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A PROSPECTIVE, SINGLE SITE, SINGLE-ARM PILOT STUDY OF CPX-351 (VYXEOS) IN INDIVIDUALS < 22 YEARS WITH SECONDARY MYELOID NEOPLASMS

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Protocol Summary

CPXSMN, A PROSPECTIVE, SINGLE SITE, SINGLE-ARM PILOT STUDY OF CPX-351 (VYXEOS) IN INDIVIDUALS < 22 YEARS WITH SECONDARY MYELOID NEOPLASMS
Principal Investigator: Raul C. Ribeiro, MD
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Brief overview: Secondary myeloid neoplasms (SMN) encompass therapy-related AML and MDS, and those with antecedent hematological disorders. These malignancies have a low incidence in the first two decades of life, have a poor prognosis and lack unified treatment approaches. There are no specific treatment guidelines for this subgroup of myeloid neoplasms in children, except that most patients are candidates for hematopoietic stem cell transplantation (HSCT). CPX-351 was FDA approved in the US for therapy-related AML and AML with myelodysplasia related changes syndrome in adults in 2017, and the approval extended to patients older than 1 year of age in 2021. The FDA approval for younger age group was based on the use of CPX-351 in a Phase II study in children with relapsed AML, and pharmacokinetic data showing similar distribution parameters in children and adults. Specific clinical efficacy and toxicity of CPX-351 data in children and adolescents with SMN are not available.
Intervention: Interventional, primary therapeutic Drug: CPX-351 (Vyxeos)
Brief outline of treatment plan: We propose to use the same dose and schedule approved for adults with secondary AML and recently expanded to include children. The CPX-351 dose for the first induction cycle is 100 units/m^2 (corresponds to 44 mg/m^2 daunorubicin and 100 mg/m^2 cytarabine) on Days 1, 3 and 5. A second induction cycle at the same CPX-351 dose is administered on Days 1 and 3 for patients with CR/CRi but positive residual disease (MRD ≥0.1%) by flow cytometry. Patients attaining complete remission and MRD < 0.1% could proceed to hematopoietic stem cell transplantation (HSCT). If HSCT cannot be performed within four weeks from the evaluation date (Day 22 of the first cycle), patients will receive a second course with lower doses of daunorubicin (29 mg/m^2) and cytarabine (65 mg/m^2).
Study design: Prospective, single site, single-arm pilot study.
Sample size and duration: 25 patients accrued over 2-3 years.
Data management: Data management and statistical analysis will be provided by the Comprehensive Cancer Center Hematological Malignancies Program and the Biostatistics Department at St. Jude Children's Research Hospital.
Human subjects: The main risk to research participants will be the potential toxicities associated with the use of investigational agent, CPX-351. The research participants will be informed of the toxicities that have been associated with the study drugs and potential side effects of procedures recommended in this study. Adverse events will be monitored, treated, and reported following institutional and federal guidelines and regulations.

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APPENDICES

1.0 OBJECTIVES

Hypothesis: CPX-351 is active and well-tolerated in individuals < 22 years with secondary myeloid neoplasms (SMN).

1.1 Primary Objective

1.1.1 Determine the composite complete remission (CR) and complete remission with incomplete peripheral blood recovery (CRi) rates, safety, and tolerability in patients under 22 years of age with SMN treated with one or two courses of CPX-351 before HSCT.

1.2 Secondary Objectives

1.2.1 Describe the toxicity profile of patients with SMN treated with one or two courses of CPX-351.
1.2.2 Describe the biologic correlates of response in patients with SMN after one or two courses of CPX-351.
1.2.3 Estimate the 3-year overall survival of patients who received one or two courses of CPX-351 followed by HSCT.

1.3 Exploratory Objectives

1.3.1 Describe the clinical correlates of the cytarabine (Ara-C) pharmacogenomic response score (ACS), the leukemia stem cell score (pLCS6) and drug resistance score (ADE-RS5) in patients receiving CPX-351.
1.3.2 Investigate how genetic lesions in leukemic cells change after CPX-351treatment.
1.3.3 Explore the feasibility of single-cell DNA sequencing (scDNAseq) for characterization and tracking of clonal repertoires.

2.0 BACKGROUND AND RATIONALE

2.1 Background

Secondary myeloid neoplasms in children and adolescents are rare and have a poor prognosis.[1] This distinct subgroup of myeloid neoplasms is heterogeneous and includes patients previously treated for malignancies or secondary to antecedent hematologic disorders, including acquired and inherited bone marrow failure, and germline cancer predisposition syndromes. Depending on the blast count at the diagnosis, the disease continuum spans from secondary myelodysplastic syndrome (sMDS) (<20% marrow blasts) to secondary acute myeloid leukemia (sAML) (≥20% marrow blasts), however, almost all patients show increase blast counts over time.

Resistant leukemia and treatment-related mortality account for most of the failures among children and adolescents with SMN. A SEER population-based study showed that the 5-year survival of sAML was around 23%.[2] An abstract presented at the ASH meeting in 2019 by the AML-BFM study group reported that the overall survival for 145 patients with

sAML treated between 1993-2018 was 28% (median age, 10.6 years; range, 1.4-26.6 years). For patients treated between 2012 and 2018, the survival was 45%, although the follow-up was short.[3] In general, the patients who were enrolled between 1993-2003 on BFM AML protocols received two intensive induction remission courses followed by two consolidation courses before proceeding to hematopoietic cell transplantation (HCT). The rate of early deaths was high among these patients (14%). After 2014, patients received only two cycles of intensive chemotherapy before transplantation. The 5yrs□overall survival (OS) rate increased from 19 ± 5% (n=70 1993□2003) to 34±7% (n=41, 2004□2011) and 45±9% (n=34, 2012□2018; $p_{\text{log rank}}$ <0.03) suggesting that profound myelosuppression might not be necessary for improved outcomes. The poor tolerance to conventional intensive AML therapy is partially explained by previous exposure to chemotherapy or previous bone marrow damage associated with immunologic, environmental, or constitutional genetic characteristics.[4-10] Secondary MDS appears to have similar outcome to the AML counterpart. A report from the European Working Group of MDS in childhood, showed that the overall survival (OS) at 5 years was 44% for 80 patients with treatment-related MDS. In this study cytogenetic characteristics were associated with outcome. Cases with structural complex karyotypes had OS < 10%.[11] Biologically, SMN harbor complex karyotypic and molecular changes typically associated with poor prognosis in *de novo* AML.

The FDA granted a breakthrough therapy designation for CPX-351 in 2016 based on pre-clinical and Phase I and II studies.[12-20] In a Phase III randomized trial, CPX-351 was compared to standard 7 plus 3 chemotherapy. CPX-351 led to a significantly higher remission rate (48% vs. 33%) and relatively low 60-day mortality (13.7%).[21] At 12 months, 42% of patients enrolled in the CPX-351 arm remained alive compared with 28% in the control arm.[21] A 5-year follow-up of this study has recently been reported.[22] Despite these excellent results, CPX-351 has not been investigated in children and adolescents with SMN. The ongoing Children's Oncology Group frontline AML trial (AAML1831) compares CPX-351 + Mylotarg to standard chemotherapy + Mylotarg in induction remission for individuals less than 22 years with *de novo* AML.[23] In this trial, patients with SMN or with a germline predisposition to leukemia are not eligible. Therefore, currently there is no available protocol for patients with SMN in this age group in the US. There is an unmet need to investigate a uniform treatment for pediatric patients with SMN prior to HSCT.

Since 2017, we have treated seven patients with CPX-351 for sAML/MDS. Four patients had a history of chemotherapy and radiotherapy for solid tumors, one chemotherapy for T-cell acute lymphoblastic leukemia, and two had AML with poor genetic features that rapidly progressed from MDS. The age at diagnosis of these patients ranged from 13 to 23 years, median 17 years. The number of courses of CPX-351 ranged from 1 to 3. All seven patients had CR or CRi. Six of the seven patients received allograft in complete morphologic remission; some with minimal residual disease level <1%. One patient was not transplanted because of logistic difficulties, and leukemia progressed before HSCT could be arranged. CPX-351 was well tolerated in all seven patients, and except for skin rashes, no severe complications attributed to CPX-351 were noted. Mortality within the first 60 days was zero. Six patients underwent transplantation. HSCT was well-tolerated, led to full engraftment, and none of the patients experienced severe toxicity. All six patients who underwent HSCT are alive for periods ranging from 1 to 3 years after

diagnosis. Based on this limited series, we suggest that CPX-351 might be as effective in children and adolescents as in adults with treatment-related AML or MDS-related AML. Ideally, HSCT should be performed as soon as possible after remission is attained. If HSCT will not be available in a timely fashion, therapy adapted to the leukemia genotype could be considered to bridge to HSCT.

