FRED HUTCHINSON CANCER RESEARCH CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE
SEATTLE CHILDREN'S

Current version: 7/07/2021
Previous version: 1/12/2021

Title of Protocol:
A randomized trial for patients with high-grade myeloid neoplasms with measurable residual disease (MRD): CPX-351 vs. immediate allogeneic hematopoietic cell transplantation

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Study Regimen: AML/MDS-EB2 patients with measurable residual disease randomized to either immediate hematopoietic cell transplantation vs. treatment with up to 2 courses of CPX-351 prior to hematopoietic cell transplantation.

IND HOLDER: Filippo Milano, MD, PhD
IND Number: 152141

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PROTOCOL SYNOPSIS

Protocol Title	*A randomized trial for patients with high-grade myeloid neoplasms with measurable residual disease (MRD): CPX-351 vs. immediate allogeneic hematopoietic cell transplantation (alloHCT)*
Protocol Number	*RG1007476*
Protocol Sponsor	*Filippo Milano, MD, PhD*
IND Number	*152141*
Trial Phase	*Phase II*
Trial Type	*Interventional*
Clinical Indication	*Patients with detectable leukemia following intensive chemotherapy*
Study Objectives	*To determine whether treatment with CPX-351 improves outcomes over immediate allogeneic transplantation among patients with measurable residual disease following intensive chemotherapy.*
Study Design	*Eligible AML/MDS-EB2 patients with MRD will be enrolled and randomized to either immediate allogeneic hematopoietic cell transplantation vs. treatment with CPX-351 for 1-2 courses prior to allogeneic hematopoietic cell transplantation.*
Population	*Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS)-EB2*
Primary Endpoints	*Overall survival*
Secondary Endpoints	*Among all patients:* • *Relapse-free survival* • *relapse* • *non-relapse mortality* • *rate of transplantation* • *type of transplant (myeloablative, reduced intensity)* *Among patients treated with CPX-351:* • *frequency of MRD prior to transplantation* • *changes in leukemia-associated mutations prior to transplantation*
Type of control	*Active Treatment*
Investigation Drug	*CPX-351 (Daunorubicin and Cytarabine) Liposome for Injection*
Dose	*Daunorubicin 44 mg/m2 and cytarabine 100 mg/m2, days 1,3,5 of a 28-day cycle. Patients may receive up to two courses of CPX-351 to be given on days 1 and 3 of the second cycle.*
Route of administration	*Intravenous*
Transplant Conditioning Regimens	*Various for patients receiving allogeneic hematopoietic cell transplantation*
Trial Blinding	*Not Blinded*
Treatment Groups	*ARM A – Early allogeneic transplantation without MRD directed therapy.* *Patients assigned to ARM A will be treated with allogeneic transplantation without intervening chemotherapy according*

	to established treatment protocols and at the direction of their physician. *ARM B – CPX-351 followed by allogeneic transplantation. Patients assigned* *to ARM B will receive CPX-351 (Daunorubicin and Cytarabine) Liposome for Injection at a dose of daunorubicin 44 mg/m2 and cytarabine 100 mg/m2 via intravenous infusion over 90 min on days 1, 3, and 5. Patients may receive up to two courses of CPX-351. Patients may receive subsequent allogeneic transplantation at the discretion of their physician.*
Treatment Schedule	*Among patients randomized to ARM A – Transplantation will be initiated by the treating physician.* *Among patients randomized to ARM B – CPX-351 will be administered on days 1,3, and 5. Patients may receive up to two courses of CPX-351 with the second course given on days 1 and 3 only. CPX-351 is followed by allogeneic transplantation at the discretion of the treating physician.*
Efficacy Assessments	*Treatment response (e.g. CR without MRD, CR, CRi, morphologic leukemia-free state [MLFS], partial remission [PR]) or treatment failure (e.g. refractory disease, death in aplasia, death from indeterminate cause, hematologic relapse, molecular relapse) as well as treatment outcome (e.g. overall survival, relapse-free survival, event-free survival, and remission duration) will be determined by peripheral blood count and bone marrow evaluation and categorized according to the 2017 European LeukemiaNet criteria.(Döhner et al., 2017)*
Number of trial subjects	*130*
Estimated duration of trial	*5 years*
Duration of Participation	*2 years following enrollment. Additional demographic details (survival) following 1yr.*

