during the Treatment Phase. In the event of persistent disease or relapse the patient may receive other anti-leukemic therapies. Administration of isolated doses of intrathecal chemotherapy is allowed.

7.5 Duration of Protocol Treatment

Patients may continue on study provided they have not met the criteria for discontinuation of therapy (See Section 5.2). The table below summarizes the expected duration of the Treatment Phase. Patients may receive up to two induction courses followed by consolidation courses. After the Treatment Phase, patients are followed until death.

8.0 Assessment of Efficacy

8.1 Evaluable for Efficacy

All patients receiving one or more doses of study therapy are evaluable for efficacy.

8.2 Overall Survival

All enrolled patients are assessed for overall survival. Overall survival is measured from the date of registration to death from any cause, patients not known to have died at last follow-up are censored on the date they were last known to be alive.

8.3 Event-free Survival

All enrolled patients are assessed for Event-free survival. Event-free survival is defined as the time from study registration to the date of induction treatment failure (persistent disease), relapse from CR or CRi, initiation of non-protocol chemotherapy (e.g. salvage therapy), or death from any cause, whichever comes first. Patients transferred to transplant will be censored for Event-free Survival at the start of conditioning therapy. Patients alive and not known to have any of these events are censored on the date they were last examined.

8.4 Response Assessment Criteria

During the Treatment Phase patients will be assessed for response according to the following criteria(15):

Complete remission (CR)[1]	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count >1.0 x 10^9/L (1000/μL); platelet count >100 x 10^9/L (100,000/μL).
CR with incomplete recovery (CRi)[2]	All CR criteria except for residual neutropenia (<1.0 x 10^9/L [1000/μL]) **and/or** thrombocytopenia (<100 x 10^9/L [100,000/μL])
Treatment failure	
Persistent Disease (PD)	Failure to achieve CR or CRi; only includes patients surviving ≥7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination
Death in aplasia	Deaths occurring ≥7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring ≥7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available
Relapse[3]	Bone marrow blasts ≥5% (confirmed); or confirmed, sustained reappearance of blasts in the blood after achievement of a CR or CRi, not considered due to recovery from chemotherapy; or development of extramedullary disease

[1]All criteria need to be fulfilled; marrow evaluation should be based on a count of 200 nucleated cells in an aspirate with spicules; if ambiguous, consider repeat exam after 5 to 7 days; flow cytometric evaluation may help to distinguish between persistent leukemia and regenerating normal marrow; a marrow biopsy should be performed in cases of dry tap, or if no spicules are obtained; no minimum duration of response required.

[2]Some patients may not achieve complete hematologic recovery prior to initiation of consolidation. CRi usually cannot be declared earlier than Day 42 to allow for adequate time for documentation of peripheral blood recovery. Consolidation usually begins no earlier than 42 days after the last induction course.

[3]In cases with low blast percentages (5-10%), a repeat marrow should be performed to confirm relapse. Appearance of new dysplastic changes should be closely monitored for emerging relapse. In a patient who has been recently treated, dysplasia or a transient increase in blasts may reflect a chemotherapy effect and recovery of hematopoiesis.

The response of patients with no post-baseline bone marrow assessment is entered as not done.

8.4.1 Timing of response assessment

In general, the patient's response to induction therapy is made on the first day when all criteria for CR or treatment failure are met. The bone marrow assessment and the

peripheral counts are not required to be performed on the same day but recovery of counts (including absence of peripheral blasts) must be performed within 14 days of the bone marrow assessment.

8.4.2 Best Response

Patients who complete the induction(s) with a response of CRi may be upgraded to a CR during or after consolidation if the patient's peripheral blood counts meet the criteria for CR after declaration of a CRi. To upgrade a response to CR both peripheral blood and bone marrow assessment must be obtained within a 14 day period and all criteria for CR must be met.

8.5 Remission Duration

Only patients achieving CR or CRi are assessed for remission duration (relapse-free survival). Remission duration is measured from the date of achievement of a remission (CR/CRi) until the date of relapse or death from any cause; patients not known to have relapsed or died at last follow-up are censored on the date they were last examined.

8.6 Morphologic Leukemia-free State

All randomized patients that have at least one evaluable post-randomization bone marrow assessment performed on or after Day 14 after the last induction are assessed for morphologic leukemia-free state. Morphologic leukemia-free state is defined as bone marrow blasts <5% AND absence of Auer rods and/or extramedullary disease.

