A Single-Arm, Open-Label Phase 2 Pilot Study of Vyxeos (CPX-351) in Adults with Relapsed or Refractory Acute Lymphoblastic Leukemia

NCT03575325

Version 3.0

December 11, 2019

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Protocol Identifying Number: 19482

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IND/IDE Sponsor: Bijal Shah, MD

IND Number: 138773

Funded by:

Jazz Pharmaceuticals, Inc.

Version Number: v.1.0

25 January 2018

Version Number: v.1.1
21 Feb 2018

Version Number: v.1.2

30 Mar 2018

Version Number: v.1.3

20 Apr 2018

Version Number: 2.0

03 Jun 2019

Version Number 2.1

04 Oct 2019

Version Number 3.0

11 Dec 2019

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LIST OF ABBREVIATIONS

AE	Adverse Event
ALL	Acute Lymphoblastic Leukemia
ANCOVA	Analysis of Covariance
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
aPTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CMS	Centers for Medicare and Medicaid Services
CNS	Central Nervous System
CR	Complete Response
CRF	Case Report Form
CRi	Complete Remission with incomplete hematologic recovery
CSF	Cerebral Spinal Fluid
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Forms
EKG	Electrocardiogram
EOT	End of Treatment
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICH E6	International Conference on Harmonisation Guidance for Industry, Good Clinical Practice: Consolidated Guidance
IND	Investigational New Drug Application
IRB	Investigational Review Board
IT	Intrathecal
LDH	Lactate Dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MRD	Minimal Residual Disease
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the ICH E6, the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed of their obligation to meet the above commitments.

Principal Investigator: Bijal Shah, MD
Print/Type Name

Signed: _____ Date: _____

STUDY SUMMARY

Title: A Single-Arm, Open-Label Phase 2 Pilot Study of Vyxeos (CPX-351) in Adults with Relapsed or Refractory Acute Lymphoblastic Leukemia.

Objectives:

Primary Objective:

1) To estimate the complete response rate following treatment with CPX-351

Important Secondary Objectives:

1) To evaluate the safety and tolerability of CPX-351
2) To estimate minimal residual disease burden following treatment with CPX-351
3) To estimate the progression free survival (PFS) following treatment CPX-351
4) To estimate the overall survival (OS) following treatment with CPX-351

Endpoint

Primary Endpoint:

1) Complete remission rate

Important Secondary Endpoints:

1) To evaluate the safety and tolerability of CPX-351
2) Minimal Residual Disease Burden
3) PFS
4) OS

Population: Patients ≥18 years old with relapsed acute lymphoblastic leukemia (ALL) who are registered at the Moffitt Cancer Center.

Phase: 2, pilot

Number of Sites enrolling participants: 1

Description of Study Agent :

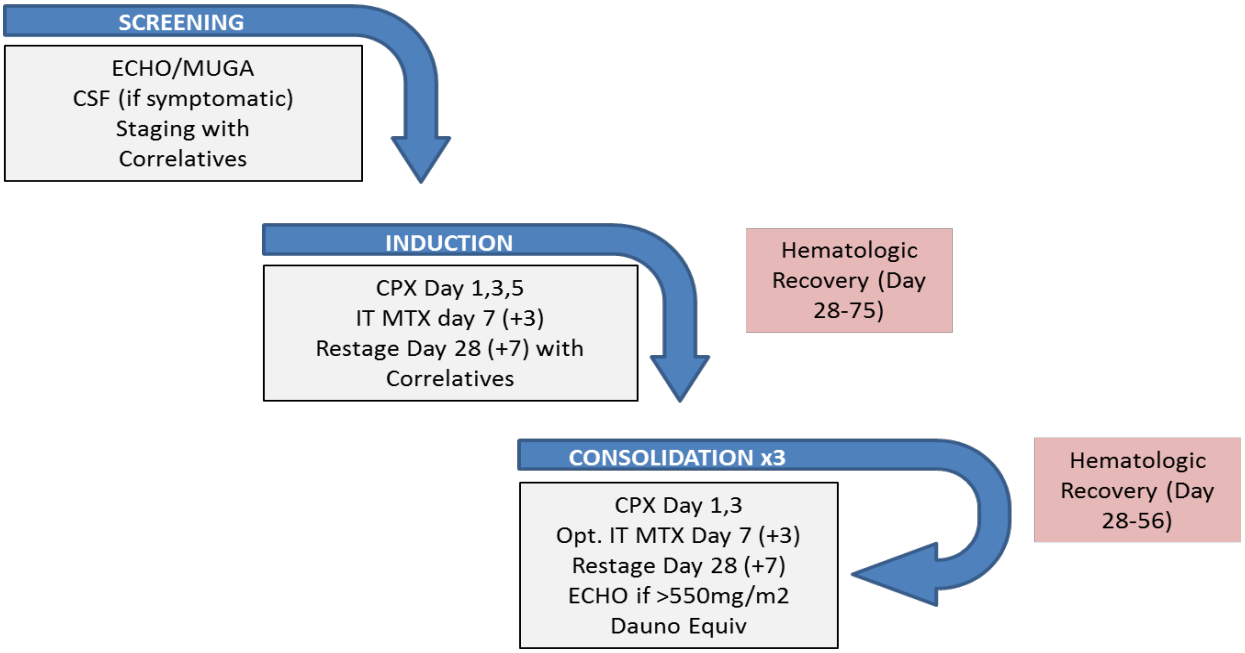
1) Induction: Vyxeos (CPX-351), 100 units/m^2 days 1, 3, & 5
2) Consolidation: Vyxeos (CPX-351) 65 units/m^2 days 1 & 3 (up to 3 cycles)

Study Duration: 24 months

Participant Duration: 30 months

SCHEMATIC OF STUDY DESIGN

Induction	CPX-351 100 u/m^2 90 minute infusion days 1,3,5
Consolidation Per section 7.3.2.11	CPX-351 65 u/m^2 90 minute infusion days 1,3 (administered 35-75 days after start of induction) for up to 3 cycles.



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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

Outcomes are poor among adults with relapsed and refractory ALL, with most chemotherapy regimens demonstrating a CR rate of ~40%, coupled with a median EFS of ~6mo. (ref 1-10). To this end, several

novel therapies have emerged, including blinatumomab (CR/CRi rate 43%, med OS 7.7mo), inotuzumab ozagomycin (CR/CRi rate 80.7%, med OS 7.7mo), and liposomal vincristine (CR/CRi rate 20%, 6mo OS 35%). (ref 11-13). While these therapies have advanced the management of relapsed ALL, relapse and shortened life expectancy remain the expectation for most patients.

Chemotherapy resistance has been related in part to the overexpression of cell proteins implicated in drug transmembrane transport, such as PGP and LRP. (ref 14-18). Attempts to intensify therapy by increasing anthracycline dose or with the use of novel anthracycline derivatives have not significantly improved outcome. (ref 8-9,19). More recently, trials using liposomal daunorubicin in conjunction with high dose cytarabine demonstrated improved clinical efficacy (CR 80%, 12mo OS 39%). Notably, in cells from those with increased expression of multi-drug resistant proteins (72% of patients), the in vitro retention of liposomal daunorubicin and subsequent apoptosis were both significantly higher, when compared with free daunorubicin. (ref 18) Recent work further suggests molar ratio of cytarabine:daunorubicin, in addition to drug uptake, may be important in yielding therapeutic response. (ref 20-21)

CPX-351 (or Vyxeos) is a liposomal formulation of cytarabine and daunrobicin at a fixed molar ratio. A randomized study of CPX-351 (vs non-liposomal cytarabine and daunorubicin) in high risk acute myeloid leukemias demonstrated a significant benefit in complete remission rates (47.7% vs 33.3%) and median overall survival (9.56 vs. 5.95 months), culminating in FDA approval August 2017.

Preclinical and early clinical data using primary ALL cell samples suggests that this benefit may extend to those with lymphoblastic leukemias. Specifically, “high sensitivity” to CPX-351 was observed in 20% of samples. (ref 22). This was further corroborated with xenograft modeling using samples derived from pediatric patients with relapsed ALL, wherein response was estimated via ORM modeling to be 100%, with response in 4 of 5 mouse groups suggestive of CR. Notably, activity was also observed in a Philadelphia chromosome positive patient derived xenograft. (ref 23) Finally, CPX-351 was also tested clinically during phase 1 testing in 3 patients with ALL, with one showing subsequent CR. (ref 24)

Based on these data, the author believes there is rationale for further study of this approach in relapsed and refractory ALL.