ABBREVIATIONS

AML	Acute myeloid leukemia
ATG	Anti-thymocyte globulin
BM	Bone marrow
CLIA	Clinical Laboratory Improvement Amendment
CPX-350	Cytarabine and daunorubicin
CR	Complete remission
CRi	Complete remission with incomplete hematologic recovery
CTCAE	NCI Common Terminology Criteria for Adverse Events
DLI	Donor lymphocyte infusion
DLT	Dose-limiting toxicity
FLAG	Fludarabine, cytarabine, G-CSF
FLAMSA	Fludarabine, amsacrine, and cytarabine
FHCRC	Fred Hutchinson Cancer Research Center
G-CLAC	G-CSF, clofarabine, and cytarabine
GVHD	Graft-versus-host disease
GVL	Graft-versus-leukemia
HCT	Hematopoietic cell transplantation
HLA	Human leukocyte antigen
LFS	Leukemia-free survival
MEC	Mitoxantrone, etoposide, and cytarabine
MLFS	Morphologic leukemia-free state
MMF	Mycophenolate mofetil
MRD	Measurable (‘minimal’) residual disease
NMDP	National Marrow Donor Program
MTD	Maximum tolerated dose
NCI	National Cancer Institute
OS	Overall survival
PBSC	Peripheral blood stem cells
RIC	Reduced-intensity conditioning
R/R	Relapsed/refractory
SCCA	Seattle Cancer Care Alliance
TBI	Total body irradiation
TRM	Treatment Related Mortality
UW(MC)	University of Washington (Medical Center)

Table of Contents

1.0 GENERAL INFORMATION

1.1 Title: A randomized trial for patients with high-grade myeloid neoplasms with measurable residual disease (MRD): CPX-351 vs. immediate allogeneic hematopoietic cell transplantation

1.2 Funding information: The study will be funded by Jazz Pharmaceuticals.

1.3 Investigator information: The study will be conducted within the Cancer Consortium, based at Fred Hutchinson Cancer Research Center.

2.0 INTRODUCTION TO THE PROTOCOL

2.1 Introduction

Acute myeloid leukemia (AML) is a disease that is fatal within the first few weeks or months after diagnosis if left untreated. Intensive induction chemotherapy soon after diagnosis is the most common treatment for fit patients and should be considered regardless of patient's age according to the 2017 European LeukemiaNet (ELN) guidelines. (Döhner et al., 2017) Though most patients will achieve a complete remission (CR), defined as morphologic blast count in the bone marrow <5% coupled with peripheral count recovery, most will ultimately relapse. Allogeneic hematopoietic cell transplantation (alloHCT) offers a chance to decrease the relapse risk in many patients.

2.2 AML with MRD

2.2.1 Detection of MRD

The 2017 ELN guidelines single out a new response category of CR without measurable residual disease (MRD) applicable to patients with a demonstrable marker of disease prior to treatment that is no longer detectable after treatment (e.g., a flow cytometric immunophenotype, a molecular marker that can be followed by quantitative polymerase chain reaction or next generation sequencing, or abnormal karyotype identified through conventional cytogenetics or fluorescence *in situ* hybridization). (Döhner et al., 2017) Sensitivities of the methodologies differ based on the characteristics of the individual tests and their operators as well as the characteristics of an individual patient's leukemic blasts.(Grimwade & Freeman, 2014) In 2018, the ELN published consensus recommendations regarding MRD monitoring in AML, suggesting that central laboratories with significant experience in the different methodologies perform testing whenever possible.(Schuurhuis et al., 2018)