9.0 Assessment of Safety

9.1 Evaluable for Safety

All patients who have received at least one dose will be considered evaluable for safety.

9.2 Adverse Events

9.2.1 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered with a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. This includes worsening of a pre-existing condition or increase in frequency of a pre-existing condition. An adverse event is considered serious if it meets any of the serious criteria listed in Section 9.2.2. To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as

"serious", which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Adverse events are to be recorded in the case report form from the start of the infusion on Day 1 until 30 days after the last dose of study drug, with the exception of serious adverse events. (See Section 9.2.2). Leukemia Department Guidelines for AE recording will be used.

The Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

Adverse events and protocol specific data will be entered into CORe/PDMS. CORe/PDMS will be used as the electronic case report form for this protocol.

Adverse drug reactions (ADRs) are all noxious and unintended responses to a medicinal product related to any dose that a causal relationship between the medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out. An unexpected ADR is any adverse reaction not identified in nature or intensity in the current Investigator's Brochure.

9.2.2 Serious Adverse Event Reporting

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

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Serious Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).
- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.
- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.
- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.
- Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

9.2.3 Communication between Investigator and Jazz Pharmaceuticals

The Investigator must send all SAEs (initial and follow up) that require collection and reporting per protocol, and which occur in a patient who received CPX-351, to Jazz Pharmaceuticals within 1 business day of their awareness of the SAE via email to AEreporting@jazzpharma.com. In addition, all other adverse events must be reported to Jazz in summary of line-item form upon Jazz's request.

Follow-up information is sent either via a new Serious Adverse Event Form (stating that this is a follow-up), or via the original one form (with the new information highlighted and a new date provided). The follow-up should describe whether the event has resolved

or continues, if and how it was treated, whether the patient continued or discontinued study participation.

The telephone and fax numbers of the local Clinical Research contact person and the contact person in the local department of Clinical Safety, specific to the site, must be listed in the investigator folder provided for each individual site and provided to the manufacturer at the start of the trial. Questions referring to a specific serious adverse event occurring in a study patient should be directed to the local Clinical Research contact person specified in the investigator folder provided for the site.

9.3 Cardiac Toxicity Monitoring

As anthracyclines are known to have an adverse effect on cardiac function, each patient's cardiac function will be monitored through the Treatment and Follow-up Phases. Any left ventricular ejection fraction below 50% will be recorded as adverse events. If the left ventricular ejection fraction is reduced >10% to below 50% from the baseline assessment at the last Treatment Phase measurement, left ventricular ejection fraction will continue to be monitored every 3 months until a return to baseline or 1 year, whichever comes first.

9.4 Laboratory Data

Laboratory data obtained according to the schedule of assessments will be recorded on the CRF or other data collection instrument. Only laboratory data requested by the protocol need be recorded unless specific findings result in a clinical event such as an adverse event or documentation of peripheral blood count recovery. These results will be collected.

9.4.1 Copper Assessment

Patients will have serum copper assessed routinely and the percentage of patients with persistent (>1 year) copper elevations will be reported. Also, patients with persistently elevated serum copper will be evaluated for clinical abnormalities associated with copper toxicity (e.g. unexplained increase in liver function tests). Serum copper elevations are laboratory values and are not reported as adverse events unless associated clinical signs and symptoms of copper toxicity.

MDACC will draw the serum copper, send it out for processing and will report the results in clinic station in approximately 4 days.

10.0 Statistical Considerations(16, 17)

General Description:

This is a Phase II study of CPX-351 Liposome Injection in newly diagnosed AML patients with high risk of induction mortality. Two dose levels (50 units/m2 and 75 units/m2) of CPX-351 will be studied initially. The primary objective is to evaluate treatment response. The efficacy outcome is CR or CRi within two induction cycles. A maximum of 30 patients will be equally randomized to the two dose levels. A Bayesian method by Thall et al (1995)

will be used for futility and toxicity monitoring. After 15 patients have been accrued to each dose level, an assessment of overall safety/dose limiting toxicities and efficacy will be made and one dose level will be chosen for further study. OR if both dose levels are safe and further dose escalation seems possible, one additional cohort with a dose of 100 units/m2 will be open. A maximum of 15 patients will be enrolled in this arm and the same toxicity and futility monitor rules will be applied. After 2 or 3 dose levels have been studied, one dose levels will be chosen for further study. An expansion cohort of 10 patients will be treated at the dose level with a favorable response and/or toxicity profile for further evaluation for safety and efficacy. Consequently, the preferred dose level has 25 patients evaluable.