2.2 RATIONALE

Remission rates vary considerably depending on population studies (children vs adults), relapsed vs refractory status, and timing of salvage (first relapse vs beyond first relapse) making it difficult to extrapolate baseline rate. If focusing specifically on 2 trials that used an induction composed of cytarabine (3gm/m2 x5d) and idarubicin (40mg/m2 on d3), complete remission rates are approximately 40% (38-44%, total n=54). (ref 4-5). Alternatively, a more recent phase 3 trial of inotuzumab vs standard of care in patients in first or second relapse shows a composite CR rate in the standard of care arm (Cytarabine+Mitoxantrone,high dose Cytarabine, and Fludarabine, Cytarabine, and GCSF, total n=109) of 29%. (ref 13). Given these data, it is determined that a baseline CR rate of 35% is likely to estimate activity with a standard salvage regimen.

Data using liposomal anthracyclines among those with relapsed ALL, including FLAD (Fludarabine, Cytarabine, Liposomal Daunorubicin) and Cytarabine+Liposomal Daunorubicin, have shown an improvement in CR rates, at 65% and 80%, respectively. (ref 18,28) Together these data suggest a CR rate of 65% should be attainable using CPX-351.

However, at this stage, a phase 2 pilot trial is proposed to explore response rates among 10 patients with ALL. Should sufficient activity be observed, consideration may be made in consultation with Jazz

Pharmaceuticals, to increase the budget to increase accrual to formally test our hypothesis, possibly in a multicenter setting.

Safety will be monitored as per standard routine, accounting for adverse events using CTCAE v. 5.0. Myelotoxicity is anticipated, in addition to associated infections. Frequency of these events will be monitored for comparison with historical rates, referencing against historical regimens noted above. Adverse events of special interest include bone marrow aplasia (hypocellular marrow without adequate count recovery >42 days from start of therapy), and *symptomatic* heart failure (accompanied by a decrease in the ejection fraction >10% from baseline greater than 42 days from start of therapy).

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

The table below summarizes adverse reactions obtained directly from the Investigator Brochure Version 13.0. The table includes pooled data from five clinical trials of 375 patients with AML. The frequency is referenced using the following MedDRA scale:

· Very common: ≥1/10 (≥10%);
· Common (frequent): ≥1/100 and <1/10 (≥1% and <10%);
· Uncommon (infrequent): ≥1/1,000 and <1/100 (≥0.1% and <1%);
· Rare: ≥1/10,000 and <1/1,000 (≥0.01% and <0.1%);
· Very rare: <1/10,000 (<0.01%), including isolated reports.

System Organ Class	All AEs/Frequency	Grade 3-5 AEs/Frequency
Blood and lymphatic system disorders	Very Common Febrile neutropenia Common Neutropenia Thrombocytopenia Anemia	Very Common Febrile neutropenia Common Neutropenia Thrombocytopenia Anemia
Cardiac disorders	Very Common Cardiotoxicity Arrhythmia Chest pain	Very Common Cardiotoxicity Common Arrhythmia Chest pain
Eye disorders	Very Common Visual impairment	Uncommon Visual impairment
Gastrointestinal disorders	Very Common Mucositis Abdominal pain Constipation Decreased appetite Diarrhea/colitis Nausea Vomiting Common Dyspepsia	Common Mucositis Abdominal pain Constipation Decreased appetite Diarrhea/colitis Nausea Uncommon Vomiting Dyspepsia
General disorders and administration site conditions	Very Common Edema Pyrexia Fatigue Chills	Very Common Fatigue Common Edema Pyrexia Uncommon Chills
Immune system disorders	Very Common Hypersensitivity	Common Hypersensitivity
Infections and infestations	Very Common Infection	Very Common Infection
Metabolism and nutrition disorders	Common Tumor lysis syndrome	Common Tumor lysis syndrome

Musculoskeletal and tissue disorders	Very Common Musculoskeletal pain	Common Musculoskeletal pain
Nervous system disorders	Very Common Headache Dizziness	Common Headache Uncommon Dizziness
Psychiatric disorders	Very Common Delirium Sleep disorders Anxiety	Common Delirium Uncommon Sleep disorders
Renal and urinary disorders	Very Common Renal insufficiency	Common Renal insufficiency
Respiratory, thoracic and mediastinal disorders	Very Common Dyspnea Pleural effusion Cough	Very Common Dyspnea Uncommon Pleural effusion
Skin and subcutaneous disorders	Very Common Pruritis Hyperhidrosis Common Alopecia Night sweats Uncommon Palmar-plantar erythrodysesthesia syndrome	Uncommon Hyperhidrosis
Vascular disorders	Very Common Hemorrhage Hypertension Hypotension	Very Common Hemorrhage Common Hypertension Hypotension

2.3.2 KNOWN POTENTIAL BENEFITS

Initial work with primary ALL cell samples suggested "high sensitivity" to CPX-351 in 20% of samples. (ref 22). This was further corroborated with xenograft modeling using samples derived from pediatric patients with relapsed ALL. Specifically, response was estimated via ORM modeling to be 100%, with response 4 of 5 mouse groups suggestive of CR. Notably, activity was also observed in a Philadelphia chromosome positive patient derived xenograft. (ref 23) Finally, CPX-351 was also tested clinically during phase 1 testing in 3 patients with ALL, with one showing subsequent CR. (ref 24) Based on these data, there is reason to believe there could be benefit to subjects with relapsed and refractory ALL.

To date we have treated 7 patients with CPX-351. We have seen CR in two patients and PR in 3 other evaluable patients following induction (ORR 83%). Relevant grade 3-5 SAE's to date include: bleeding, veno-occlusive disease of the liver, and fungal pneumonia.

3 OBJECTIVES AND PURPOSE

Primary Objective:

To estimate the complete response rate following treatment with CPX-351. Complete remission will be evaluated by bone marrow biopsy at day 28 of therapy in all patients with medullary disease receiving at least one full cycle of CPX-351. For patients with extramedullary disease, CT or PET/CT will be used for response. Cheson criteria (2003) will be used to evaluate remission status, specifically complete remission (CR), complete remission with incomplete hematologic recovery (CRi/CRh), partial response (PR), and aplasia.

Important Secondary Objectives:

Additional clinical endpoints will include

1) To evaluate safety and tolerability of CPX-351 using CTCAE 5.0.
2) Minimal residual disease assessment (MRD) by 10 color flow cytometry and next generation sequencing (NGS) by Clonoseq (for Philadelphia negative leukemia) or PCR for BCR-ABL (for Philadelphia positive leukemia).
3) Progression Free Survival as defined by Cheson Criteria, namely progression, failure to respond, or death, as assessed from time of first treatment.
4) Overall Survival as defined by Cheson Criteria, namely death due to any cause as assessed from time of first treatment.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

This is an open label, single arm, single center, phase 2 pilot study of Vyxeos induction & consolidation in relapsed and refractory ALL.

4.2 STUDY ENDPOINTS

4.2.1 PRIMARY ENDPOINT

- We will measure remission rate at day 28 to address the primary endpoint of complete remission (with or without complete hematologic recovery), as defined by Cheson Criteria (ref 27). For those with extramedullary disease, Lugano criteria will be used to assess response (ref 32). This is a standard assessment of drug efficacy for phase 2 clinical trial design in acute leukemias, as response correlates closely with progression free- and overall survival (PFS and OS).

4.2.2 SECONDARY ENDPOINTS

Secondary Endpoints will include

1) Evaluation of safety of CPX-351 using CTCAE 5.0.
2) Minimal residual disease assessment (MRD) by 10 color flow cytometry and next generation sequencing (NGS) by Clonoseq (for Philadelphia negative leukemia) or PCR for BCR-ABL (for Philadelphia positive leukemia) at day 28. Using a threshold of 0.01% leukemic cells, MRD provides a better estimate leukemic cell sensitivity to therapy, and correlates with both PFS and OS.
3) Progression Free Survival as defined by Cheson and Lugano Criteria (ref 27 and 32), namely progression, failure to respond, or death, as assessed from time of first treatment. The 12 month progression free survival rate with standard chemotherapeutic approaches in relapsed ALL is estimated at 10%. PFS is a standard estimate that accounts for response rate, response duration, and mortality to estimate therapeutic benefit in clinical trials.
4) Overall Survival as defined by Cheson and Lugano Criteria (ref 27 and 32), namely death due to any cause as assessed from time of first treatment. The 12 month overall survival for those with standard chemotherapeutic approaches in relapsed ALL estimated at 20%. Overall survival is a standard criteria for assessing therapeutic benefit in clinical trials.