2.1.1 Anthracycline cardiotoxicity

Although cardiotoxicity is a concern with the use of anthracycline drugs, and some patients may reach a cumulative doxorubicin equivalent dose of 530 mg/m^2 in our study, available data showed that CPX-351 is not particularly cardiotoxic. In the Phase I/II COG study of CPX-351[18], myelosuppression was the limiting toxicity. Cardiac toxicity was not seen in doses up to 100 u/m^2. One patient had transient cardiotoxicity at a higher dose (135 u/m^2). Eligibility criterion was 450 mg/m^2 (daunomycin/doxorubicin equivalence) for entry in the COG study. Moreover, one third of the patients had relapsed after hematopoietic stem cell transplantation. The liposomal CPX-351 encapsulation is likely the main reason cardiotoxicity is less common. Intensification of chemotherapy with another liposomal encapsulated daunorubicin, DaunoXome® (DNX), has proven effective and tolerable in the context of pediatric AML studies conducted by the International Berlin-Frankfurt-Münster Study Group. In the relapse setting, DNX plus fludarabine and cytarabine (FLAG-DNX) demonstrated enhanced leukemic efficacy and similar cardiotoxicity compared to FLAG alone (no anthracycline).[24] Although, data on cardio protection of CPX-351 are still sparce, the encapsulation of the compounds in lipid particles appears to limit the release of the drugs in non-hematopoietic organs. Pre-clinical studies informed that the free drug distribution is mainly in the bone, marrow, spleen, and liver. CPX-351 (without dexrazoxane) is currently being studied in newly diagnosed pediatric AML by COG in Phase III randomized study (NCT04293562). Finally, currently, most children and adolescents who receive anthracycline for treating their primary cancer also receive dexrazoxane. Dexrazoxane significantly reduced short-term cardiac dysfunction, although it is not known whether the beneficial effect of dexrazoxane extends to long-term cardiac complications. Therefore is plausible that the lifetime exposure recommended for doxorubicin equivalents might change. In summary, the lipidic formulation of daunorubicin in CPX-351, the relatively low doxorubicin equivalent dose (65 mg/m^2 per course) in our study, the pre-clinical and clinical data suggesting less cardiotoxicity of anthracycline in lipid formulation, CPX-351 efficacy in this potentially fatal disorder, and prior approval by FDA for this indication, support further studies of CPX-351.

2.2 Rationale for this Study

The results of Phase I and Phase II trials of CPX-351 in individuals younger than 22 years with relapsed AML conducted by Children's Oncology Group (COG) revealed a response rate of 68.3% (CR + CR without platelet recovery) and no toxic deaths during induction or after HSCT (18,19). The study cohort comprised patients with poor prognosis including two-thirds of whom had relapsed within the first year from the diagnosis, and 10 of 38 patients relapsed after HSCT. The response rates (efficacy) in this trial compared favorably to those reported by the same group treating children and adolescents with relapsed AML using standard regimens.[25,26] However, cases of SMN were not enrolled on this trial. To our

knowledge, no other studies are currently active or under development to include children and adolescents with SMN. Our study will provide unavailable data for this patient population on the response to upfront treatment with CPX-351.

Because the bone marrow and other organs of many patients with SMN have been exposed to prior high doses of chemotherapy and/or radiotherapy or harbor intrinsic genetic abnormalities of the hematopoietic system, they are prone to develop severe toxicity after intensive salvage conventional induction therapy. Advantages of liposomal preparations include prolonged time in circulation due to protection of the drug from enzymatic inactivation, circumvention of drug efflux transporters responsible for drug resistance and altered bio-distribution of the liposome formulation favoring accumulation of the drug in the bone marrow and spleen while sparing of normal tissue.[27] Related to anthracycline-induced toxicity, which is potentially devastating for children treated with conventional AML induction therapy, anthracycline liposomal delivery systems have the potential to decrease cardiotoxicity,[28,29] although this benefit is not yet well studied for CPX-351.[20,30,31]

In summary, efficacy, and toxicity data of CPX-351 in children with SMN, can provide critical information for improving the outcome of this rare subgroup of myeloid neoplasms.

2.3 Rationale for Correlative Studies

2.3.1 Pharmacogenomics

Considering that CPX- 351 needs to be activated to the active metabolite ara-CTP for its antileukemic effect, we anticipate that genetic profiling of patients based on ACSS score will be helpful in predicting response and developing strategies to adapt cytarabine dosing in future studies.[32] Our transcriptomic based pediatric six-gene leukemic stem cell score (pLSC6) is predictive of overall outcome and HSCT related outcome.[33] We have also recently developed a drug resistance score that is composed of 5 genes of relevance to cytarabine and daunorubicin that is predictive of outcome.[34] Based on these results we propose to evaluate these transcriptomic variables in response to CPX-351 as well as response to HSCT.

2.3.2 Genomic characteristics of pediatric SMN

Secondary myeloid neoplasms in children have distinct genetic lesions, as defined previously.[11,35] It is not known how CPX-351 alters the architecture of aberrant hematopoietic clones. This study offers the opportunity to determine how genetic lesions in leukemic cells change after CPX-351 on clonal hierarchy and size. This can be achieved using standard genomic methodologies established at our institution. Standard genomic methods (whole exome / genome / transcriptome sequencing) will be used to identify genetic alterations at diagnosis. However, the technical limitation of these approaches is the inability to call somatic mutations with low allelic burden, thus potentially missing small, mutated clones that decrease in size after CPX-351. This provides a rationale to apply targeted ultra-deep sequencing panels with a sensitivity of at least 0.1%, or other approaches such as PCR or cell free DNA sequencing. Another possibility is to explore the feasibility of single-cell sequencing (scDNAseq) for characterization and tracking of clonal

repertoires in response to CPX-351 treatment. In addition, this method can resolve the question if distinct mutations are found in the same or separate cells, and which is the dominant one that is CPX-351 responsive.

Overall, we believe that the results from these investigations will open up opportunities for the clinical utility of genomic, pharmacogenomic and transcriptomic data in pediatric SMN.

3.0 ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

3.1 Inclusion Criteria

According to institutional and NIH policy, the study will enroll research participants regardless of gender and ethnic background. Institutional experience confirms broad representation in this regard.

3.1.1 Patients must be $\geq$ 1 year and < 22 years of age at the time of enrollment.

3.1.2 Patient must have one of the following diagnoses:

Treatment-related MDS/AML: Patients with solid organ or hematopoietic neoplasms previously treated with alkylating agents, ionizing radiation, topoisomerase inhibitors, antimetabolites, thiopurines, mycophenolate mofetil, fludarabine, and anti-tubulin agents (vincristine, vinblastine, vindesine, paclitaxel, and docetaxel usually in combination), who develop MDS, or AML are candidates for the CPXSMN protocol. If the bone marrow has between 5% and 20% blasts (higher-risk MDS), patients are discussed with the hematopoietic stem cell transplantation (HSCT) team for consideration to receive chemotherapy before HSCT. If the consensus is that cytoreduction before HSCT is necessary, and the cumulative dose of doxorubicin equivalent is < 500 mg/m^2 (in cases of cardio protection) or $\leq$ 400 mg/m^2 (cases without cardio protection), patients are eligible for the CPXSMN protocol.

OR

Secondary MDS/AML: Patients with primary MDS in transformation to AML (refractory cytopenia with an excess of blasts), acquired aplastic anemia evolving to AML, myeloid neoplasms arising from inherited bone marrow failure syndromes (including severe congenital neutropenia, Schwachman-Diamond syndrome, MECOM syndrome) or MDS/AML predisposition syndromes (including germline predisposition in *GATA2*, *RUNX1*, *SAMD9/SAMD9L*, *ERCC6L2*, *NF1*, *ETV6*, *ANKRD26*, *ERCC6L2, TP53* or *CEBPA* genes) are eligible for the CPXSMN trial. If the bone marrow has between 5% and 20% blasts (higher-risk MDS), patients are discussed with the HSCT team for consideration to receive chemotherapy before HSCT. If the consensus is that cytoreduction before HSCT is necessary, the patients are eligible for the CPXSMN protocol.

3.1.3 Patients must have a performance status corresponding to an Eastern Cooperative Oncology Group (ECOG) score of 0, 1 or 2. Use Karnofsky for patients > 16 years of age and Lansky for patients ≤ 16 years of age. See Appendix I for performance status correlations.