Additional 25 patients will be added to the arm with the dose of 100 units/m2, the sample size for this arm will be up to 40, and the maximum number of patients for all three arms will be up to 80.

Multc Lean software version 2.1 developed in the Department of Biostatistics MDACC was used for the study design.

The study will monitor futility and toxicity, and the monitoring rules will be applied to each dose separately. If study in one of the two doses is stopped early due to futility or excessive toxicity, all the future patients will be enrolled in the open dose arm (i.e., without randomization). In the case the lower dose (50 units/m2) has been shown to be too toxic at an interim look (thus stop further patient enrollment) the higher dose (75 units/m2) will also stop accrual.

Futility monitoring:

Treatment success is defined as achievement of CR or CRi. We will accrue a maximum of 15 patients at each dose level and test the null hypothesis that the true response is at most 5% (meaning little or no activity) versus the alternative hypothesis that the true response is 30% or greater, independently in each treatment arm. Assuming that the success rate is binomially distributed, this design with 15 patients in each arm has 87% power and a one-sided type I error rate of 0.05 using an exact test.

To monitor futility, the study in one dose level will be stopped early if the data in that dose level suggest that:

$$\Pr(\theta_E < 0.25 + \theta_S \mid \text{data}) > 0.95$$

That is, if at any time during the study we determine that there is a greater than 95% chance that the response rate is less likely to improve by 25% than the null hypothesis in one dose level, we will terminate the enrollment in that dose. Here θ_E represents the response rate for experimental drug in this study and θ_S represents the response rate for the standard practice (null hypothesis). We assume that θ_S has a distribution of beta (10, 190) to reflect the information on 200 patients for the historical data, which has a mean response rate of 0.05 and a variance of 0.0002. We also assume that θ_E has a non-informative flat prior beta(0.1, 1.9) with mean of 0.05 and variance of 0.0158.

The futility stopping boundaries for the overall response based on these assumptions and monitoring condition is shown in table 7. For example, accrual will be stopped in one dose if there is no response observed in the first 5 patients treated in that dose.

The toxicity monitoring/efficacy summary will be submitted to the IND Office Medical Monitor (Dr. Agueda Cohen) for every 5 subjects treated in each Arm (per Table7, 8, 11 & 12).

Table 7: Stopping boundaries for futility monitoring for the initial cohort of each dose (n=15)

# Patients (in complete cohorts of 5) (inclusive)	**Stop the enrollment in one dose if there are this many responses total in that dose:**
	# Responses (inclusive)
5	0
10	0-1
15	Always stop with this many patients

Toxicity monitoring

In addition, toxicity will be monitored closely. Denote the probability of toxicity by T_E, where toxicity is defined as DLTs. We assume toxicity rate beta (0.66, 1.34) for the experimental treatment in this study. Our safety monitoring rule is described as follows: $Pr(T_E > 0.33 \mid data) > 0.85$. That is, we will stop the enrollment in one dose if, at any time during the study, we determine that there is more than 85% chance that the DLT rate is more than 33% in that dose. Stopping boundaries corresponding to this stopping rule is listed in table 8. For example, the enrollment in one dose will be stopped if the first 4 of the first 5 patients experienced toxicity in that dose.

Table 8: Stopping boundaries for toxicity monitoring for the initial cohort of each dose (n=15)

# Patients (in complete cohorts of 5) (inclusive)	**Stop the enrollment in dose level if there are this many DLTs total in that dose level:**
	# DLTs (inclusive)
5	4-5
10	6-10
15	Always stop with this many patients

The operating characteristics for this design are presented in table 9. If the enrollment in one dose continues until 15 patients are evaluated in each arm and 5 CR or CRi are observed, then the 95% credible interval for the response rate would be (11.6%, 47.7%).

Table 9: Operating characteristics of the initial cohort of each dose (n=15)

True CR+CRi Rate	True DLT Rate	Early Stopping Probabilities	Average sample size treated
0.05	0.1	0.931	6.47
0.05	0.2	0.932	6.46
0.05	0.33	0.938	6.39
0.05	0.5	0.959	6.12
0.1	0.1	0.784	88.13
0.1	0.2	0.787	8.10
0.1	0.33	0.804	7.94
0.1	0.5	0.872	7.30
0.2	0.1	0.462	11.1
0.2	0.2	0.468	11.0
0.2	0.33	0.512	10.66
0.2	0.5	0.681	9.33
0.3	0.1	0.229	13.01
0.3	0.2	0.237	12.95
0.3	0.33	0.30	12.5
0.3	0.5	0.543	10.67
0.3	0.6	0.735	9.08
0.4	0.1	0.098	14.11
0.4	0.2	0.108	14.04
0.4	0.33	0.181	13.50
0.4	0.5	0.465	11.42
0.4	0.6	0.691	9.61