Notably, an individual patient may have MRD detectable by one method but not another. One analysis suggested that discordance between flow and cytogenetic detection of MRD was found in 22% of patients; most importantly, however, detection of MRD by any means was a negative prognostic marker.(Fang et al., 2012) Detection of certain molecular abnormalities can be predictive of relapse, while persistence of others after chemotherapy (the so-called DTA mutations, including *DNMT3A*, *TET2*, and *ASXL1*) is not.(Jongen-Lavrencic et al., 2018)

2.2.2 Predictive significance of MRD

The best treatment strategy for patients who achieve a CR after induction but have persistent MRD is not clear. Such patients with detectable flow, molecular, or cytogenetic abnormalities have a higher likelihood of relapse than those who obtain CR without MRD after induction therapy.(X. Chen et al.,

2015; Y. Chen et al., 2011; Freeman et al., 2013; Marcucci et al., 2004; Othus et al., 2016; Terwijn et al., 2013) Patients not receiving HCT had an 80% probability of relapse within 1 year if a conventional complete response (CR) was accompanied by MRD vs. 20% if it was not.(X. Chen et al., 2015) Though alloHCT may be curative in some such patients, the presence of pre-HCT MRD is indicative of worse outcomes post-transplant, with particularly poor relapse-free survival.(Mortland et al., 2013; Walter, Gooley, et al., 2011; Walter et al., 2015) Araki at al. observed patients receiving HCT in morphologic remission accompanied with MRD had rates of relapse, relapse-free survival, and survival at 1 year of 60%, 20%, and 30-40% respectively, significantly worse compared to those seen in patients in morphologic remission without MRD (15%, 75% and 80% respectively).(Araki et al., 2016)

Some pediatric AML investigators have proposed "risk adapted" strategies, which refine treatment in light of response to initial therapy,(Rubnitz et al., 2010) but these methods have not been widely adopted in adult AML. Additionally, no therapy has yet been shown to reliably and effectively eradicate MRD, either pre- or post-HCT. It should be noted that few studies have specifically targeted the MRD-positive population, though many are ongoing or planned since this is such a difficult population to treat.(Percival & Estey, 2019)

Eradication of MRD pre-HCT has the potential to improve post-HCT outcomes.

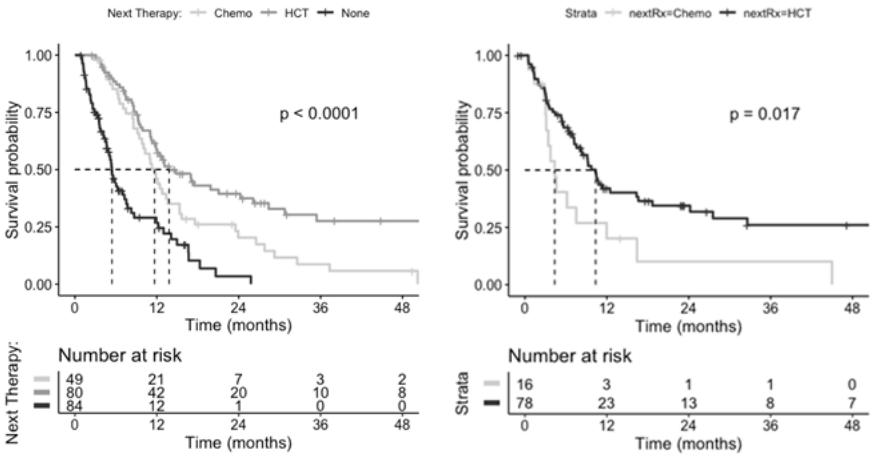
2.2.3 Eradication of MRD at FHCRC/UW

Attempts to reduce MRD in patients with AML at FHCRC/UW have been largely unsuccessful. A recent retrospective analysis by J. Appelbaum found that MRD, generally present after one course of intensive initial induction, was eliminated in 10/28 patients (36%; 95% CI 19-56%) given additional intensive chemotherapy, and remained in all 21 patients given a less intense treatment as first attempt to eliminate MRD.(Appelbaum et al., 2019) Furthermore, it is unclear whether reduction of MRD will *per se* improve post HCT outcome. Dr. Appelbaum's analysis identified 16 patients subsequently transplanted (7 of 16 in CR/CRp) of whom only 2 survived longer than 1 year, with survival similar regardless of whether MRD was eliminated (7 pts) or not (9 pts) by chemotherapy.