4.2.3 EXPLORATORY ENDPOINTS

Exploratory Endpoints will include:

1) An exploration of the activity of CPX-351 ex vivo using live image capture in a reconstituted tumor micro-environment (EVOS system) as a potential biomarker for response.

2) An assessment of apoptotic threshold as a measure of tumor cell fitness (dynamic BH3 profiling). These data may additionally support combinatorial approaches using clinical inhibitors of apoptosis.
3) An assessment of the T-cell repertoire using multi-parameter flow, and tumor/stromal PDL1 expression. These data will similarly be used to support potential future combinatorial strategies.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

Patients must meet all of the following criteria to be eligible for study entry:

1. Be willing and able to provide written informed consent/assent for the trial.

2. Be ≥ 18 years of age on day of signing informed consent.

3. Able to adhere to the study visit schedule and other protocol requirements.

4. Pathologically confirmed B- or T-cell acute lymphoblastic or mixed phenotype acute leukemia, with ≥5% peripheral blood or bone marrow lymphoblasts and/or extramedullary disease >1x1cm.

5. Relapsed or refractory acute lymphoblastic leukemia after at least 1 prior cycle of therapy. Patients with Philadelphia chromosome positive disease must have failed at least two prior tyrosine kinase inhibitors.

6. Eastern Cooperative Oncology Group (ECOG) performance status 0-1.

7. Cardiac ejection fraction ≥ 50% by echocardiography or MUGA.

8. Must be at least 2 weeks out from any prior systemic chemotherapy, blinatumumab, radiation, and/or other investigational agents, and have recovered to grade 1 from any toxicity related to prior therapy. Glucocorticoids are permitted up to 1 day prior to the first dose.

9. Serum bilirubin and creatinine ≤ 1.5x upper limit of normal (ULN). AST and ALT must be ≤3x ULN, unless there is suspected liver involvement.

10. Females of childbearing potential (FCBP) must have a negative serum pregnancy test at screening. A FCBP is considered when a sexually mature female: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 12 consecutive months.

11. A FCBP must agree to use of two methods of highly effective contraception, be surgically sterile, or abstain from heterosexual activity for the course of the study through 30 days after the last dose of study treatment.

12. Male subjects must agree to use an adequate method of contraception starting with the first dose of study therapy through 30 days after the last dose of study therapy. Men must agree to not donate sperm during and after the study

5.2 PARTICIPANT EXCLUSION CRITERIA

Any of the following is a criterion for exclusion from the study:

1. Clinical evidence of active central nervous system (CNS) leukemia.

2. Any major surgery or radiation therapy within four weeks.

3. Any active infection requiring systemic therapy, including HIV, Hepatitis B, and/or Hepatitis C.

4. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator (including but not limited to severe graft-versus-host disease, unstable angina, pulmonary hypertension, active/prior veno-occlusive disease of the liver, or severe CHF (NYHA III-IV).

5. Patients with active (uncontrolled, metastatic) second malignancies are excluded.

6. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit through 30 after the last dose of trial treatment.

7. Hypersensitivity to cytarabine, daunorubicin, or liposomal products.

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

9. Patients with prior cumulative anthracycline exposure of greater than 368 mg/m^2 daunorubicin (or equivalent).

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

Recruitment of the 10 participants for this trial will occur from new or established patients treated at the Hematology Clinic at Moffitt Cancer Center. Screen failure is anticipated to be less than 10%. The target accrual is expected to be reached in 24 months. All faculty members in the Lymphoma Section will be sub-investigators on the trial. They will be trained on the trial design, study agents, and eligibility criteria. This will equip each investigator to discuss the trial with potential participants as appropriate. The trial will be listed on the Moffitt Cancer Center website (www.moffitt.org) for the availability of local physicians to refer patients who could be eligible for the trial.

All investigators will be made aware of the emphasis to enroll women and minorities. The design of the trial and eligibility criteria are not restrictive relative to women and minorities. According to the National Cancer Institute Surveillance, Epidemiology, and End Results Program, there are approximately 6000 new cases of ALL per year, of which nearly half will occur among those over age 20 years. (ref. 29) In a currently enrolling Moffitt trial (MCC18329) for patients with ALL, 50% of participants were young adults (age 18-39), 25% were female, and 100% were Hispanic/Portuguese. The sex, ethnicity, and race of each participant on this trial will be captured in the Oncore electronic data base for reporting purposes.

The multi-modal aspect of this disease will require knowledge of the barriers to recruitment and retention present to young adults, older adults, and those who may be Hispanic. The young adult population can be challenging to interest in trial participation. The article by Pearce (ref. 30) provides understanding of communication strategies, the need of young adults to feel autonomous, and the importance young adults place on being treated in a specialist care setting to facilitate trial participation.

The article could be utilized as a resource for trial staff. Once consented, the young adult participant will have contact with the research nurse frequently during trial participation to maintain continuity and provide support, which Pearce describes as a way to impact retention.

Potential participants will be assessed prior to screening for any perceived barriers related to the clinical trial process. Referrals will be made to a social worker, supportive care services, financial services, or the research nurse to address barriers prior to consent.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur that may jeopardize participant safety. In addition, a subject may be withdrawn by the investigator if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent
- Unacceptable adverse events
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in the Schedule of Events and Section 7.3 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring. Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status and toxicity up to 30 days. Follow-up rules will apply unless participant initiates a non-study cancer treatment, withdraws consent, or becomes lost to follow-up (unsuccessful attempts to contact for 3 months). After documented disease progression, each subject will be followed 12 months for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

Every effort will be made to maintain contact with a subject who withdraws early. If the subject remains at MCC for further care, clinic visits may be tracked and the condition of the subject followed. If the subject is not continuing care at MCC, contact information will be updated at the end of treatment visit, including accurate phone numbers, and email address. The importance of the follow-up period for AEs and SAEs will be stressed to the subject.

All subjects receiving at least 1 day of therapy will be evaluable for safety. Replacement of participants is permitted for those who screen fail or drop out prior to completing initial response assessment at day 28 of treatment, in order to meet the accrual goal of this study.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to investigator, funding agency, the IND/IDE sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
4. Plans to modify or discontinue the development of the study drug
5. Determination of futility

In the event Jazz Pharmaceuticals, Inc. decides to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

6 STUDY AGENT

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

Study agent will be supplied by the manufacturer or IND/IDE sponsor to the Moffitt Research Pharmacy.

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

- CPX-351 (Vyxeos) is stored as a dry powder at 2ºC to 8ºC
- CPX-351 (Vyxeos) is provided by Jazz Pharmaceuticals.
- CPX-351 is FDA approved and commercially marketed for patients with Acute Myeloid Leukemia
- For this trial we will be using investigational supply for this trial in Acute Lymphoblastic Leukemia.

6.1.3 PRODUCT STORAGE AND STABILITY

Vials of CPX-351 should be refrigerated prior to use (at 2-8°C) and should be utilized prior to expiration as indicated on the individual vials. If reconstituted product is not diluted into an infusion bag immediately, store in refrigerator at 2ºC to 8ºC for up to 4 hours.

6.1.4 PREPARATION, PRODUCT STORAGE AND STABILITY

The appropriate number of vials of CPX-351 (cytarabine:daunorubicin) Liposome Injection should be removed from the refrigerator, and allowed to equilibrate to room temperature for 30 minutes prior to reconstitution.