If three dose arms continue until 15 patients in each arm evaluated, the toxicity profile and response will be summarized for them. The dose chosen for further study will be the dose with the highest CR+CRi rate, provided that toxicity profile are also acceptable. The probability of correctly selecting the superior dose for different success rate scenarios in the three arms is summarized in table 10 below [Simon R et al 1985].

Table 10: Probability of the best arm being chosen

True Response rate in Superior Dose	True Response rate in inferior Dose 1	True Response rate in inferior Dose 2	Probability of the best arm is chosen

30%	10%	10%	86%
30%	15%	15%	71%
40%	25%	25%	63%
40%	30%	25%	56%
40%	30%	30%	50%
50%	25%	25%	83%
50%	30%	25%	78%
50%	30%	30%	73%
50%	40%	30%	59%
60%	25%	25%	94%
60%	30%	25%	91%
60%	30%	30%	89%
60%	40%	30%	79%
60%	50%	30%	63%

The arm with a favorable toxicity profile and/or a favorable might be chosen for further evaluation by expanding a cohort of 10 additional patients in that arm. We will use the same criteria (e.g. expansion will be stopped if $Pr(T_E > 0.33 \mid data) > 0.85$) to monitor toxicity for the expansion cohort by using a total sample size of 25 for the winner arm. The stop boundaries are summarized in the following table. If 10 or more patients out of the 1st 20 patients experienced DLT, the trial will be stopped and no expansion cohort will be evaluated. If 10 or more patients out of 20 patients experienced DLT (by adding 5 more patients to the 15 patients in that arm), the expansion will be stopped.

Table 11. Toxicity monitoring for the expanded cohort at a dose of 75 units/m^2

# Patients (in complete cohorts of 5) (inclusive)	**Stop the arm with expansion cohort if there are this many dose limiting toxicities total:**
	# Toxicities (inclusive)
5	4-5
10	6-10
15	8-15
20	10-20
25	Always stop with this many patients

Expansion of arm with the dose of 100 units/m^2

Up to additional 25 patients will be enrolled in this arm, thus total sample size for this arm is up to 40. Assuming that the success rate is binomially distributed, 40 patients will have has 99% power to detect a difference of 5% vs. 30% in success rate at a one-sided type I error rate of 0.05 using an exact test.

Similar futility and toxicity monitoring rules listed above will also be applied for the expansion of arm with 100 units/m^2. That is, the trial will be stopped early if

$$Pr(\theta_E < 0.25 + \theta_S \mid data) > 0.98 \text{ for futility}$$

or

$$Pr(T_E > 0.33 \mid data) > 0.95 \text{ for toxicity}$$

where θ_E represents the response rate for experimental drug in this study and θ_S represents the response rate for the standard practice (null hypothesis). T_E represents DLT rate. For trial simulation, we use the same assumption for prior distributions listed above for each parameter. That is, θ_S has a prior distribution of beta (10, 190), θ_E has a non-informative flat prior beta(0.1, 1.9), and T_E has a prior distribution of beta (0.66, 1.34).

Table 12 and 13 present the corresponding stopping boundary and operating characteristics (OCs).

Table 12: Stopping boundaries for futility and toxicity of the expansion cohort with the dose of 100 units/m^2

N patients evaluated	Stop for toxicity if >= this number of patients experience DLT	Stop for futility if <= this number of patients achieve CR/Ci
5	4-5	0
10	7-10	0
15	9-15	0-1
20	11-20	0-2
25	13-25	0-3
30	15-30	0-4
35	17-35	0-5
40	19-40	0-6

Table 13: OCs of the expansion cohort with the dose of 100 units/m^2

True CR+CRi Rate	True DLT Rate	Early Stopping Probabilities	Average sample size treated
0.05	0.1	0.9965	8.16
0.05	0.2	0.9965	8.14
0.05	0.33	0.9969	7.99
0.05	0.5	0.999	7.26
0.1	0.1	0.9294	13.16
0.1	0.2	0.930	13.10
0.1	0.33	0.9372	12.63
0.1	0.5	0.9803	10.19
0.2	0.1	0.5119	25.54
0.2	0.2	0.5156	25.39
0.2	0.33	0.5654	24.00