If MRD is resistant to elimination or if its disappearance is irrelevant, it may be preferable to proceed directly to HCT despite the deleterious influence of pre-HCT MRD on post HCT outcome. Dr. Appelbaum's review found survival from the time of initial chemotherapy was longer in the 80 patients who received HCT as first treatment for MRD than in the 49 who received chemotherapy (**figure 1A**, median 13.8 mos. vs 11.6 mos., p = 0.01). Survival was not statistically different in the 16/49 patients who received HCT after first receiving chemotherapy than in the 80 patients who received HCT as first therapy for MRD despite the "guarantee time" the 16 enjoyed. Furthermore, survival dated from HCT was shorter in the 16/49 patients who received chemotherapy followed by transplant than in those given HCT as initial treatment for MRD (**figure 1B**, p= 0.02, l median 4.4 vs. 10.4 mos.) Regardless of elimination of MRD only 2 of the 16 (12.5%, 2-38%) survived longer than 1 year vs. 23 of the 80 (29%, 19-40%) given HCT without a prior attempt to eliminate MRD.

FIGURE 1. Panel A: Survival from start of initial treatment for MRD: chemotherapy 49 patients, HCT without prior effort to eliminate MRD 80 patients, no treatment 84 patients**. Panel B:** Survival from HCT in 16 patients who received chemotherapy to eliminate pre-HCT MRD and in 78 patients who went directly to HCT (no prior attempt to reduce MRD)

A) **B)**



If efforts to reduce pre-HCT MRD were only intermittently successful, and if reduction was irrelevant, such efforts would serve only to delay HCT, if for example infectious complications arose; indeed, as noted above, only 16/49 patients who received chemotherapy to reduce MRD prior to HCT subsequently received HCT. Alternatively, attempts to reduce pre HCT MRD might make successful HCT less likely if, for example, blast numbers increased during the delay. This possibility might partially explain the infrequency (16/49) with which patients who initially received therapy to reduce MRD subsequently received HCT.

However, the above data were collected retrospectively with a variety of different induction attempts. These limitations motivate the planned clinical trial. Patients with high-grade myeloid neoplasms who have MRD will be randomized between two approaches: direct to alloHCT (Arm A) vs. treatment to eliminate MRD followed by alloHCT (Arm B).

2.3 **CPX-351**

CPX-351 (brand name Vyxeos) is a liposomal formulation of cytarabine and daunorubicin at an optimal, fixed molar ratio. The drug was approved by the Food and Drug Administration in 2017 for treatment of secondary AML in patients aged 60-75. Approval was based on a longer survival and event-free survival with CPX-351 in a trial randomizing such patients between standard 7+3 and CPX-351.(Lancet et al., 2018) Of particular interest is the disproportionately better survival with CPX-351 than 7+3 considering only patients receiving HCT (HR 0.46, 95% CI 0.24-0.89) than considering all patients (HR 0.69, 95% 0.52-0.90).(Lin et al., 2018) Possible explanations for the improved survival outcomes for patients after CPX-351 include HCT's greater sensitivity to an absence of MRD and a distinctive ability of CPX-351 to reduce MRD.

2.4 **Dose Rationale**

For patients randomized to Arm B of the clinical trial, they will receive induction dosing of CPX-351 at 100 units/m2 on days 1, 3, and 5 of a 28-day cycle. Patients may receive up to two courses of CPX-351. Patients who receive the second course will receive induction dosing of CPX-351 at 100 units/m2 on days 1 and 3 of a 28-day cycle.