Note: Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

3.1.4 Concomitant medications restrictions

See Section 4.2.5 or Appendix II for concomitant therapy restrictions for patients during treatment.

3.1.5 Organ function requirements

3.1.5.1 Adequate renal function defined as:

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

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

The threshold creatinine values in this table were derived from the Schwartz formula for estimating GFR (REF) utilizing child length and stature data published by the CDC.

3.1.5.2 Adequate liver function defined as (unless it is related to leukemic involvement):

- Direct bilirubin ≤ 1.5 x upper limit of normal (ULN) for age and institution. At institutions that do not obtain a direct bilirubin in patients with a normal total bilirubin, a normal total bilirubin may be used as evidence that the direct bilirubin is not > 1.5 x the ULN.
- SGPT (ALT) ≤ 3.0 x ULN for age and institution

3.1.5.3 Adequate cardiac function defined as:

- Shortening fraction of ≥ 27% by echocardiogram, or
- Ejection fraction of ≥ 50% by radionuclide angiogram or echocardiogram, and

- Corrected QT (QTcB) interval < 500 msecs

3.1.5.4 Central nervous system function defined as:

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

3.1.6 Prior therapy

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

a. Myelosuppressive chemotherapy: Must not have received myelosuppressive chemotherapy within 3 weeks of entry onto this study (excluding hydroxyurea). Cyto-reduction with hydroxyurea can be initiated and continued for up to 24 hours prior to the start of CPX-351.
b. Biologic (anti-neoplastic agent): At least 7 days since the completion of therapy with steroids, retinoids or hypomethylating agents. Note: For agents that have known adverse events occurring beyond 7 days after administration (i.e. monoclonal antibodies), this period must be extended beyond the time during which acute adverse events are known to occur.
c. Radiation therapy (RT): ≥ 2 weeks for local palliative RT (small port); ≥ 6 months must have elapsed if prior craniospinal RT or if ≥ 50% radiation of pelvis; ≥ 6 weeks must have elapsed if other substantial BM radiation. Note: Patients must have received ≤ than 13.6 Gy prior radiation to mediastinum. Patients with prior cumulative doxorubicin equivalent > 400 mg/m^2 and prior radiation (any dose) to the mediastinum are not eligible for the protocol.
d. Hematopoietic stem cell transplantation: No evidence of active graft vs. host disease for at least 4 weeks. For allogeneic HSCT patients, ≥ 3 months must have elapsed since HSCT.
    - Must have received no more than 1 prior autologous or allogeneic stem cell transplant.
    - Patients must be off all systemic immunosuppressive therapy for at least 2 weeks, excluding hydrocortisone for physiologic cortisol replacement.
e. Intrathecal cytotoxic therapy:
    - No waiting period is required for patients having received intrathecal cytarabine, methotrexate, and/or hydrocortisone.
    - At least 14 days must have elapsed since receiving liposomal cytarabine (DepoCyte) by intrathecal injection.
f. Growth factors:
    - Patients must not have received hematopoietic growth factors for 7 days prior to CPX-351.

- Patients must not have received pegfilgrastim for 14 days prior to CPX-351.

3.1.7 HIV disease

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

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

3.1.8 Residual or relapsed solid malignancy

Patients with residual or relapsed solid malignancy (for example osteosarcoma) at the time of the diagnosis of SMN are not excluded from this trial and the treatment individualized to integrate the management of the two malignancies.

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

3.2 Exclusion Criteria

3.2.1 Patients with *de novo* AML (i.e., patients eligible for St. Jude or COG frontline AML trials).

3.2.2 Patients with any of the following:

- Constitutional trisomy 21 or with constitutional mosaicism of chromosome trisomy 21
- Patients with Fanconi Anemia (FA)
  Note: Very rarely patients with FA may not display any of the classic constitutional signs and symptoms associated with the disease. Myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML) could be the first manifestation of a patient with FA.[36] For patients presenting with MDS/AML without a clear etiology—such as history of chemotherapy or radiation (indicative of treatment-related AML), or any well-defined genetic origins (e.g., mutations in *GATA2, SDAM9, SDAM9/L, RUNX1*, or *ETV6*)—genetic testing for FA should be considered, especially if the disease progression is not acutely rapid. In cases where AML is advancing rapidly, consideration for enrolling the

patient should follow a thorough discussion with the principal investigator of the protocol.

- DNA repair syndromes
- Dyskeratosis congenita (telomeropathy)
- Wilson disease or other copper-related metabolic disorders
- Mixed phenotype acute leukemia
- Philadelphia chromosome-positive myeloid neoplasms (AML or CML)
- Acute promyelocytic leukemia (APL), or
- Juvenile myelomonocytic leukemia (JMML) and related RASopathy disorders in chronic phase.

3.2.3 Patients who have received greater amount of doxorubicin equivalents as stated in Section 3.1.2 parameters are not eligible. For the purposes of determining eligibility for this protocol, the following cardiotoxicity multipliers will be used to determine doxorubicin equivalents.[37-39]

- Doxorubicin (reference): 1
- Daunomycin: 0.5
- Epirubicin: 0.5
- Idarubicin: 5
- Mitoxantrone: 10

3.2.4 Patients who are currently receiving another investigational drug.

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

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

3.2.7 Patients with known active HBV and HCV infections.

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

3.2.9 Pregnancy and breast feeding

- Female patients who are pregnant are ineligible due to risks of fetal and teratogenic adverse events as seen in animal/human studies.
- Lactating females who are breastfeeding an infant/child
- Female patients of childbearing potential are not eligible unless a negative pregnancy test result has been obtained.
- Sexually active patients of reproductive potential are not eligible unless they have agreed to use an effective contraceptive method for the duration of their study participation and for at least 6 months after the last dose of protocol therapy.

3.3 Research Participant Recruitment and Screening

This is a single site study to be conducted at St. Jude Children's Research Hospital.

3.4 Enrollment on Study at St. Jude

A member of the study team will confirm potential participant eligibility and complete the 'Participant Eligibility Checklist' in OnCore. A research participant-specific consent form will be generated. The entire signed consent/assent form must be scanned into the Electronic Health Record by the study team designee.

4.0 TREATMENT PLAN

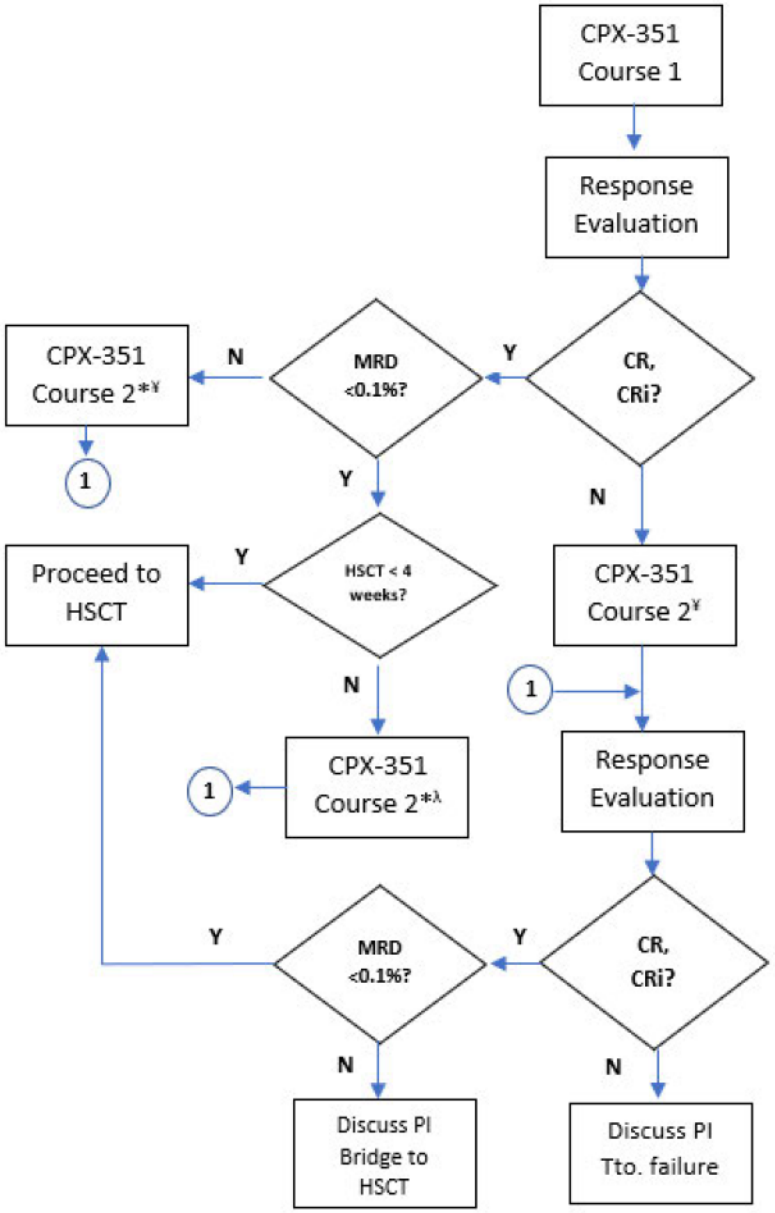
4.1 Design and Study Overview (Figure 1)

This is a prospective, single site, single-arm pilot study to assess the safety and efficacy of CPX-351 (liposomal daunorubicin and cytarabine) for the treatment of pediatric secondary myeloid neoplasms.