0.2	0.5	0.8637	16.36
0.3	0.1	0.2029	33.50
0.3	0.2	0.209	33.30
0.3	0.33	0.2903	31.31
0.3	0.5	0.7774	20.23
0.4	0.1	0.0817	37.19
0.4	0.2	0.0887	36.96
0.4	0.33	0.1824	34.70
0.4	0.5	0.7436	22.12
0.4	0.6	0.9612	14.02

Statistical methods:

Data analysis will be performed using SAS or S-plus, as appropriate. For each arm, the CR/CRi rate and the DLT rate will be estimated with95% confidence intervals. The intent-to-treat patients will be used for the primary efficacy analysis, patients who lost-to-follow up in the first 2 cycles will be treated as failures. Patients who received at least one dose of the treatment drug will be evaluable for toxicity outcomes. In addition, Fisher's exact test will be used to compare the response rate and toxicity rate between the two dose levels. For the exploratory objectives, Fisher's exact test will be used to compare immune responses between different doses of CPX-351. Two-sample t test or ANOVA or Fisher's exact test will be used to check the association between Troponin – T and cardiotoxicity, and t test or ANOVA will be used to compare the apoptosis of ex-vivo cultured leukemia cells.

11.0 References

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12.0 APPENDIX 1: Patient Evaluation Flow Sheet – Treatment Phase

Evaluations (except screening) to be conducted for each INDUCTION[1] and CONSOLIDATION:

Day:	Screening	1 ±2	2 ±2	3 ±2	4 ±2	5 ±2	6 ±2	7 ±2	10 ±2	14 ±3	21 ±3	28 ±3	35 ±3	42 ±3	Weekly[3] ±3	End of Phase/Early Termination
Informed Consent[4]	x															
Medical History	x															
Physical Exam	x									x				x		x
Vital Signs	x									x				x		x
ECOG Performance Status	x															x
ECG	x															x
Registration	x															
Hematology	x	x		x		x		x	x	x	x	x	x	x	x	x
Biochemistry	x	x		x		x		x	x	x	x	x	x	x	x	x
Pregnancy Test[9]	x															
Copper levels	x											x[8]				x
Bone Marrow Aspiration/Biopsy	x											x[5]				
Chest X-ray	x															
Echocardiography/MUGA	x	x[10]												x		x
Response Assessment										x[6]						
Cytogenetics/Molecular Markers	x											x				
Adverse Events		Assess throughout Induction and Consolidation														x
Concomitant Medications		Assess throughout Induction and Consolidation														x
CPX-351 Administration		x		x		x[2]										
Immune markers*	x	x[7]														
Cardiac markers*	x	x	x				x			x						x
Pharmacodynamics*	x	x	x													

*Optional

[1]The first induction may end prematurely if a second induction is necessary, see Section 0. The schedule of evaluations for the first induction is followed until the second induction starts, then the evaluations are followed as indicated in the flow sheet, beginning with Day 1

[2] Second inductions and consolidations of are CPX-351 on Days 1 and 3, see Section 4.6

[3]Continue weekly evaluations until confirmation of response (CR/CRi) or persistent disease is declared

[4] Within 30 days prior to start of screening, if informed consent was collected, 30 days elapse and the patient is still not screened he/she must sign another ICF

[5]Required after each induction; (in case the Day 28 (+/-7 days) bone marrow is non-evaluable or assessment of aplasia is equivocal, a repeat evaluation may be performed 5-10 days later, at the discretion of the treating physician, in order to determine effect and need for second induction); as needed thereafter to confirm response/persistence/relapse in second inductions & consolidations

[6]Induction(s) only; see Section 8.4.1 for details on when response is assessed.

[7]At start of induction, induction 2 (when applicable), each consolidation

[8]Copper levels to be done before start of next cycle
[9]Women of childbearing potential
[10]Prior to every other course of therapy after cumulative dose of DNR exceeds 300 mg/m^2; prior to every course of treatment after the cumulative DNR dose exceeds 500 mg/m^2, or 30 days after the last course.