Then, reconstitute each vial with 19 mL of Sterile Water for Injection using a sterile syringe and immediately thereafter start a 5-minute timer. Carefully swirl the contents of the vial for 5 minutes while gently inverting the vial every 30 seconds. Do not heat, vortex, or shake vigorously. After reconstitution, let rest for 15 minutes. After reconstitution (but before final dilution), the concentration is 5 u/mL.. The reconstituted product should be an opaque, purple, homogeneous dispersion, essentially free from visible particulates. Gently invert each vial 5 times prior to withdrawing the reconstituted product for further dilution. If the reconstituted product is not diluted into an infusion bag immediately, store in refrigerator at 2ºC to 8ºC for up to 4 hours.

Aseptically withdraw the calculated volume of the reconstituted product from the vial(s) with a sterile syringe and transfer it to an infusion bag containing 500 mL of 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. There may be residual product remaining in the vial. Discard unused portion. Gently invert the bag to mix the solution. The dilution of the reconstituted product results in a deep purple, translucent, homogeneous dispersion, free from visible particulates. If the diluted infusion solution is not used immediately, store in refrigerator at 2ºC to 8ºC for up to 4 hours. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Only solutions without visible particles should be used.

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.

6.1.5 DOSING AND ADMINISTRATION & ROUTE OF 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.

6.1.7 STARTING DOSE AND DOSE ESCALATION SCHEDULE

Patients will receive induction with CPX-351 at a dose of 100 u/m2 administered intravenously over 90 minutes on days 1, 3 and 5 of a 28 day cycle.

This may be followed by consolidation with CPX-351 at a dose of 65 u/m2 administered intravenously over 90 minutes on days 1 and 3 of a 28 day cycle.

6.1.8 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

It is the intention of the study to treat every patient at full dose. Doses may be delayed due to toxicities (for example hypersensitivity reactions). Any doses missed or delayed due to toxicity may be administered as soon as the patient has recovered from the toxicity.

6.1.9 DURATION OF THERAPY

All patients completing induction will be evaluable for efficacy. Consolidation may be started at the time of hematologic recovery (28-75 days after start of induction) for those achieving a complete remission or complete remission with incomplete hematologic recovery. Consolidation cycles may be repeated at the time of hematologic recovery for a maximum total of 3 consolidation cycles.

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

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. Institutions should maintain records that document adequately that the patients were provided the doses specified by the protocol and reconcile all investigational product received from the Sponsor. Records of storage conditions (temperature logs) must be kept for the entire period that CPX-351 is maintained at the institution.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY-SPECIFIC PROCEDURES

The Schedule of Events - Section 7.3.7 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the PI and/or Jazz Pharmaceuticals for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.2 STANDARD OF CARE STUDY PROCEDURES

The majority of procedures and laboratory tests on this trial will performed per standard of care for ALL. Standard of care screening testing will include: bone marrow biopsy, echocardiogram/MUGA, EKG, serum pregnancy test, physical examination, CBC/differential, serum chemistry, vital signs, CSF/IT chemotherapy. During hospitalization, laboratory testing, supportive care measure, and procedures will be following standard of care as for any other ALL treatment.

7.1.3 Informed Consent

Prior to any study procedure that is not considered standard of care, informed consent must be obtained and documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form. The dated signature of the person conducting the consent discussion must also appear on the consent form.

7.1.4 Inclusion/Exclusion Criteria

Prior to any trial treatment, all inclusion and exclusion criteria will be reviewed and signed by the PI or Sub-Investigator to ensure the subject qualifies for the trial.

7.1.5 Demographics and Medical History

Demographical information collected will include birth date, age, sex, race, and ethnicity. A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions and any condition diagnosed that is considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

7.1.6 Baseline Symptoms

Baseline symptoms are defined as those present at the closest time before the start of study drug administration.

7.1.7 Eastern Cooperative Oncology Group (ECOG) Performance Status

The investigator or qualified designee will assess ECOG status (see Appendix B) as specified in the Schedule of Events.

7.1.8 Concomitant Medications Review

The investigator or qualified designee will record medication, if any, taken by the subject starting at screening until end of trial treatment. All medications related to reportable SAEs should be recorded as defined in the SAE section of the protocol.

7.1.9 Adverse Event Review

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Schedule of Events and more frequently if clinically indicated. Adverse events will be collected from the time of consent until 30 days after last dose or until subsequent anti-leukemic therapy for those failing to show adequate response.

7.1.10 Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history.

7.1.11 Vital Signs

The investigator or qualified designee will take vital signs at screening and prior to the administration of each dose of trial treatment. Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.1.12 Laboratory Procedures

At screening laboratory procedures will include a CBC with differential, CMP (albumin, BUN, creatinine, alkaline phosphatase, ALT, AST, CO2, calcium, chloride, glucose, potassium, sodium, total bilirubin, total protein), LDH, uric acid, magnesium, phosphorus, coagulation studies, and urinalysis with microscopy. Laboratory testing on day one and during the hospitalization period, EOT, and follow-up will be at physician discretion per standard of care.

7.1.13 Bone Marrow Biopsy

A bone marrow biopsy will be performed at screening and at day 28. If determined the subject has extramedullary disease (as permitted in inclusion criterion #4), biopsy of the involved area will be the method by which correlative samples will be obtained, provided this can be safely obtained. If extramedullary disease is confirmed and the bone marrow biopsy is shown to be negative prior to trial participation, no bone marrow biopsy will be required at day 28. If subject discontinues treatment early or progression of disease is suspected, a bone marrow biopsy will be done at the discretion of the investigator. During follow-up, bone marrow biopsies will be performed per investigator discretion.

7.1.14 Correlative Studies

Three different tests will be performed during the course of the trial. At screening, samples will be collected from bone marrow aspirate and peripheral blood. If determined the subject has extramedullary disease (as permitted in inclusion criterion #4), biopsy of the involved area will be the method by which correlative samples will be obtained. Biopsy will involve at least 6 core biopsies from the disease sites. If the biopsy of an extramedullary site is considered to put the subject at considerable risk, the subject may still participate in the trial without collection of the correlative samples. At the day 28 time point, the samples will be obtained from peripheral blood. Correlatives will be performed at investigator's discretion if early study termination. For sampling requirements, see Section 7.2.

7.1.15 CSF for ALL Involvement/IT Chemotherapy

If a potential study subject demonstrates clinical signs or symptoms of CNS involvement, a CSF sample will be obtained through a spinal tap. If CNS involvement is confirmed, the subject will not be eligible for study participation. Provided there are no contra-indications, prophylactic intrathecal methotrexate will be administered per standard of care, 7-10 days after initiation of CPX dosing.

7.1.16 Imaging

If a study subject is found to have extramedullary disease as permitted by inclusion criterion #4, the method for baseline disease assessment will be CT or PET/CT scan per standard of care. This method will also be used for determining response assessment on Day 28.

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

- **Hematology**: hemoglobin, hematocrit, white blood cells (WBC) with differential count, platelet count.
- **Metabolic Panel**: albumin, BUN, alkaline phosphatase, CO2, calcium, chloride, glucose, potassium, sodium, total protein, LDH, uric acid, magnesium, phosphorus, creatinine, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST).

- **Urinalysis**: complete urinalysis with microscopic evaluation
- **Pregnancy test:** to be done within 24 hours prior to study intervention and results must be available prior to administration of study product.
- Laboratory testing on day one and during the hospitalization period, EOT, and follow-up will be at physician discretion per standard of care.

7.2.2 OTHER ASSAYS OR PROCEDURES

1. To explore the activity of CPX-351 ex vivo using live image capture in a reconstituted tumor micro-environment (EVOS system) as a potential biomarker for response: Primary patient ALL cells will be seeded into a 384-well plate onto reconstructed TME, which includes extracellular matrix (collagen 1) and human bone marrow derived stromal cells (BMSC). CPX-351 dosed at 5 different concentrations will be added to the media, and plate is then continuously imaged for 96 hours. A digital image analysis algorithm detects tumor cells based on cell size and membrane motion. Changes in viability are quantified by area under curve (AUC). HBL-2 cells are used as a control.

2. To identify potential drug candidates for future combinatorial approaches, including assessment of the T-cell repertoire, PDL1 expression by conventional immunohistochemistry, and BH3 profiling.
   T-cell assessment: Leukocytes will be isolated from BMBx aspirate sample by Ficoll-Hypaque gradient separation from RBCs (and other stromal elements, in the case of BMBx aspirate samples). Cells will be enumerated after 2 washes in PBS and allocated for flow cytometric analysis or for *ex vivo* culture in stimulation conditions. Cells will be aliquoted into 200,000 cell fractions in preparation for subsequent analysis. Leukocytes from each patient sample will be stained with fluorochrome-conjugated antibodies according to each of 3 analysis panels. Panels have been designed to assess lymphocytes subsets, effector/memory maturation, and exhaustion.