Patients will receive up to 2 cycles of CPX-351 for remission induction, and then will proceed to allogeneic HSCT or other therapies as per institutional practice. The second CPX-351 cycle is not given before 29 days from the first cycle. If a patient attains remission and has negative MRD after the first course of CPX-351, and HSCT can occur within 3 to 4 weeks from the evaluation date of the first course, the patient can proceed to HSCT without receiving the second course of CPX-351.

In cases in which HSCT cannot be performed within 3 to 4 weeks from the evaluation date of the second course of CPX-351, interim chemotherapy could be used. For example, another course (third course) of CPX-351 (cytarabine 65 mg/m^2, daunorubicin 29 mg/m^2) Days 1 and 3 for patients with cumulative doxorubicin equivalents of $\leq$ 400 (without cardioprotection) or < 500 mg/m^2 (with cardioprotection), *FLT3*-ITD inhibitors, or venetoclax alone or in combination. In these instances, participants will be evaluated for the primary study objectives (response and toxicity to the two cycles of CPX-351). They will continue in the study for follow-up to assess the secondary and exploratory objectives). According to St. Jude guidelines, informed consent is required for the interim chemotherapy and HSCT (non-protocol treatment plan or other study protocol.

Figure 1 – Management According to Morphologic Response and Residual Disease by Flow Cytometry



**Patients with a cumulative doxorubicin-equivalent dose exceeding >400 mg/m² (without cardioprotection) or ≥ 500 mg/m² (with cardioprotection) after the Cycle 1, will not receive a second course of CPX-351. Instead, the subsequent treatment options will be deliberated with the protocol Principal Investigator.*
¥Cycle 2 starts on or after Day 29 of the first cycle with two doses (daunorubicin 44 mg/m² and cytarabine 100 mg/m²).
λCycle 2 starts between Days 29-36 of the first cycle with two doses (daunorubicin 29 mg/m² and cytarabine 65 mg/m²).

4.2 Dosing and Schedule

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

4.2.1 Cycle 1 Therapy

Agent	Dose (Dauno)	Dose (cytarabine)	Route	Number of doses	Schedule	Notes
CPX-351	44 mg/m^2/dose, or 1.5 mg/kg if patient is < 10 kg	100 mg/m^2 or 3.3 mg/kg if patient is < 10 kg	IV over 90 minutes	3	Days 1, 3 and 5	Calculate dose based on the Daunorubicin component. See Sections 4.2.3 and 4.2.5 for additional administration details.
Intrathecal Therapy	See Section 4.2.4	IT		1	Day 1	

4.2.2 Cycle 2 Therapy*

Agent	Dose (Dauno)	Dose (cytarabine)	Route	Number of doses	Schedule	Notes
CPX-351	44 mg/m^2/dose, or 1.5 mg/kg if patient is < 10 kg	100 mg/m^2 or 3.3 mg/kg if patient is < 10 kg	IV over 90 minutes	2	Days 1 and 3	Calculate dose based on the Daunorubicin component. See Sections 4.2.3 and 4.2.5 for additional administration details.
Intrathecal Therapy	See Section 4.2.4	IT		1	Day 1	

**Patients in CR, CRp or CRi and MRD <0.1% and impossibility of undergoing bone marrow transplantation in 3 to 4 weeks from the response evaluation will receive a Cycle 2 at lower dose (daunorubicin 29 mg/m^2 and cytarabine 65 mg/m^2), Days 1 and 3.*

4.2.3 Treatment details

CPX-351 (Daunorubicin 44 mg/m² and Cytarabine 100 mg/m²) in Cycle 1

CPX-351 (Daunorubicin 44 mg/m² and Cytarabine 100 mg/m²) in Cycle 2, for patients with PR or CR/CRi with MRD ≥ 0.1%.

CPX-351 (Daunorubicin 29 mg/m² and Cytarabine 65 mg/m²) in Cycle 2, for patients with CR/CRi with MRD < 0.1%.
IV administration over 90 minutes, and hospital inpatient.

Cycle 1, Days 1, 3, and 5
Cycle 2, Days 1 and 3

100 units/m² of CPX-351 corresponds to 44 mg/m² of daunorubicin and 100 mg/m² of cytarabine.

Patients will be admitted to the hospital to receive CPX-351. It should be administered by central venous or peripherally inserted central (PICC) catheter. Do not use an in-line filter. Flush the line with NS or D5W after infusion. Do not mix with or administer with any other medications. If infusion-related reactions occur, premedication with antihistamines and/or corticosteroids may be necessary (see Section 4.4.1).

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

4.2.4 Intrathecal chemotherapy

Lumbar puncture and intrathecal (IT) chemotherapy will be given on Day 1 of each cycle, for all patients, but may be delayed or anticipated if clinically indicated. The patients without evidence of CNS leukemia will not receive further IT therapy during Cycle 1. Patients with CNS disease (defined as the presence of any blasts in the cerebrospinal fluid) will undergo weekly ITMHA starting on Day 8 until the cerebrospinal fluid is leukemia-free. Leucovorin rescue (5 mg/m²/dose, max 5 mg, PO or IV) may be given at 24 and 30 hours after each triple intrathecal treatment.

Intrathecal therapy dosing

Patient Age	Methotrexate	Hydrocortisone	Cytarabine	Volume*
< 1 year	6 mg	12 mg	18 mg	6 ml
≥ 1 to < 2 years	8 mg	16 mg	24 mg	8 ml
≥ 2 to < 3 years	10 mg	20 mg	30 mg	10 ml
≥ 3 years	12 mg	24 mg	36 mg	12 ml

**Institutional standard volumes can be used*

4.2.5 Concomitant therapy restriction

See Appendix II for drugs, food and supplements that may interact with daunorubicin and cytarabine.

4.2.5.1 Myeloid growth factor support

Myeloid growth factors should not be used as prophylaxis. They can be administered to patients in the following clinical scenarios: (1) prolonged neutropenia that is clinically significant; (2) clinical or culture proven bacteremia during neutropenia, or with invasive fungal infection. For initiation of growth factor outside of scenarios (1) or (2) above, notify the Principal Investigator.

4.2.5.2 Corticosteroid therapy

Corticosteroids should not be used as anti-emetic therapy. Corticosteroid therapy is not permissible except for the following indications: (1) as treatment or prophylaxis for anaphylactic reactions; (2) As a treatment or prophylaxis for symptoms of Ara-C syndrome including fever, rash, or conjunctivitis (see Section 4.3.2); (3) As treatment for rash possibly related to CPX-351 (see Section 4.4.5); (4) cases with hyperinflammatory presentation of secondary myeloid neoplasm and (5) suspected or confirmed adrenal insufficiency.

4.2.5.3 Hepatotoxic medications

Concomitant use with hepatotoxic agents may impair liver function and increase the toxicity of CPX-351. Monitor hepatic function more frequently if co-administration with hepatotoxic medications is necessary.

4.2.5.4 Duration of therapy

The CPXSMN trial includes one or two cycles for remission induction. Patients in remission following Cycle 1 or Cycle 2 should proceed to HSCT. In situations where for any reason the HSCT is expected to be delayed for more than 3 to 4 weeks, the patient should be discussed with protocol PI for the consideration of alternatives as a to bridge to HSCT, including one course of abbreviated CPX-351 (daunorubicin 29 mg/m^2 and cytarabine 65 mg/m^2; Days 1 and 3).

4.3 Supportive Care

4.3.1 Central venous access

It is recommended that all patients have at least a single lumen central venous access line or a PICC catheter placed prior to the beginning of therapy.

4.3.2 Conjunctivitis prophylaxis

Administer artificial tears (e.g., hydroxymethylcellulose, hypromellose, polyvinyl alcohol), 2 drops in each eye every 4 hours while awake, beginning immediately before the first dose of CPX-351 and continuing until 24 hours after the last dose. May also consider administering steroid eye drops such as 0.1% dexamethasone or 1% prednisolone ophthalmic solution, 2 drops in each eye every 6 hours.