13.0 APPENDIX 2: Patient Evaluation Flow Sheet –Follow-up

	Monthly for 1 Year (±10 Days)	Every 2-3 months for 1 year	Every 3-4 months for 1 year	Early Termination
Patient status report	**X**[1]			**x**
Hematology		**x**		**x**
Biochemistry		**x**		
Copper levels	only if abnormal[2]			
Bone Marrow Aspiration/Biopsy			**x** For patients in CR, consider performing if peripheral blood counts fall below 1000/µL for ANC or 100,000/µL for platelets for >1 month with no obvious explanation for the drop or at any time there is suspicion of relapse; for patients in CRi perform if counts fall significantly below peak recovery. If the peripheral blood counts in a patient with a CRi recover to CR levels (≥1000/µL for ANC or ≥100,000/µL for platelets), consider performing a bone marrow evaluation to confirm full CR. Following the first year of follow up, aspirations will be done as clinically indicated; record relapse information, including any bone marrow evaluations. Not required following the initiation of new therapy and or relapse.	
Echocardiography or MUGA scan			**x** Perform every 3-4 months if last Treatment Phase LVEF was reduced >10% from baseline and is less than 50%. Continue assessments until LVEF returns to baseline or until 1 year from start of therapy.	

[1] Survival followed until death

[2] >20% above upper limit of normal. To be performed monthly until return to within 20% of ULN, or until 1 year from the last dose, or the initiation of new therapy, or relapse (whichever is earlier).

14.0 APPENDIX 4: Performance Status – ECOG

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction (Karnofsky 90-100)
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work) (Karnofsky 70-80).
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours (Karnofsky 50-60).
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours (Karnofsky 30-40).
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair (Karnofsky 10-20).

15.0 APPENDIX 5: Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

This 80 page document can be obtained as a pdf file from http://ctep.cancer.gov.
The publication date is May 28, 2009

16.0 APPENDIX 6: Immune resonstitution after chemotherapy with CPX-351

Immune reconstitution is believed to constitute a major element that determines the risk of relapse and the possibility of late infectious complications. Immune reconstitution after standard cytarabine and anthracycline combination has not been well studied and not at all after therapy with CPX-351. To this effect we propose the following:

To determine how CPX-351 influences the recovery of functional pathogen-specific and leukemia-specific immune responses.
We will conduct analyses of T cell phenotype, estimate antigen-specific T cell precursor frequencies (by intracellular cytokine flow cytometry and peptide/dextramer staining) and determine antigen-specific cytokine production capacity (cytokine bead array assay). We will evaluate antigen-specific T cell recovery following treatment with CPX-351 by analyzing 1) Immunophenotypic recovery of Th1, Th2, Th17 and regulatory T cells; 2) The number and function of recovering CMV and Flu-specific T cells capable of responding functionally to in vitro stimulation with overlapping pools of peptide by intracellular cytokine detection; 3) The number and function of T cells capable of responding to leukemia-associated antigens including proteinase 3 (PR3), Wilms tumor antigen (WT1) and cancer testis antigens (e.g. PRAME) will be assessed following stimulation with overlapping pools of peptide and intracellular cytokine assay.

To determine how CPX-351 influences the recovery and function of NK cells
To evaluate if CPX-351 will impact on NK phenotype and function, we will conduct longitudinal analyses of NK phenotype and function during CPX-351 therapy. Quantitative 10-color flow cytometric analysis will be performed to 1) characterize the functional phenotype of NK cells using an extended panel of antibodies against activating (NKp30, NKp44, NKp46, NKG2D, NKG2C, DNAM1) and inhibitory receptors (NKG2A and KIRs); 2) assess the effector function of NK product against MHC class I-deficient leukemia targets and primary AML cells from the recipient by intracellular cytokine assay for interferon-gamma (IFN-γ) production, chemokine production including CCL3 (MIP1α) and CCL4 (MIP1β) and cytotoxicity, including CD107a degranulation and 51chromium release assay; Confocal microscopy will be used to directly assess the immune synapse formation with leukemia targets.

Sample collection and distribution
60 cc of blood (Green top tubes- heparin) and 5 cc in purple top (EDTA) to be collected at 3 time points:

1- Before induction with CPX-351
2- At recovery from induction, just prior to consolidation #1
3- At recovery from 1st cycle of consolidation just prior to consolidation #2

Samples will be sent to Dr. Katy Rezvani's lab manager, P14.3144; tel: 713-745-0027.

17.0 APPENDIX 7: Early Detection of CPX-351-induced cardiotoxicity using high sensitivity troponin T

Chemotherapy is an effective treatment in most cancers. Yet all these drugs have a various range of side effects. Chemotherapy-induced organ injury remains a major issue in cancer patient care. Chemotherapy-induced cardiotoxicity is the most serious one, particularly associated with the use of anthracyclines, ultimately impacting the long-term cancer survivors and survival time. CPX-351 is a liposomally encapsulated combination of cytarabine and daunorubicin. The liposomal encapsulation may lead to a decrease in cardiac toxicity. However, the CPX-351-induced cardiotoxicity has not been evaluated.