3. PDL1 expression: Will be determined on blasts/stroma using Dako antibody on formalin fixed paraffin embedded bone marrow core biopsy slides per routine.

4. BH3 Profiling: Briefly following membrane permeablization with digitonin, BH3 mimetic peptides targeting BIM, BAD, HRK, and MS-1 will be used to interrogate the contribution of BCL2, BCLXL, and MCL1 to mitochondrial outer membrane permeablization (MOMP). The collapse of the transmembrane mitochondrial potential is used as a surrogate for MOMP, and estimated by fluorescence following rhodamine dye accumulation.

5. Sampling of extramedullary disease will involve CT guided or excisional biopsy collection of 6 core biopsy samples (if possible), to be divided evenly between the two investigators' laboratories assigned to perform the above tests. This will be done in accordance with the Moffitt Total Cancer Care (TCC) protocol.

7.3 STUDY SCHEDULE

7.3.1 SCREENING

Screening Visit (Day -28 to -1)

- Obtain and review medical history to determine eligibility based on inclusion/exclusion criteria.

- Written informed consent to be obtained prior to any screening procedures
- Review medications history (including over-the-counter drugs, vitamins, herbs, alcohol) to determine eligibility based on inclusion/exclusion criteria.
- Perform physical examination needed to determine eligibility based on inclusion/exclusion criteria
- Vital signs measurements
- Evaluation of ECOG performance status
- Twelve-lead ECG and Echocardiogram or MUGA.
- Serum beta-human chorionic gonadotropin (β-hCG) pregnancy test for women of childbearing potential
- Clinical laboratory testing including urinalysis, serum chemistry, hematology, and coagulation parameters. Clinical assessments (laboratory tests, ECHO/MUGA, EKG) performed as part of the subject's routine clinical evaluation and not specifically for this study need not be repeated after signed informed consent has been obtained provided the assessments fulfill the study requirements and are performed within 28 days of starting study treatment.
- Bone marrow biopsy and peripheral blood sample will need to be repeated in order to collect correlative samples.
- CT or PET/CT scan of affected areas for subjects determined to have extramedullary disease or for staging purposes prior to treatment.
- Spinal tap if subject is determined to be symptomatic for CNS involvement

7.3.2 ENROLLMENT/BASELINE/STUDY VISITS

7.3.2.1 Induction Baseline Visit (Day 1)

- In-patient admission
- Verify subject still meets inclusion/exclusion criteria.
- Review results of serum pregnancy test, if applicable.
- Assess and record baseline symptoms prior to study drug administration.
- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- LDH, uric acid, magnesium, phosphorus
- Initiation of induction therapy- study drug administration (CPX-351 intravenously)

7.3.2.2 Induction Day 2

- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- LDH, uric acid, magnesium, phosphorus

7.3.2.3 Induction Day 3

- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- LDH, uric acid, magnesium, phosphorus
- Study drug administration (initiation of CPX-351 intravenously)

7.3.2.4 Induction Day 4

- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- LDH, uric acid, magnesium, phosphorus

7.3.2.5 Induction Day 5

- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- LDH, uric acid, magnesium, phosphorus
- Study drug administration (initiation of CPX-351 intravenously)

7.3.2.6 Induction Day 6

- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- LDH, uric acid, magnesium, phosphorus

7.3.2.7 Induction Day 7

- CBC with differential

- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- LDH, uric acid, magnesium, phosphorus
- Peripheral blood sample for correlative studies
- CSF sample/IT chemo (if no contraindications, will be performed day 7-10 see Section 7.1.15)

7.3.2.8 Induction Day 10

- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- LDH, uric acid, magnesium, phosphorus
- CSF sample/IT chemo (if not performed on day 7, 8, or 9)
- Continuous hospitalization until determined to have acceptable bone marrow recovery

7.3.2.9 Induction Day 28

- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- LDH, uric acid, magnesium, phosphorus
- Bone marrow biopsy, if bone marrow biopsy is positive at screening
- CT or PET/CT scan of affected areas for response assessment for subjects with extramedullary disease
- Peripheral blood sample for correlative studies

7.3.2.10 Induction Day 29 +

- Continuous hospitalization until determined to have acceptable bone marrow recovery
- Laboratory testing continues per standard of care until hospital discharge

7.3.2.11 First Consolidation Therapy Day 1

- Subject has documented response (CR,Cri, or PR, see Appendix A), or in the opinion of the principal investigator is showing clinical benefit with adequate count recovery (ANC > 500/□Land platelets > 50,000/□).
- CBC with differential

- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- Day 1 must be given 28 days-75 days after start of induction
- Study drug administration (initiation of CPX-351 intravenously)

7.3.2.12 First Consolidation Therapy Day 3

- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- Study drug administration (initiation of CPX-351 intravenously)

7.3.2.13 First Consolidation Day 28

- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- LDH, uric acid, magnesium, phosphorus
- Bone marrow biopsy, if bone marrow biopsy is positive at screening
- CT or PET/CT scan of affected areas to assess response for subjects with extramedullary disease

7.3.2.14 Second, and Third Consolidation Therapy Day 1

- Subject has documented response (CR,CRi, or PR, see Appendix A), or in the opinion of the principal investigator is demonstrating clinical benefit with adequate count recovery (ANC > 500/□Land platelets > 50,000/□)
- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- Echocardiogram will be repeated for any patient with >550mg/m2 daunorubicin (or daunorubicin anthracycline equivalent) exposure. Therapy will only be permitted if the ejection fraction remains >50% and without >10% decrease in ejection fraction from baseline.
- Day 1 must be given 28 days-56 days after start of the previous consolidation
- Study drug administration (initiation of CPX-351 intravenously)

7.3.2.15 Second and Third Consolidation Day 3

- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- Study drug administration (initiation of CPX-351 intravenously)

7.32.16 Second and Third Consolidation Day 28

- CBC with differential
- Chemistry profile (ALT, AST, alkaline phosphatase, total bilirubin, calcium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin)
- LDH, uric acid, magnesium, phosphorus
- Bone marrow biopsy, if bone marrow biopsy is positive at screening
- CT or PET/CT scan of affected areas to assess response for subjects with extramedullary disease

7.3.2.17 End of Treatment Visit

- Visit occurs 30 days (+10 days) after the last dose of study drug
- May occur earlier if initiating subsequent antineoplastic therapy
- Assess adverse events

7.3.3 FOLLOW-UP

After the end of treatment assessment, all subjects will be followed with procedures and physical examinations per standard of care.

7.3.4 FINAL STUDY VISIT

When it is determined the study is to be final closed, a Final Study Visit will be performed. This visit will occur at the next regularly scheduled standard of care Follow-Up Visit after trial closure is confirmed. Any AE that is ongoing will be considered ongoing at the Final Study Visit.

Final instructions will be provided to the subject and the subject will be informed if results will be available or any planned presentation or publication of study data.

7.3.5 EARLY TERMINATION VISIT

If a subject terminates study participation for any reason prior to completion of the protocol treatment plan, any subsequent procedures or evaluations will be per physician discretion as part of standard of care. Evaluation of AEs will continue as outlined in Section 7.1.9.

7.3.6 UNSCHEDULED VISIT

Any visit not included on the Schedule of Events Table that includes examination in the Moffitt hematology clinic, inpatient hospitalization or any unexpected extended hospitalization, laboratory

evaluations or management of adverse events, will be considered an Unscheduled Visit. It will be documented in the subject's medical record and included on the adverse event log. Adverse event or serious adverse event data will be collected in Oncore. No separate Oncore form for an unscheduled visit will be used to collect additional data.