4.3.3 Prophylaxis for *Pneumocystis jiroveci* pneumonia

All participants should receive prophylaxis for *Pneumocystis jiroveci* pneumonia according to institutional guidelines.

4.3.4 Prophylaxis for fungal infections

Because patients with leukemia are at high risk for fungal infections, all participants should receive antifungal prophylaxis according to institutional guidelines.

4.3.5 Prophylaxis for bacterial infections

Because patients undergoing treatment for leukemia are at high risk for bacterial infections, all participants should receive antibiotic prophylaxis according to institutional guidelines. We recommend starting prophylactic antibiotics when the ANC $\leq$ 500 and falling or predicted to fall and continued until the ANC $\geq$ 100 on three consecutive days and rising.

4.3.6 Management of febrile neutropenia

All patients with fever $\geq$ 38.3°C on a single occasion or $\geq$ 38.0°C that persists for one hour should be hospitalized and treated immediately with broad spectrum antibiotics according to institutional guidelines.

Other supportive care should be done as per institutional standard.

4.4 Dose Modifications for Toxicities

4.4.1 Allergy to CPX-351

For hypersensitivity reactions, interrupt the infusion immediately. For mild symptoms, initiate the infusion at half the prior rate of the infusion once symptoms resolve. For moderate symptoms, do not restart the infusion. For subsequent doses premedicate with anti-histamines and/or corticosteroids prior to initiating infusion at same rate. Permanently discontinue CPX-351 for severe or life-threatening symptoms.

4.4.2 Cardiac toxicity

If ejection fraction falls <50% (or if EF is unevaluable, SF < 24%), hold Cycle 2 and repeat ECHO in 1-2 weeks. If EF remains <50% (or if EF is unevaluable, SF < 24%), the patient will not receive Cycle 2.

4.4.3 Transaminases

If the ALT or AST are > 10 x ULN, attempts should be made to identify the cause and notify the PI. In most cases, the therapy will proceed without modification.

4.4.4 Hyperbilirubinemia

In severe liver dysfunction, the half-life of daunorubicin is prolonged and the AUC may be more than 3-fold that of patients with normal hepatic function. However, there are no available dose adjustment guidelines in the literature. Therefore, the dose reductions implemented for daunorubicin will be utilized as outlined in the table below.

Direct Bilirubin	**CPX-351**
≥ 2 and < 3	50% of the calculated dose
≥ 3 and < 5	25% of the calculated dose
≥ 5 mg/dL	Hold dose and notify PI

Full dose of CPX-351 may resume when the direct bilirubin has fallen to < 1.2 mg/dL.

4.4.5 Rash with CPX-351

A maculopapular rash occurring within one week of treatment initiation occurs in the majority of patients treated with CPX-351. Grade 3 rashes occurred in 39.6% of children participating in the COG AAML1421 study after receiving CPX-351. The rash typically responded to topical or systemic corticosteroids. Subjects experiencing a rash from CPX-351 should first receive topical corticosteroids.

Pruritus may be treated with antihistaminic (diphenhydramine). If extensive or refractory to topical therapy, a 3 - 5 day course of systemic prednisone or methylprednisolone may be used. Rash is not an indication to dose-reduce or discontinue CPX-351.

4.4.6 Hand-foot syndrome

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

5.0 DRUG INFORMATION

5.1 CPX-351

Source and pharmacology: Cytarabine is metabolized intracellularly by deoxycytidine kinase to its active form cytosine arabinoside triphosphate. The active metabolite damages DNA during the S-phase by inhibiting alpha-DNA polymerase, inhibiting DNA repair through an effect on beta-DNA polymerase, and by incorporation into DNA in place of cytosine. Daunorubicin is an anthracycline antibiotic that damages DNA by intercalating DNA strands resulting in uncoiling of the helix. Daunorubicin also inhibits polymerase activity which affects gene expression regulation and the formation of oxygen free radicals.

Cytarabine:daunorubicin liposome (CPX-351) is a 5:1 fixed molar ratio of the two drugs within a liposome. This ratio has shown synergy and improved activity *in vitro* in comparison to the agents given separately. The liposome is composed of distearylphosphatidylcholine (DSPC), distearylphosphatidylglycerol (DSPG), and cholesterol in a 7:2:1 molar ratio. Daunorubicin is complexed with copper gluconate (0.9 mg/mL) to stably encapsulate it and then suspended in sucrose. Cytarabine:daunorubicin liposomes accumulate in the bone marrow. The drug is preferentially taken up within the cytoplasm of leukemic cells then released causing efficient and rapid leukemic cell death.

Pharmacokinetics: Cytarabine is converted to the inactive metabolite uracil arabinoside by a pyrimidine nucleoside deaminase. Daunorubicin hydrochloride is extensively and rapidly metabolized in the liver and other tissues, producing the active metabolite daunorubicinol. Further metabolism via reduction cleavage of the glycosidic bond, 4-O demethylation, and conjugation with both sulfate and glucuronide also occur. Following a single daunorubicin hydrochloride dose, 25% is eliminated in an active form by urinary excretion and an estimated 40% is eliminated by biliary excretion.

Liposomal encasement markedly decreases clearance and results in prolonged half-life. The terminal half-life for cytarabine is generally 1-3 hours but is prolonged to 37-42 hours for the liposomal product. The terminal half-life of daunorubicin is 45 minutes in the initial phase and 18.5 hours in the terminal phase but is prolonged to 22-25 hours for the liposomal product.

Hepatic Impairment: No clinical trials have been conducted to evaluate the effect of hepatic impairment on the pharmacokinetics of cytarabine:daunorubicin liposome.

Renal Impairment: No clinical trials have been conducted to evaluate the effect of renal impairment of cytarabine:daunorubicin liposome.

Drug Interactions: Please refer to labelling of cytarabine and daunorubicin as the risk of drug interactions are expected to be substantially similar for CPX-351. No formal drug-drug interaction trials have been conducted with CPX-351.

Concomitant use of cardiotoxic agents may increase the risk of cardiotoxicity.

Concomitant use with hepatotoxic agents may impair liver function and increase the toxicity of CPX-351.

Formulation and stability: CPX-351 is a purple lyophilized formulation. One unit contains 1 mg cytarabine and 0.44 mg daunorubicin. Each 50 mL vial contains 100 units, corresponding to 100 mg cytarabine and 44 mg daunorubicin. Store vials refrigerated at 5°C (±3°C) in an upright position.

Components of CPX-351 (cytarabine:daunorubicin) Liposome Injection

Component	Mw	Amount per vial	Amount per unit
Cytarabine, USP/PhEur	243	100 mg	1 mg
Daunorubicin HCl USP/PhEur (reported as the free base)	528	44 mg	0.44 mg
Distearoylphophatidylcholine	790	454 mg	4.5 mg
Distearoylphosphatidylglycerol	801	132 mg	1.3 mg
Cholesterol, HP	387	32 mg	0.3 mg
Copper gluconate, USP	454	92 mg	0.9 mg
Triethanolamine, NF, PhEur	149	7 mg	0.07 mg
Sucrose, NF, PhEur	342	2054 mg	20.54 mg

CPX-351 should be reconstituted with 19 mL sterile water for injection to a final concentration of 5 units/mL. Do not heat CPX-351 for injection. Carefully swirl contents of the vial for 5 minutes while gently inverting every 30 seconds. After reconstitution, let rest for 15 minutes. Repeat vial inversion 5 times prior to withdrawing drug for dilution. The drug should be further diluted with 0.9% Sodium Chloride or D5W and dispensed in non-DEHP polypropylene or polyolefin bags or non-DEHP syringes. See suggested final dilution volumes below. CPX-351 is stable for 4 hours refrigerated at 2□C to 8□C (36□F to 46□F) after the final dilution if not used immediately. If stored refrigerated for 4 hours, must use immediately. Unused drug should be discarded.

Suggested final dilution volumes for preparation:

CPX-351 Dose (Dauno)	D5W or 0.9% NaCL final volume (mL)
4.4 to < 44	100 mL
≥ 44 to < 110	250 mL
≥ 110 to ≥ 176	500 mL

Supplier: Drug will be supplied by Jazz Pharmaceuticals.