Identification of chemotherapy-induced cardiac toxicity has been dependent on imaging modalities, such as echocardiogram or MUGA scan. Despite their usefulness, routine cardiac imaging alone lacks sensitivity for early detection of subclinical cardiac disease. Multiple cardiac biomarkers have been evaluated extensively in both animal models and clinical studies such as creatine kinase (CK), creatine kinase – MB fraction (CK-MB), troponin T (cTnT), troponin I (cTnI), brain natriuretic peptide (BNP), C-reactive protein (CRP), and interleukin family member (ST2).[5, 6] In acute coronary syndromes, troponin is one of the most well established biomarkers used to guide diagnosis and treatment. However, the utility of biomarkers in monitoring myocardial damage secondary to chemotherapy is still controversial.

Both cTnT and cTnI have been demonstrated in multiple clinical studies to be sensitive and specific markers for detecting myocardial injury after chemotherapy administration. The level of troponin released into circulation has a good predictive value for subsequent decrease in LV ejection fraction and adverse cardiac events long before the impairment is observed by echocardiogram or symptoms have developed. Elevation in troponin has shown a positive predictive value of 50-84%, and a negative predictive value of 90-99%.[5, 7] Furthermore, the peak value and the duration of elevated troponin level have a strong relationship with the degree of LV dysfunction. In contrast, BNP, NT-proBNP, CK, CK-MB and CRP have shown to be less sensitive and specific in detecting subclinical heart failure in this group of patients.[8, 9]

Recent advances in assay technology have led to more sensitive and precise cardiac troponin T assays that can detect TnT level as low as 0.005 μg per liter.[10] With increasing in sensitivity, high-sensitive troponin T (hs-TnT) has superiority over standard troponin test in early diagnosis of acute myocardial infarction.[11] In chronic stable angina, higher levels of hs-TnT has been shown to be associated with the higher incidence of cardiovascular death and heart failure in the future.[12] Recently, hs-TnT has been evaluated as a peri-operative risk stratification in non-cardiac surgery patients.[13] Interestingly, they found that hs-TnT level above universal cut-off were associated with a 2.6-fold increase in in-hospital mortality and morbidities from cardiovascular system.

To our knowledge, no prior study has ever evaluated hs-TnT during CPX-351 chemotherapy administration. Therefore, our primary objective is to assess whether hs-TnT obtained during the course of CPX-351 chemotherapy could allow us to monitor the development of acute cardiotoxicity.

- Primary objective:

To test the feasibility of using high-sensitivity troponin T to monitor acute CPX-351-induced cardiotoxicity. In addition, BNP and cardiac troponin I will be measured.

- Roche high sensitivity troponin T assay is a sandwich chemiluminescent assay. High sensitivity troponin T assay gives detection limit of 5 ng/L and the 99th percentile of cutoff 14 ng/L. It can be run on MODULAR ANALYTICS E170, Elecsys 2010, cobas e411, cobas e 601 and cobas e 602 analyzers.
- This high sensitivity troponin T assay will be measured on Cobas e601 using Roche reagents including calibrators and controls.

Sample collection and distribution
3 cc of blood (Green top tubes- heparin) at the following time points:

1- Before the start of each cycle with CPX-351 (baseline)
2- Approximately 24 hrs after the end of the first dose (Day 1 + 1) (for each cycle)
3- Approximately 25 hrs after the end of the dose given on day 5 (Day 5 + 1) (for each cycle)
4- Approximately on day 14 (±4 days) for each cycle
5- End of treatment

Samples will be sent to Dr. Qing H. Meng's lab, on R4.1446, Clinical Chemistry Laboratory, Department of Laboratory Medicine; c/o Dr. Qing H. Meng, tel: 713-792-6320; fax: 713-792-4793; email: qhmeng@mdanderson.org.

18.0 APPENDIX 8: Pharmacodynamic Investigations During CPX-351 Therapy

The objectives of the pharmacokinetic and pharmacodynamic investigations during this clinical trial are as follows:

(1) To test ex vivo cytotoxicity of combination of Pim kinase inhibitor(s) and CPX-351 on circulating leukemia cells.
(2) To investigate effect of Pim kinase inhibitor on phosphorylated and total protein levels in pim kinase signaling pathway.