7.3.7 SCHEDULE OF EVENTS TABLE

Procedures: Day:	**Screening[a]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**10**	**28**	**29+[i]**	**End of Treatment/ Early Term.[g]**	**Follow-Up**
Informed consent	X												
Demographics and Medical History	X												
Baseline Symptoms[b]		X											
day Performance Status	X												
Concomitant Medication Review	X	Assess throughout Induction and Consolidation											
Review Adverse Events[c]		Assess throughout Induction and Consolidation										X	
CPX-351 Administration[d]		X		X		X							
Physical Examination	X												
Serum pregnancy test[e]	X												
Vital signs (temp, pulse, resp, BP, weight)	X	X		X		X	Per SOC						
Height	X												
12-lead EKG	X												
Echocardiography/MUGA	X										X[l]		
CBC with differential	X	X	X	X	X	X	X	X	X	X	Per SOC		
Serum chemistry testing [f]	X	X	X	X	X	X	X	X	X	X	Per SOC		
LDH, uric acid, magnesium, phosphorus	X	X	X	X	X	X	X	X	As needed				
Coagulation studies (PT/INR, aPTT, fibrinogen)	X												
Urinalysis with microscopy	X												
Bone marrow biopsy[k]	X									X			
CT or PET/CT Scan[m]	X									X			
Correlative studies[h]	X[1,2,3]									X[3]			
CSF for ALL involvement/IT chemo[j]	X							X					
Follow-Up													X

a. Screening assessments will be performed within 28 days of the dose of study drug Day 1. Any standard of care procedure performed within 28 days of Day 1, may be used for screening/eligibility even if performed prior to consent.
b. Baseline symptoms are defined as those present at the closest time before the start of study drug administration.
c. Adverse event data will be captured from the time of consent until 30 days after the last dose of CPX-351.

d. CPX-351 induction therapy will only be administered to a subject following hospital admission. One induction will be administered. Patients with a CR, CRi, PR or determined by the PI to be experiencing clinical benefit after induction, may receive up to 3 consolidation treatments, all administered as an outpatient. First consolidation must be administered no earlier than 35-75 days after day 1 of induction. Second and third consolidations will be administered no earlier than 35-56 days after the start of the previous consolidation.
e. Serum pregnancy test will be performed on females of childbearing potential (FCBP). A FCBP is considered when a sexually mature female: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 12 consecutive months.
f. Serum chemistry testing will include the following: albumin, BUN, creatinine, alkaline phosphatase, ALT, AST, CO2, calcium, chloride, glucose, potassium, sodium, total bilirubin, total protein.
g. The EOT visit will occur 30 days (+10 days) after the last dose of study drug or before the start of subsequent antineoplastic therapy if that occurs sooner.
h. Correlative studies will be performed as below:
   1. BH3 protein- bone marrow/core biopsy
   2. Ex vivo drug sensitivity assay- bone marrow/core biopsy
   3. T-cell profile- bone marrow/core biopsy/peripheral blood
   4. PDL1 immunohistochemistry- bone marrow/core biopsy
i. After induction day 28 and through consolidation therapy, laboratory testing will be continued as necessary per standard of care.
j. Only obtained at screening if symptomatic. Per standard of care, CSF (if no contraindications) will be obtained 7-10 days after initiation of CPX dosing and IT methotrexate will be administered. See Section 7.1.15.
k. Bone marrow biopsy will be at screening and day 28 of induction therapy. During consolidation therapy, a bone marrow biopsy will be performed on day 28 (+7 days) after the start of each consolidation per standard of care.
l. Echocardiogram will be repeated for any patient with >550mg/m2 daunorubicin (or daunorubicin anthracycline equivalent) exposure. Therapy will only be permitted if the ejection fraction remains >50% and without >10% decrease in ejection fraction from baseline.
m. CT or PET/CT scan will be utilized at screening and Day 28 as response assessment for subjects with extramedullary disease or for staging purposes as determined by the investigator.

7.4 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

All subjects should be maintained on the same medications throughout the study period, as medically feasible.
The investigator should instruct the subject to notify the study staff about any new medications he/she takes after the start of the study drug. All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) administered after the subject starts treatment with study drug must be recorded:

- Administration of pegfilgrastim or filgrastim following initiation of protocol therapy will occur for all patients. It is suggested dosing start at Day 6.
- Administration of erythropoietin or darbopoietin is allowed;
- Patients must be instructed not to take any additional medications (including herbal supplements and over-the-counter products) during the trial without prior consultation with the investigator. All medications taken within 30 days of screening should be recorded. If concomitant therapy must be added or changed, the reason and name of the drug/therapy should be recorded;
- In general, the use of any concomitant medication/therapies deemed necessary for the care of the patient are allowed, including drugs given prophylactically (e.g. antiemetics).

7.5 JUSTIFICATION FOR SENSITIVE PROCEDURES

7.5.1 PRECAUTIONARY MEDICATIONS, TREATMENTS, AND PROCEDURES

- Subjects will not routinely be pre-medicated for hypersensitivity or infusion-related reactions initially during the first infusion of the first treatment course. If a reaction occurs, pre-medications for subsequent infusions will be at investigator discretion. Infusion reactions will be treated per Moffitt institutional standards.
- In previous clinical trials, no drug or food interactions were identified as being associated with CPX-351.

7.6 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

- An evaluation of prior exposure to anthracyclines should be performed during screening. Per the study Exclusion Criteria, subjects with prior cumulative anthracycline exposure of greater than 368 mg/m2 daunorubicin will be excluded.
- High dose corticosteroids used for management of ALL symptoms are permitted up to one day prior to the first dose of study drug. Corticosteroids required as pre-medication for CT scans or for short-term use as treatment for adverse events, are permitted in consultation with the PI.
- Other anti-cancer treatment is not permitted during the treatment phase of the study.

7.7 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

- Subjects may be pre-medicated for nausea and vomiting at investigator discretion.
- Patients may receive ongoing supportive and palliative care (e.g. pain control) as clinically indicated throughout the study.
- 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 per Moffitt institutional standard of care.
- Growth factor support will be administered according to Moffitt protocol.
- The use of transfusion support (RBCs and platelets) will be according to standard of care.

7.8 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

- Growth factor support with granulocyte stimulating factor will be administered according to Moffitt protocol for the treatment of cytopenias
- The use of transfusion support (RBCs and platelets) will be according to standard of care as rescue therapy for anemia or thrombocytopenia.
- Corticosteroids and antihistamines may be administered per Moffitt protocol for the treatment of hypersensitivity reactions should they emerge.

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

An adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Schedule of Events and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up

period. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

Adverse events and suspected adverse reactions are considered "serious" if, in the view of either the investigator or sponsor, they result in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious 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.

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS

Unanticipated problems (UPs) involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

All AEs will be graded using the CTCAE 5.0 criteria.

For AEs not included in the protocol-defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or

incapacitating.

8.2.2 RELATIONSHIP TO STUDY AGENT

For all collected AEs, the investigator will evaluate the participant and will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Unrelated** – The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.2.3 EXPECTEDNESS

The PI will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews with study participants presenting for medical care, or upon review by a study monitor. All AEs, including local and systemic reactions not meeting the criteria for SAEs, will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of event. All AEs occurring during the study must be documented appropriately, regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at anytime during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of AEs will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as "intermittent" require documentation of onset and duration of each episode in the medical record.

Adverse events for the purpose of this study will be will be reported from the time period beginning when the consent form is signed until 30 days after study drug discontinuation. Events will be followed for outcome information until resolution or stabilization is achieved. Any AE present at the time of final study closure will be considered ongoing.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

Adverse event documentation will be performed by entry into the Moffitt OnCore electronic database. Reporting of adverse events to the FDA for IND renewal will occur annually by a report obtained from the AEs collected in OnCore. Reporting to Jazz Pharmaceuticals will be as noted in the section on AEs of special interest.

All AEs will be documented on a paper log with columns for the replication of the fields in the OnCore AE data form. The treating physician/investigator will review the log for accuracy and assign CTCAE grade and the attribution of a study agent to the AE.

Adverse events for the purpose of this study will be reported from the time period beginning when the consent form is signed until 30 days after study drug discontinuation.

8.4.2 SERIOUS ADVERSE EVENT REPORTING

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the adherence to be stable. Other supporting documentation of the event may be requested by Jazz Pharmaceuticals, Inc. and should be provided as soon as possible. Moffitt as the study sponsor will be responsible for notifying FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

8.4.3 UNANTICIPATED PROBLEM REPORTING

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report UPs to their IRB and to Jazz Pharmaceuticals, Inc. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;

- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB and to the study sponsor within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the study sponsor within 2 business days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures) and the supporting agency head (or designee), within 7 days of the IRB's receipt of the report of the problem from the investigator.