Toxicity: See table below

Incidence	Toxicities	
Common (>20% of patients)	• Neutropenia • Thrombocytopenia • Anemia • Febrile neutropenia • Nausea, vomiting, diarrhea/colitis, constipation • Decreased appetite • Abdominal pain • Edema	• Rash • Fatigue • Headache • Cough, dyspnea • Fever, chills • Hemorrhage • Insomnia • Hypotension • Infection • Musculoskeletal pain
Occasional (4-20% of patients)	• Dizziness • Dyspepsia • Anxiety, delirium • Pruritus • Hypoxia • Pleural effusion • Mucositis • Hypertension • Tachycardia, atrial fibrillation	• Hypokalemia • Hyperhidrosis • Renal failure • Respiratory failure • Cardiotoxicity • Chest pain • Visual impairment • Night sweats
Rare (≤ 3% of patients)	• Alopecia • Palmar-plantar erythrodysesthesia • Hypersensitivity reaction • Tumor lysis syndrome • Increased blood copper levels	
Pregnancy & Lactation	Based on animal data CPX-351 can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies of CPX-351 in pregnant women. Cytarabine and daunorubicin are reproductive and developmental toxicants in multiple species (mice, rats, and/or dogs). Patients should be advised to avoid becoming pregnant while receiving CPX-351. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, apprise the patient of the potential harm to a fetus. Advise women and men of reproductive potential to use effective contraception during treatment and for 6 months following the last dose of CPX-351. It is unknown whether CPX-351 is excreted in human milk. CPX-351 is not recommended for nursing mothers. Because of possible drug excretion in human milk and because of the potential for serious adverse reactions in nursing infants, lactating women not to breastfeed during treatment with CPX-351 and for at least 2 weeks after the last dose.	

**Due to the long plasma half-life of CPX-351, time to recovery of neutrophils and platelets may be prolonged.*
***Visual impairment includes photophobia, photopsia, photosensitivity reaction, retinal tear, uveitis, blurred vision, reduced visual acuity, and vitreous floaters.*

Dosage and route of administration: See Treatment and Dose Modifications sections of the protocol. The drug should be infused over 90 minutes via non-DEHP tubing. Do NOT administer through an inline filter.

Accountability: Because this study is being conducted under an IND, participating pharmacies will be required to submit Drug Accountability Logs at the time of monitoring documenting receipt and shipment of drug supply, dispensing/ordering of supply, and destruction of unused study medication and/or damaged or expired drug.

6.0 EVALUATIONS, TESTS, OBSERVATIONS

6.1 Pre-Treatment Clinical Evaluations

- Physical exam with vital signs, height, weight, and BSA
- Complete blood count with differential
- Chemistry profile: glucose, electrolytes, BUN, creatinine, LDH, uric acid, bilirubin, SGOT, SGPT, calcium, phosphorous, magnesium, total protein, and albumin
- EKG, echocardiogram or MUGA
- Bone marrow evaluation for morphology, immunophenotyping to confirm diagnosis and to establish a leukemia-associated phenotype for subsequent MRD studies, genomics, and proteomics. For St. Jude cases, left over bone marrow and peripheral blood sent to biorepository.
- For patients with elevated leukocyte counts and high blast percentages, or patients too ill to undergo bone marrow aspirate, all diagnostic studies may be performed on blood rather than bone marrow.
- Lumbar puncture with CSF cell count and differential and intra-thecal chemotherapy (single-procedure consent).
- Pregnancy test for females of childbearing potential
- HLA typing of the patient, siblings, and parents if not previously done.

6.2 Evaluations Before and During Therapy

Required studies	Baseline/Pre-Treatment (within 7 days)	Cycle 1	Cycle 2	End of Therapy Evaluations within ± 7 days
Clinical				
Physical exam, vital signs	X	Day 1	Day 1	Day 22, Day 29 or at the time of hematologic recovery
CBC, with differential	X	Day 1 and twice a week for the first week and then weekly	Day 1 and twice a week for the first week and then weekly	Day 22, Day 29 or at the time hematologic recovery
Tumor lysis panel (potassium, calcium, phosphorus, uric acid, BUN, creatinine, and LDH)	X	Twice a week for the first week and then weekly	Twice a week for the first week and then weekly	NA
ECG	X		Prior to Cycle 2	Day 22, Day 29 or at the time hematologic recovery
Echocardiogram or MUGA	X		Prior to Cycle 2	Day 22, Day 29 or at the time of hematologic recovery
Bone marrow biopsy and aspirate[λ]	X	Day 22		Day 22, Day 29 or at the time hematologic recovery
Lumbar puncture, CSF cell count and cytology	See Section 4.2.4	Cycle 1 – See Section 4.2.4 Cycle 2 – Day 1		N/A
Laboratory Research[¥]				
Pharmacogenomics	--	--	--	PB and BM Day 22, Day 29 or at the time hematologic recovery
Genomic studies	X	PB and BM end of Cycle	PB and BM end of cycle	PB and BM Day 22, Day 29 or at the time hematologic recovery

Abbreviations: BM, bone marrow; PB, peripheral blood; NA, not applicable.
[λ]*MRD studies will be performed locally or centrally. Bone marrow aspirate and biopsy centrally reviewed (at diagnosis, prior the second course and end of therapy)*
[¥]*Research: specimens will be collected only during routine clinical testing (bone marrow and peripheral blood). On each occasion, the amount of peripheral blood may not exceed the lesser of 50 ml or 3 ml per kg in an 8-week period and collection may not occur more frequently than 2 times per week. Bone marrow for research: Each aspirate will consist of approximately 2 mL of marrow. A single extra aspirate (~2 mL) will be obtained from children under the age of 8 years, two additional aspirates (up to ~4 mL) will be obtained from those between the ages of 9 and 13, and up to three aspirates (up to ~6 mL) will be obtained from patients who are 14 years and older. Follow up research studies can be collected at the discretion of the investigator during routine clinical testing.*
Pre-cycle tests obtained within 7 days of starting the chemotherapy.

6.3 Research Studies

6.3.1 Pharmacogenomics

Genomic DNA (peripheral blood) will be obtained from patients before HSCT for genotyping of 10 SNPs that are part of the ACS10 score. Genotype score will be developed for each patient followed by generation of polygenic scores to classify patients into low or high score groups. The score will be evaluated for association with the clinical outcome endpoints. Similar association will be performed for transcriptomic based pLSC6 and drug resistance scores. We request RNA/specimen prior to HSCT for testing gene expression levels of 11 genes that are part of the two scores. We have all the necessary equipment, protocols, and resources to test both DNA based pharmacogenomics and RNA based transcriptomic score along with funds to support this.

6.3.2 Genomic characterization of SMN

Standard high throughput genomic methods (whole exome/genome sequencing, RNA sequencing) will be used to identify the mutations and other genomic lesions in bone marrow and peripheral blood sample at diagnosis. To identify somatic mutations with low allelic burden, we will apply targeted deep-sequencing or alternative approaches such as ddPCR or cell free amplification. For characterization and tracking of clonal repertoires, scDNAseq will be performed in bone marrow or blood specimens at diagnosis and after CPX-351 treatment. These methods required equipment and funds are available to us.

6.4 Evaluations after Completion of Therapy

When a participant discontinues the study treatment, a final visit will be conducted. Following discontinuation of the study treatment, the participant will be treated according to the investigator's discretion. Participants will be monitored for adverse events for 30 days post protocol treatment, or until additional anti-SMN therapy is received (whichever occurs first).

If a participant discontinues from the study due to an adverse event considered related to study treatment, a follow-up visit should be conducted no later than 30 days after the last dose of protocol therapy. Safety assessments are recommended at least every 30 days, until all toxicities resolve, return to baseline, or become clinically satisfactory, stable, or are considered irreversible, or until patient receives other anti-SMN therapy.

7.0 EVALUATION CRITERIA

7.1 Response Evaluation during the First Course of CPX-351

A bone marrow aspirate and biopsy will be obtained on Day 22 of Cycle 1. Complete morphologic remission response (CR) is defined as less than 5% blasts without Auer rods by morphological evaluation of the bone marrow (M1 marrow). Patients in morphologic CR without hematological recovery (white cell blood count $\geq 1.0 \times 10^9$/L, unsupported platelet count $\geq 30.0 \times 10^9$/L and absolute neutrophil count $\geq 0.3 \times 10^9$/L) are classified as

morphological CR and incomplete recovery (CRi) of absolute neutrophil, white blood cell, and/or platelet counts. Patients in CR or CRi and MRD <0.1% after the first cycle are candidates for hematopoietic stem cell transplantation. These patients will receive the second course of CPX-351(daunorubicin 29 mg/m^2 and cytarabine 65 mg/m^2) when they meet the criteria for hematological recovery and a hematopoietic stem cell transplant will not be available within 3 to 4 weeks from the evaluation date. If hematological recovery is not observed by Day 42 another bone marrow evaluation will be obtained. Partial response (PR) is defined as 5% to 25% blasts. Patients in PR, CR, or CRi, but with MRD ≥ 0.1% blasts with a leukemia-associated phenotype by flow cytometry will proceed to the second course (two doses) of CPX-351 (daunorubicin 44 mg/m^2 and cytarabine 100 mg/m^2, on Day 29 of Cycle 1. For treatment decisions, in case of discrepancy between the number of blasts by morphological and by flow cytometric analysis, the number of blasts by flow cytometry will be used. Lack of response is defined as an absolute increase of 20% of marrow blasts from the baseline value, 25% or more blasts, circulating leukemia blasts or development of extramedullary disease. These patients are considered to have treatment failure and taken off study.