Only patients entering at MD Anderson Cancer Center will be studied. All patients with ≥5,000 WBC/μl of peripheral blood will be requested to participate in the cellular pharmacodynamics studies. Patients will sign consent forms to enter these investigations.

Once a patient has consented to participate in the investigation, please contact Ms. Vrushali Datar (pager 713-606-2212) or Yuling Chen (pager 713-404-2550) in The Laboratory of Cellular and Molecular Pharmacology (Dr. Gandhi, phone, 713 792-3336) to coordinate scheduling of blood samples. To facilitate preparedness for laboratory studies, please plan on initiating therapy in the subsequent morning if the clinical situation allows and please inform us as soon as there is a possibility of patient entering the clinical trial.

Blood samples will be collected at the following times from patients in the Dose Escalation Phase: Total of 2 samples will be collected.

Cycle 1.	Day 1: 0 (pre-dose),	Two green top tubes (2 × 10mL)
	24 h	One green top tube (1 × 10mL)

For the pre-dose (sample 1), two tubes of blood (10mL each) will be collected in a Vacutainer green top tube. For the remaining sample (sample 2), one tubes of blood (10mL) will be collected in a Vacutainer green top tube. The samples will be mixed and immediately put on ice-bath. Samples will be brought to Dr. Gandhi's laboratory in the Tan zone (T1.3839). The blood will be centrifuged and plasma will be separated, transferred to storage tubes and immediately frozen, and stored until analysis. After removal of plasma, the blood cells will be resuspended in PBS and AML blasts will be isolated by Ficoll-Hypaque density-gradient centrifugation procedures. Cells will be used for the following laboratory endpoints.

NOTE: We may not be able to do all studies in every patient. This will be decided based on diagnosis and number of leukemia cells recovered from the peripheral blood sample.

Effect of ex-vivo combination with Pim kinase inhibitors on AML blasts during CPX-351 therapy: Pre-treatment leukemia cells or cells isolated during CPX-351 therapy will be cultured ex-vivo with varying concentrations of Pim kinase inhibitors to evaluate the effect of combined DNA damage and anthracyclines with Pim kinase inhibition on AML blasts.

Induction of apoptosis will be measured using flow cytometric analysis of Annexin-V and PI binding assay. Cells will be saved as pellets for future testing of phosphorylated and total protein levels in pim kinase signaling pathway.

19.0 APPENDIX 9: AML with MDS-Associated Karyotype

With myelodysplastic syndrome-related cytogenetic abnormalities:

- Complex karyotype (defined as 3 or more chromosomal abnormalities).
- Unbalanced: -7 or del(7q); -5 or del(5q); i(17q) or t(17p); -13 or del(13q); del(11q); del(12p) or t(12p); del(9q); idic(X)(q13).
- Balanced: t(11;16)(q23;p13.3); t(3;21)(q26.2;q22.1); t(1;3)(p36.3;q21.2); t(2;11)(p21;q23), t(5;12)(q33;p12); t(5;7)(q33;q11.2); t(5;17)(q33;p13); t(5;10)(q33;q21); t(3;5)(q25;q34)

20.0 APPENDIX 10: European Leukemia Net (ELN) Classification

Table 4. Standardized reporting for correlation of cytogenetic and molecular genetic data in AML with clinical data

Genetic group	Subsets
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD (normal karyotype)
	Mutated *CEBPA* (normal karyotype)
Intermediate-I*	Mutated *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL*
	Cytogenetic abnormalities not classified as favorable or adverse†
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
	t(6;9)(p23;q34); *DEK-NUP214*
	t(v;11)(v;q23); *MLL* rearranged
	−5 or del(5q); −7; abnl(17p); complex karyotype‡

Frequencies, response rates, and outcome measures should be reported by genetic group, and, if sufficient numbers are available, by specific subsets indicated; excluding cases of acute promyelocytic leukemia.

*Includes all AMLs with normal karyotype except for those included in the favorable subgroup; most of these cases are associated with poor prognosis, but they should be reported separately because of the potential different response to treatment.

†For most abnormalities, adequate numbers have not been studied to draw firm conclusions regarding their prognostic significance.

‡Three or more chromosome abnormalities in the absence of one of the WHO designated recurring translocations or inversions, that is, t(15;17), t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), inv(3) or t(3;3); indicate how many complex karyotype cases have involvement of chromosome arms 5q, 7q, and 17p.