8.4.4 EVENTS OF SPECIAL INTEREST

1. Cardiotoxicity: Current studies estimate the risk of cardiotoxicity to be low, perhaps related to the liposomal formulation. However, given that patients will have likely had prior anthracycline exposure (up to a maximum of 368 mg/m2 daunorubicin or equivalent), we will obtain baseline echocardiogram or MUGA to ensure adequate ejection fraction. Followup echocardiogram or MUGA will be performed in all patients with symptomatic heart failure, defined as follows: worsening 1) dyspnea on exertion, 2) orthopnea, and/or 3) paroxysmal nocturnal dyspnea - with following concomitant findings of fluid overload on exam not well explained by prior therapy (such as hydration related to an episode of sepsis), including: 1) development of a third heart sound, 2) jugular venous distention with positive hepatojugular reflex, 3) pulmonary rales, and/or 4) lower extremity edema (ref 31). Follow-up echocardiogram or MUGA prior to each cycle of consolidation will be performed for all patients exceeding 550 mg/m2 daunorubicin (or equivalent) . Patients with an ejection fraction of <50% or a decrease of >10% from baseline will discontinue consolidation therapy. The development of symptomatic heart failure and/or decrease in ejection fraction to <50%, or >10% from baseline will be reported to Jazz Pharmaceuticals.

2. Bone marrow aplasia: Liposomal delivery of chemotherapy may facilitate increased deposition within hematopoietic stem cells, culminating in delay or failure to recover hematopoietic cell reconstitution following therapy. In particular, a hypocellular marrow without adequate count recovery >42 days from start of induction or consolidation cycles will be reported to the sponsor.

8.4.5 REPORTING OF PREGNANCY

If a pregnancy occurs in a female subject enrolled into the study, or a female partner of a male subject within 30 days of completing the CPX-351 infusion, the pregnancy must be reported to the key sponsor contact. Information regarding the pregnancy and/or the outcome may be requested by the key sponsor.

In addition to reporting any pregnancies occurring during the study, investigators will monitor for pregnancies that occur after the last dose of CPX-351 through 3 months for female subjects and for 6 months for the female partner of the male subjects.

The pregnancy should be reported to the key sponsor contact within 24 hours of the investigators knowledge of the pregnancy event.

If a lactation case occurs while the female subject is taking protocol required therapies report the lactation case to the key sponsor contact.

In addition to reporting a lactation case during the study, investigators should monitor for lactation cases that occur after the last dose of protocol required therapies through 3 months.

8.5 STUDY HALTING RULES

Safety assessments will consist of monitoring and recording all adverse events and serious adverse events, the regular monitoring of hematology and blood chemistry parameters and regular physical examinations. Adverse events will be evaluated continuously throughout the study. Safety and tolerability will be assessed according to the NIH/NCI Common Terminology Criteria for Adverse Events version (CTCAE v5.0) that is available at: http://evs.nci.nih.gov/ftp1/CTCAE/About.html
The Protocol Monitoring Committee (PMC) at Moffitt monitors its assigned ongoing research protocols for: adverse event reporting, data and safety monitoring, and internal audit findings. The PMC, upon review of any agenda item, may approve the study for continuation, require revisions, suspend or close a protocol. The PMC meets monthly and reviews accrual, patterns and frequencies of all adverse events, protocol violations and when applicable, internal audit results.

Investigators of studies which are designated to be reviewed by the PMC for data and safety monitoring, shall provide a statistical report of the study's progress and summary of adverse events and deviations based on the phase of the study and the associated risk of the study or more often if applicable. The PI will be notified for recommendations or notifications after the PMC review in regards of continuation or stopping the clinical trial.

8.6 SAFETY OVERSIGHT

Serious Adverse Events: Serious Adverse Events (SAEs) from this protocol will be reported concurrently to the IRB (per IRB guidelines) and Jazz Pharmaceuticals, Inc. within 24 hours of staff awareness of the event. The Protocol Monitoring Committee (PMC) will review these SAEs in accordance with their policy. The data and safety plan will define dose limiting toxicities and criteria for stopping the trial according to rules set forth by this protocol. This trial will be continuously monitored by the PI and the research team. A final safety and monitoring report will be submitted to the PMC. This protocol will be subject to periodic internal audits based on risk or as recommended by the PMC.

9 CLINICAL MONITORING

Data will be captured in OnCore, Moffitt's electronic Clinical Trials Database. For each participant enrolled, the electronic CRF must be completed by the assigned data manager or other authorized study staff. Any paper forms should be typed or filled out indelible ink and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. This also applies to records for those patients who fail to complete the study. If a patient stops dosing or terminates from the study, the dates and reasons must be noted on the CRF. If a patient terminates from the study because of a DLT, thorough efforts should be made to clearly document the outcome.

Regulatory documents and case report forms will be monitored internally according to Moffitt Cancer Center Monitoring Policies. Monitoring will be performed regularly by the MCC Clinical Monitoring

Core for accuracy, completeness, and source verification of data entry, validation of appropriate informed consent process, reporting of SAEs, adherence to the protocol, Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

The following study is a phase 2 pilot trial. Exploratory analyses of remission rate, safety, progression free survival, and overall survival are planned.

10.2 STATISTICAL HYPOTHESES

- All Endpoints are exploratory, and may be used to facilitate design of an expansion or subsequent phase 2 study.
- Primary Efficacy Endpoint:
  - Complete Remission and Complete Remission with incomplete hematologic recovery will serve as the primary endpoint.
- Secondary Efficacy Endpoint(s):
  - Safety
  - Minimal Residual Disease Remission Rate
  - Progression Free Survival
  - Overall Survival

10.3 ANALYSIS DATASETS

- All patients receiving at least one dose of CPX-351will be evaluable for safety.
- Efficacy analyses will be restricted to those receiving a full cycle of induction therapy.

10.4 DESCRIPTION OF STATISTICAL METHODS

10.4.1 GENERAL APPROACH

This will be a phase 2 pilot trial to assess efficacy of CPX-351 in relapsed and refractory acute lymphoblastic leukemia. If 4 or more CR/CRi are observed in the first 10 patients, Jazz Pharmaceuticals will consider an increase in the budget to facilitate accrual to a total of 19 patients (ie, 9 additional subjects), possibly in a multi-center setting. This would be sufficient to test our hypothesis that CPX-351 will increase the CR/CRi rate from a historical standard of 35% to 65% (as summarized in section 2.2) using a power of 90% and type I error rate of 0.1.

10.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

As noted above, this is a phase 2 pilot trial. However, expansion to a phase II trial will depend on demonstration of CR/CRi amongst 4 of the initial 10 treated patients. For illustration only, we have provided Simon Two-Stage Calculation, using power of 90% and Type I error rate of 0.1.

N1	R1	PET	N	R	Ave N	Alpha	Beta
10	**3**	**0.514**	**19**	**9**	**14.38**	**0.084**	**0.095**

Currently, we see approximately 50 new ALL patients per year, with around 70 in active followup. Given this, we can anticipate enrollment of the first 6 patients over a 12 month interval.

10.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

- Minimal Residual Disease (MRD) will be studied as a dichotomous endpoint using a cutoff of $1x10^{-4}$ cells/transcripts as the lower limit for residual leukemia, and presented as the percentage of patients reaching this landmark as their best response. MRD assessment will be obtained with each bone marrow biopsy assessment, as described below.
- Progression Free Survival and Overall Survival will be reported as per Cheson and Lugano Criteria, and analyzed using a standard Kaplan-Meier approach. Patients will undergo bone marrow biopsy evaluation after each cycle of therapy per standard of care to facilitated assessment. Patients achieving CR/CRi at the end of consolidation with marrow involvement will have follow-up bone marrow biopsy evaluation every 3 months in the first year following their last cycle of therapy, and every 6 months in the subsequent 2 years following their last cycle. Patients who remain in remission greater than 3 years from their last cycle of therapy will have bone marrow biopsy evaluation performed as needed. Patients without marrow involvement will have follow-up imaging (CT or PET/CT) every 3 months in the first year following their last cycle of therapy, and every 6 months in the subsequent 2 years following their last cycle. Patients progressing to allogeneic transplant following therapy will be censored at the time of conditioning.
- Safety will be continually assessed during the course of induction and consolidation cycles. Safety assessment will continue at a minimum of every 3 months for those who do not progress to allogeneic transplant or alternative therapy.