7.2 Response Evaluation during the Second Course of CPX-351

A bone marrow aspirate and biopsy will be obtained between Days 22-28 of Cycle 2. For patients with M1 marrow and negative MRD after Cycle 1, bone marrow evaluation after Cycle 2 may be performed on Day 28, whereas bone marrow evaluation will be done on Day 22 for the remaining patients. The same criteria for response evaluation for Cycle 1 is used for Cycle 2.

7.3 Toxicity Evaluation Criteria

Common Terminology Criteria for Adverse Events v5 (CTCAE): This study will utilize the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the current version of the CTCAE can be downloaded from the Cancer Therapy Evaluation Program home page (http://ctep.info.nih.gov). Additionally, toxicities will be reported on the appropriate data collection screens.

8.0 OFF-TREATMENT AND OFF-STUDY CRITERIA

8.1 Off-Treatment Criteria

- No response to therapy
- Relapse
- Subsequent malignancy
- Development of unacceptable toxicity during treatment
- Refusal of further protocol therapy by participant, parent, or guardian
- Completion of protocol therapy and 30 days evaluation period or participant receives other anti-SMN therapy, at the discretion of the PI

8.2 Off-Study Criteria

- Death
- Lost to follow up
- Withdrawal of consent

9.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

9.1 Adverse Events (AE)

Adverse events will be monitored from the time of first study intervention or treatment and will be collected throughout the study.

9.2 Definitions

Adverse Event (AE): Any untoward medical occurrence associated in a study participant after the first treatment or intervention on study. Adverse Events will be graded by the NCI CTCAE version 5.0

Serious Adverse Event (SAE): Any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health, and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Unanticipated problem (UP): An event which was not expected to occur, and which increases the degree of risk posed to research participants. Such events, in general, meet all of the following criteria:

- unexpected
- related or possibly related to participation in the research, and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. An unanticipated problem involving risk to subjects or others may exist even when actual harm does not occur to any participant.

The following events are expected and will not be considered serious adverse events or unanticipated problems and will not reported in an expedited manner, unless an aggregate analysis indicates that such events are occurring more frequently than expected. This will

be monitored closely by the PI and study team. These events will be reported with Annual Report:

- Hospitalization for known consequences of the underlying disease.
- Grade 3 or 4 infection or fever ≤ 7 days in duration.
- Grade 3 nausea, vomiting or diarrhea that is adequately managed with supportive care (i.e., does not require tube feeding, total parenteral nutrition, or prolonged hospitalization) or that resolves to < Grade 3 within 72 hours.
- Grade 3 or 4 electrolyte or laboratory abnormalities correctable with supportive therapy or that resolve to < Grade 3 within 72 hours.
- Grade 3 or 4 tumor lysis syndrome must resolve in ≤ 7 days without evidence of end-organ damage.

9.3 Handling of Adverse Events (AEs) and Deaths

9.3.1 Reporting of AEs and Deaths

AEs will be evaluated and documented by the clinical staff and investigators throughout inpatient hospitalizations and each outpatient visit. The study team is responsible for reviewing documentation related to AEs and entering directly into the protocol-specific database for all non-hematologic adverse events Grade 2 or higher. The data to be recorded are:

1) the event description
2) the NCI CTCAE v5.0 code and grade
3) the onset date and the resolution date (or ongoing if it has not resolved at time of off study)
4) action taken for event
5) patient outcome
6) attribution of AE to protocol treatment/interventions,
7) if AE was expected or unexpected

AEs that are classified as serious, unexpected, and at least possibly related will be notated as such in the database as unanticipated problems (UPs). These events will be reported expeditiously to the St. Jude IRB within the timeframes as described above. All serious adverse events will be notated as SAEs in the database.

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The adverse event is clearly related to the study treatment.
- Probable – The adverse event is likely related to the study treatment.
- Possible – The adverse event may be related to the study treatment.
- Unlikely – The adverse event is doubtfully related to the study treatment.
- Unrelated – The adverse event is clearly NOT related to the study treatment.

Cumulative summary of Grade 2-5 non-hematologic events will be reported as part of the progress reports to IRB at the time of continuing review. Specific data entry instructions for AEs and other protocol-related data will be documented in protocol-specific data entry guidelines, which will be developed and maintained by study team and clinical research informatics. The study team will meet regularly to discuss AEs. The PI will review AE reports generated from the research database, and corrections will be made if applicable. Once the information is final the PI will sign and date reports, to acknowledge his review and approval of the AE as entered in the research database.

9.3.2 Reporting AEs and SAEs

The St. Jude PI, upon awareness of an event, will determine the seriousness of AEs and ensure that (all SAEs and UPs are entered into the IRB electronic submission system within 10 days. All Grade 2-5 AEs, serious or not, will be recorded in the TrialMaster database and reported to the St. Jude IRB at the time of continuing review.

9.3.3 Reporting of Unanticipated Problems

The St. Jude PI will refer to St. Jude Human Research Protection Program (HRPP) Policy 01.720 for specifics on the reporting of unanticipated problems to the St. Jude IRB.

The St. Jude Regulatory Affairs Office, upon receipt, reports UPs on study to the FDA within mandated regulatory timelines per 21 CRF 312 or 812.

9.4 Reporting to Drug Manufacturer (Jazz Pharmaceuticals)

Serious adverse events and unanticipated problems as defined above will be reported by email no later than one (1) calendar day of knowledge of the event for fatal and life-threatening events to Jazz at:

- [redacted]

10.0 DATA COLLECTION, MONITORING AND CONFIDENTIALITY

10.1 Data Collection

Electronic case-report forms (e-CRFs) will be completed by the St. Jude Clinical Research Associates or the site Study Coordinator. Data from the participant's record will be entered directly into a secure study-specific database. Instructions for data entry are outlined in the database.

Data management will be supervised by PI and the Hematological Malignancies Manager of Clinical Trials Operations. Protocol-specific data. All protocol-specific data and all non-hematologic Grade 2-5 adverse events will be recorded by the clinical research associates into a protocol-specific database, AEs should be entered weekly, especially during the tolerability phase. All questions will be directed to the Principal Investigator (PI) or designee and will be reviewed at regularly scheduled working meetings.

Regular summaries of toxicity and protocol events will be generated for the PI and the Department of Biostatistics to review.

10.2 Study Monitoring

This study will be monitored according to a study specific clinical trial monitoring plan (CTMP). The CTMP is based on the current St. Jude Children's Research Hospital Institutional Plan for Data and Safety Monitoring of Clinical Trials (DSMP) which outlines the monitoring strategy based on the risk of the study with an emphasis on participant safety, data quality, and human subjects protections. Additionally, the CTMP is a supplement to the protocol, Clinical Trial Operations SOPs and study specific materials which will aid in the monitoring of this study.

The Investigator will permit study-related monitoring by providing direct access to source data and to the participants' medical histories. The Investigator will also maintain an Investigator Site File with essential documents according to ICH and all applicable regulatory guidelines and make the file accessible for monitoring visits as needed.

The Monitor will visit the study site at regular intervals during the study. Visits may be performed onsite, remotely, or in a hybrid fashion (both onsite and remote) as needed for the sponsor (SJCRH) to fulfill their obligations. Site monitoring is conducted according to applicable ICH and GCP guidelines to ensure the protection of participants' rights and well-being, protocol adherence, quality of data (accurate, complete, and verifiable), study treatment accountability, compliance with regulatory requirements, and continued adequacy of the investigational site and its facilities.

10.3 Confidentiality

Study numbers will be used in place of an identifier such as a medical record number. No research participant names will be recorded on the data collection forms. The list containing the study number and the medical record number will be maintained in a locked file and will be destroyed after all data have been analyzed. The medical records of study participants may be reviewed by the St. Jude IRB, FDA, Jazz Pharmaceuticals, and clinical research monitors.