2.5 **Other Agents**

This protocol will be an umbrella protocol in terms of HCT regimens. That is, patients randomized to Arm A (direct to HCT) or patients in Arm B who receive CPX-351 and then undergo HCT will be able to

receive any HCT regimen deemed appropriate by the treating physician including myeloablative or reduced intensity conditioning.

2.6 Risks/Benefits

AlloHCT carries inherent risks, including infection, graft-versus-host disease, and relapse of the original disease. Simultaneously, however, alloHCT represents the best chance of cure for many patients with AML and high-grade myeloid neoplasms. The goal of this clinical trial is to optimize the chance of post-HCT success for patients with evidence of pre-HCT MRD.

3.0 OVERVIEW OF CLINICAL TRIAL

3.1 Study Objectives

3.1.1 Primary Objectives

To determine whether treatment with CPX-351 improves overall survival over immediate alloHCT among patients with MRD following intensive chemotherapy.

3.1.2 Secondary Objectives

- To determine whether treatment with CPX-351 improves **relapse-free survival** over immediate alloHCT among patients with MRD following intensive chemotherapy.
- To measure the **rate of transplantation** following treatment with CPX-351 among patients with MRD following intensive chemotherapy.
- To determine whether treatment with CPX-351 improves **non-relapse mortality** over immediate alloHCT among patients with MRD following intensive chemotherapy.
- To compare, among patients with MRD following intensive chemotherapy, the frequencies of **types of transplant** received following treatment with CPX-351 vs. immediate transplantation.
- To measure the **frequency with which the burden of disease is decreased or eliminated** following treatment with CPX-351 among patients with MRD following intensive chemotherapy.

3.2 Study Population

Adults with high grade myeloid neoplasms and MRD following intensive chemotherapy with an intent to undergo alloHCT are invited to participate.

3.3 Study Design

This study is a phase II open-label randomized trial for patients with high-grade myeloid neoplasms with MRD following intensive chemotherapy, evaluating the efficacy of immediate alloHCT(ARM A) versus treatment with CPX-351 followed by alloHCT (ARM B). We intend to enroll 130 patients in order to treat 65 patients with alloHCT and 65 patients with CPX-351 followed by alloHCT.

Patients will have already received one cycle of intensive chemotherapy and remain MRD positive within 90 days of treatment. Patients must have an identified donor available prior to study enrollment.

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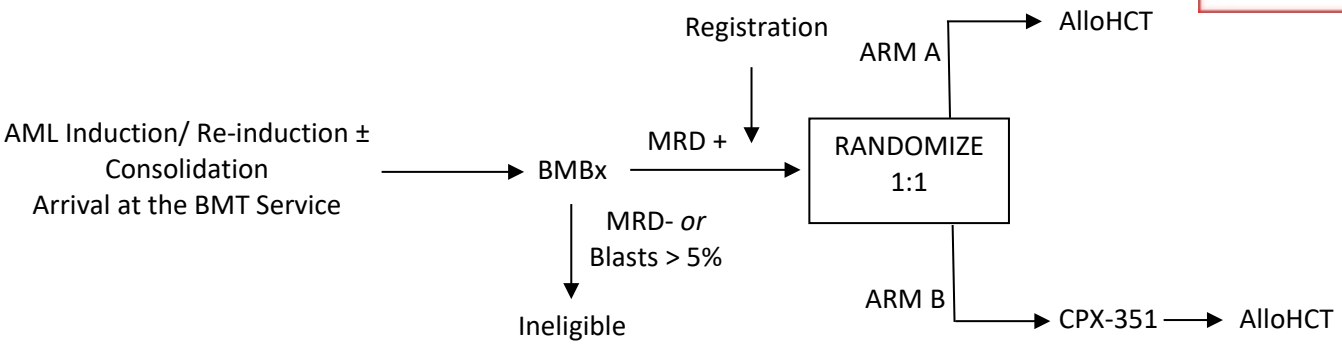

3.3.1 Primary Endpoint

The primary endpoint of the study is survival from the time of randomization. The survival (in days) of subjects in the two arms will be compared using the log-rank test.