10.4.4 SAFETY ANALYSES

Using summary statistics, safety data will be coded using CTCAE 5.0 criteria, with each AE being counted only once for a given participant. The severity, frequency, duration, outcome, and relationship of AEs to study agent, as defined by the treating investigator, will be presented by System Organ Class (SOC) and preferred term groupings. Adverse events leading to premature discontinuation from the study drug, adverse events of special interest, and serious treatment-emergent AEs will be presented in a table.

10.4.5 ADHERENCE AND RETENTION ANALYSES

Patients will be hospitalized to receive induction therapy, and remain so until sufficient recovery permits safe hospital discharge, in accordance with standard of care. Consolidation therapy will be administered as an outpatient. Patients will be monitored as an outpatient in accordance with standard of care until recovery or study discontinuation. Study discontinuation due to adverse events, related or unrelated to study drug, will be assessed.

10.4.6 BASELINE DESCRIPTIVE STATISTICS

Planned baseline statistics include age, ethnicity, gender, genetic risk group (Philadelphia positive, Philadelphia-like, MLL rearranged, p53 mutant, etc…), baseline marrow blast burden, number of prior therapies, response to prior therapies (i.e., relapsed vs refractory), and prior exposure to selected therapeutic modalities (including allogeneic transplant, CAR T-cell therapy, blinatumomab, and inotuzumab). Demographics will be presented using summary statistics.

10.4.10 TABULATION OF INDIVIDUAL RESPONSE DATA

Responses will be presented in aggregate only.

10.4.11 EXPLORATORY ANALYSES

As previously noted, all endpoints are presently exploratory in nature, and may be used to support expansion of the present study, and/or development of a separate study.

10.5 SAMPLE SIZE

Moffitt is a high volume center for ALL, with approximately 50 new cases referred to our center yearly, of whom ~10% are anticipated to be refractory to therapy, and ~50% will relapse within 2 years of therapy. The current inclusion and exclusion criteria developed within this protocol, as well as prior enrollment to protocols targeting ALL, support a screen failure rate of less than 10%. Similarly, internal experience with CPX-351 supports a drop-out rate (ie, failure to complete induction) of less than 10%. Accordingly, we anticipate full accrual of 10 evaluable patients within 24 months.

As previously noted, this is a phase 2 pilot study designed to assess the feasibility and provide a preliminary assessment of safety and efficacy to support a more formal phase 2 study.

10.6 MEASURES TO MINIMIZE BIAS

10.6.1 ENROLLMENT

Patients with relapsed ALL will be enrolled from the Malignant Hematology clinic at the Moffitt Cancer Center. Patients will not be blinded to therapy. Given the exploratory nature of this study, stratification of patients will not be performed. Patients who are unable to complete a full course of induction may be replaced to facilitate assessment of overall remission rate.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The PI and other appropriate study staff are responsible for maintaining appropriate medical and research records for this trial, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of participants. Representatives of Jazz Pharmaceuticals and federal regulatory agencies may examine records for the purpose of quality assurance reviews, evaluation of the study safety, progress of the trial, and data validity.

Source documentation in both electronic and paper form shall be retained for at least two years after the final closure of the trial. These include hospital records, clinical research subject charts (with paper AE logs), research laboratory notes, electronic CRFs, and pharmacy dispensing records.

12 QUALITY ASSURANCE AND QUALITY CONTROL

Quality control procedures will be implemented beginning with the OnCore data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the staff for clarification/resolution.

Following written SOPs, the Moffitt internal monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements (e.g., good laboratory practices (GLP), good manufacturing

practices (GMP)).

The investigational site will provide direct access to source data/documents, and reports for the purpose of monitoring and auditing, and inspection by local and regulatory authorities.

All staff will be trained by the PI through a site initiation presentation, power point training for training on the initial protocol for those staff unable to attend the initiation presentation, and ongoing self-study as protocol amendments are approved. Training will be documented on a signature log that will be filed in the electronic regulatory binder.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

13.2 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

13.3 INFORMED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study agent, study procedures, and risks will be given to the participant and written documentation of informed consent will be required prior to starting intervention/administering study product.

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing.

The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any

procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.4 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The Moffitt study monitor, other authorized representatives of Moffitt, representatives of the IRB or pharmaceutical companies supplying study products may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at Moffitt. This will not include the participants' contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by Moffitt research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Moffitt.

13.4.1 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS, OR DATA

Evaluation of correlative biomarkers will be performed on fresh tissue, with the exception of PLD1 immunohistochemistry. Patients consenting to the TCC platform may have additional unused material archived for future use – however, this will occur outside the context of this protocol, and will be governed independently.

A database will be created to store the clinical and analytical data for this project. This database will be located on a password-protected hard drive, and additionally, the database will be itself password-protected to ensure maximal security with regards to maintained privacy of patient-related information. Specific information included in this database will entail patient name, age, gender, medical record number, clinical diagnosis, molecular phenotype, treatment history, and survival. Database access will be limited to the PI and Co-PIs described above.

Patients will be selected according to established inclusion criteria as identified above. Patients will be identified primarily according to clinical documentation of disease status

included in clinical notes contained in the patient record; however, appropriate patients may also be identified in the context of clinical evaluation up front with names and medical record numbers **referred to** prior to scheduling the bone marrow biopsy procedure to facilitate sample identification and collection.

13.5 FUTURE USE OF STORED SPECIMENS

Data collected for this study will be analyzed and stored at the Moffitt Cancer Center. Permission to transmit data will be included in the informed consent.

For patients consenting to the Moffitt Total Cancer Care (TCC) protocol, de-identified biological samples will be stored within the Moffitt TCC biosample repository with the same goal as the sharing of data with Jazz Pharmaceuticals. These samples could be used for research into the causes of acute lymphoblastic leukemia, its complications and other conditions for which individuals with acute lymphoblastic leukemia are at increased risk, and to improve treatment. The Moffitt TCC biosample repository will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the masking of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage will not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the Moffitt TCC repository.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. All paper source documents will be scanned into the electronic medical record of each subject for storage. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into OnCore, a 21 CFR Part 11-compliant data capture system provided by Moffitt. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

14.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the drug combination studied in this protocol. No records will be destroyed without the written consent of the sponsor, if applicable.

14.3 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations as defined by the Moffitt Clinical Trials Office standard. All deviations must be addressed in study source documents. Protocol deviations must be sent to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

14.4 PUBLICATION AND DATA SHARING POLICY

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine.

The PI will provide to the Protocol Information Specialist at Moffitt Cancer Center, details as requested for registering and reporting results for this clinical trial on ClinicalTrials.gov. At the conclusion of the trial, the PI will make study results available to the research community and the public-at-large.

Authorship in publications will be determined by the PI depending on participation, enrollment, and significant contribution during the trial process and manuscript elaboration.

15 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership and the Moffitt Cancer Center have established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

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APPENDIX

Appendix A Response Criteria

Complete remission (CR)[a]	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); independence from red cell transfusions
CR with incomplete recovery (CRi)[b]	All CR criteria except for residual neutropenia (<1.0 x 10^9/L [1000/µL]) **or** thrombocytopenia (<100 x 10^9/L [100,000/µL])
Partial Response (PR)	Greater than 50% decrease in blasts, and/or decrease to less than 25% blasts with recovery of neutrophils (>1.0 x 10^9/L), and platelets (>100 x 10^9/L)
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 (blasts in peripheral blood, extramedullary leukemia, or persistence in the bone marrow)
Relapse[c]	Bone marrow blasts ≥5%; or reappearance of blasts in the blood after achievement of a CR or CRi; or development of extramedullary disease

[a] Bone marrow assessment required to confirm CR. 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.

[b] Bone marrow assessment required to confirm CRi. Some patients may not achieve complete hematologic recovery prior to initiation of consolidation. CRi cannot be declared earlier than Day 35 to allow adequate time for documentation of peripheral blood recovery. Consolidation may begin no earlier than 35 days after the last induction course.

[c] 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.

Appendix B
ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. 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).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	