11.0 STATISTICAL CONSIDERATIONS

11.1 Simon's Two-Stage Design for Primary Endpoint (CR/CRi)

Primary Objective: *Determine the composite complete remission (CR) and complete remission with incomplete peripheral blood recovery (CRi) rates, safety, and tolerability in patients under 22 years of age with SMN treated with one or two courses of CPX-351 before HSCT.*

The Simon's two-stage minimax design with a maximum enrollment of 25 patients will be used to evaluate this endpoint (Table 1). The design has a 4.29% level for the null hypothesis that the population rate of achieving CR or CRi (CR+CRi) before the end of the

second course of therapy is less than or equal 45% and 80.42% power for the alternative that the population CR+CRi rate is greater than or equal 70%. It will stop early and declare protocol therapy to have an undesirable CR+CRi rate if 5 or less of the first 12 patients achieve CR+CRi. If 6 or more of the first 12 patients achieve CR+CRi, then up to 13 additional patients will be enrolled to have a total of 25 patients. If 15 or fewer of the 25 patients achieve CR+CRi, then the therapy will be declared undesirable. Otherwise, if 16 or more of the 25 patients achieve CR+CRi, the therapy will be declared promising and worthy of further evaluation in a future trial.

Table 1: Simon's Two-Stage Design for CR+CRi		
	Outcome Probabilities	
Outcome	CR+CRi Rate = 45%	CR+CRi Rate = 70%
Stop for ≤ 5/12 CR+CRi	0.5269	0.0386
Stop for ≤ 15/25 CR+CRi	0.4301	0.1572
Obtain ≥ 16/25 CR+CRi	0.0429	0.8042

The design parameters of an unacceptable CR+CRi rate of 45% and a promising CR+CRi rate of 70% were chosen based on the published experience[3] (http://doi.org/10.1182/blood-2019-131911) with 145 therapy related AML patients. Of these patients, 47% achieved CR and 39% had no evidence of leukemia.

We plan to pause enrollment at any time the outcome of the most recent enrollee may satisfy the stopping criteria of this two-stage design for CR+CRi. We will check the data at the time of each new patient enrollment to determine whether the study is in such a situation.

11.2 Three-Stage Design for Tolerability Monitoring

It is also critical to monitor the tolerability of the therapy of this protocol. For this purpose, we define a *tolerability success* as a patient completing two courses of therapy without experiencing a grade 4 or 5 non-hematologic toxicity. We consider a *tolerability success rate* (TSR) less than 70% to be unacceptable. We will monitor tolerability with the exact three-stage binomial design shown in Table 2 below. We will stop enrollment and declare the therapy to be intolerable if none of the first 5 patients, 5 or fewer of the first 12, or 14 or fewer of 25 patients successfully tolerate the first two courses of protocol therapy. If none of these criteria are met and 15 or more of 25 patients successfully tolerate the first two courses, then the therapy will be declared tolerable. This monitoring rule has a 20% probability of declaring the therapy tolerable if the population TSR is 50% and an 88.8% probability of declaring the therapy tolerability if the population TSR is 70%.

We plan to pause enrollment at any time the outcome of the most recent enrollee may satisfy the tolerability monitoring stopping rule. We will check the data at the time of each new patient enrollment to determine whether the study is in such a situation.

Table 2. Three Stage Design for Tolerability Monitoring				
	Outcome Probabilities as a Function of TSR			
Outcome	TSR = 50%	TSR = 60%	TSR = 70%	TSR = 80%
Stop for ≤ 0/5 successes	0.0312	0.0102	0.0024	0.0003
Stop for ≤ 5/12 successes	0.3579	0.1496	0.0370	0.0038
Stop for ≤ 14/25 successes	0.4093	0.2746	0.0729	0.0044
Obtain ≥ 15/25 successes	0.2015	0.5655	0.8877	0.9915

11.3 Statistical Analysis Plan for Secondary Objectives

Objective 1.2.1: *Describe the toxicity profile of patients with SMN treated with one or two courses of CPX-351.*

We will define categories for the toxicity events (respiratory, cardiac, etc.) and for each category we will determine the highest grade experienced by each patient. We will then report the number of patients with each grade in each category.

Objective 1.2.2: *Describe the biologic correlates of response in patients with SMN after one or two courses of CPX-351.*

We will use logistic regression modeling explore biologic correlates (genomic, transcriptomic, methylomic, pharmacologic, cytogenetics, etc.) as predictors of response. For genomic associations, we will use false discovery rate methods to address multiple testing.

Objective 1.2.3: *Estimate the 3-year overall and event-free survival of patients who received one or two courses of CPX-351 followed by HSCT.*

We will use the Kaplan-Meier method to estimate overall survival and event-free survival of all patients. We define OS as the time elapsed from protocol enrollment to death and censor times of living patients at last follow-up. We define EFS as the time elapsed from protocol enrollment to death, relapse, discontinuation of therapy due to excessive toxicity or resistant disease, or development of an additional malignancy and censor times for patients free of these events at last follow-up. To explore the impact of transplant, we will fit Cox models with transplant as a time-dependent covariate (with value 0 prior to transplant and 1 after transplant).

11.4 Statistical Analysis Plan for Exploratory Objectives

Exploratory Objective 1.3.1: *Describe the clinical correlates of the cytarabine (Ara-C) pharmacogenomic response score (ACS), the leukemia stem cell score (pLCS6) and drug resistance score (ADE-RS5) in patients receiving CPX-351.*

We will use the rank-sum test to compare each of these scores between responders and non-responders. Also, we will use Cox regression models to associate each of these scores with EFS and OS.

Exploratory Objective 1.3.2: *Investigate how genetic lesions in leukemic cells change after CPX-351treatment.*

This will be a descriptive analysis.

Exploratory Objective 1.3.3: *Explore the feasibility of single-cell DNA sequencing (scDNAseq) for characterization and tracking of clonal repertoires.*

This will be a descriptive analysis.

12.0 OBTAINING INFORMED CONSENT

12.1 Consent Prior to Research Interventions

Initially, informed consent will be sought for the institutional banking protocol (TBANK) and for other procedures as necessary. During the screening process for eligibility, informed consent is required before any research tests are performed.

The process of informed consent and assent for CPXSMN will follow institutional policy. The informed consent process is an ongoing one that begins at the time of diagnosis and ends after the completion of therapy. Informed consent should be obtained by the attending physician or his/her designee, in the presence of at least one non-physician witness. Throughout the entire treatment period, participants and their parents receive constant education from health professionals at St. Jude and are encouraged to ask questions regarding alternatives and therapy. All families have ready access to chaplains, psychologists, social workers, and child life specialists, in addition to that provided by the primary clinicians involved in their care.

12.2 Consent at Age of Majority

The age of majority in the state of Tennessee is 18 years old. Research participants must be consented at the next clinic visit after their 18th birthday. If an affiliate is located in a country or state where a different age of majority applies, that location must consent the participants according to their local laws.

12.3 Consent When English is Not the Primary Language

When English is not the patient, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information documented in the participant's medical record. Either a certified interpreter or the telephone interpreter's service will be used to translate the consent information. The process for obtaining an interpreter and for the appropriate use of an interpreter is outlined on the Interpreter Services, OHSP, and CTO websites.

13.0 REFERENCES

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APPENDIX I: PERFORMANCE STATUS CRITERIA

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

APPENDIX II: DRUGS, FOOD AND SUPPLEMENTS INTERACTIONS

Drugs that may interact with daunorubicin:

- Some antibiotics and antifungals (clarithromycin, erythromycin, itraconazole, ketoconazole, rifampin)
- Some antiepileptics (carbamazepine, phenobarbital, phenytoin, fosphenytoin)
- Some antiretrovirals (lapatinib, lopinavir; nelfinavir, ritonavir, saquinavir, telaprevir, tipranavir)
- Some heart medications (amiodarone, carvedilol, digoxin, dronedarone, quinidine, propafenone, verapamil)
- Other agents, such as atorvastatin, clozapine, cyclosporine, dexamethasone, ivacaftor, leflunomide, lumacaftor, natalizumab, nefazodone, progesterone, ranolazine, rifampin, tacrolimus, tofacitinib, and trazodone

Food and supplements that may interact with daunorubicin:

- Echinacea
- Grapefruit, grapefruit juice, Seville oranges, star fruit
- St. John's Wort
- Drinks, food, supplements, or vitamins containing "flavonoids" or other "antioxidants"

Drugs that may interact with cytarabine (by vein):

- Clozapine, flucytosine, leflunomide, natalizumab

Food and supplements that may interact with cytarabine:

- Echinacea