3.3.2 Secondary Endpoints

- **Relapse-free survival** from the time of MRD identification and (among patients transplanted) from the time of transplantation. The following comparisons will be made: (1) number of days elapsing between the date of MRD identification and the date of death or relapse, (2) among transplanted patients, the number of days elapsing between the date of transplantation and the date of death or relapse.

- **Rate of transplantation** following treatment with CPX-351 among patients with MRD following intensive chemotherapy. The proportion of patients having begun transplant conditioning 60 days and 180 days following enrollment will be compared using the chi-squared test. The **time to transplant** among the two arms will be compared by comparing the number of days elapsed between enrollment and the initiation of transplant conditioning by the log-rank test.

- Frequencies of the **types of transplant** received. Among the patients receiving transplantation, the proportion of patients in each arm receiving myeloablative transplant conditioning will be compared using the chi-squared or Fisher's exact test. Among patients receiving transplantation, the proportion of patients in each arm receiving donor stem cells from a haploidentical donor, an unrelated donor, or from cord blood unit will each be compared using the chi-squared or Fisher's exact test.

3.4 Estimated Accrual

We estimate that we will enroll approximately 30 patients per year, to complete accrual in 5 years.

3.5 Name of Sponsor/Funding Source

This trial is supported by funding from Jazz Pharmaceuticals. This trial is conducted under an IND held by Sponsor-Investigator: Filippo Milano, MD, PhD.

4.0 SAFETY CONSIDERATIONS

4.1 Stopping Rules

This trial may be discontinued at the discretion of the principal investigator following low accrual or infrequent transplantation following treatment with CPX-351. See statistical considerations, section 17 for more details.

5.0 SELECTION OF STUDY POPULATION

5.1 Eligible Disease and Stage

AML other than acute promyelocytic leukemia (APL), MDS-EB2, or another high-risk myeloid neoplasm (≥ 10% blasts in the blood or marrow), having completed at least one cycle of chemotherapy intended to induce remission (see section 5.1.2 regarding requirements for prior regimens).

5.1.1 Measurable or Non-Measurable Disease

Subjects must have MRD, defined as the presence of original disease detected by multi-parameter flow cytometry and cytogenetic/molecular assessment within 90 days of chemotherapy intended to induce remission (see section 5.1.2 regarding requirements for prior regimens):

- Abnormal cells identified by multiparameter flow cytometry, present at a frequency of between 0% and 5% of total nucleated cells, judged in the opinion of the hematopathologist to represent continued presence of malignant cells.
- Abnormal karyotype; present in any number of metaphase cells.
- Abnormal fluorescence in-situ hybridization; judged in the opinion of the hematopathologist to represent continued presence of malignant cells.
- The presence of any leukemia associated mutation as detected by DNA sequencing, except mutations in *DNMT3A, TET2,* or *ASXL1.* This includes (but is not limited to) the following genes*: CBL (CDS), CSF3R (Exons 14, 15, 17), EZH2 (Exons 15-20), FBXW7 (CDS), FGFR1 (Exons 4, 11-17, partial 18), FLT3 (p.D835H), GATA1 (Exons 2-3), GATA2 (Exons 3-5), HRAS (Exon 1-2), IDH1(p.R132), IDH2 (Exon 4), JAK2 (Exon 12, 14, 16), KIT (8-18), KMT2A (CDS), KRAS (CDS), MAP2K1 (Exons 2, 3, 6), MPL (Exon 10), MYD88 (Exon 3-5), NOTCH1 (Exons 20, 26, 27), NPM1 (Exon 12), NRAS (CDS), PDGFRA (Exons 12-18), PHF6 (CDS), PTEN (CDS), RB1 (CDS), RUNX1 (Exon 4-8), SF3B1 (Exon 14-16), SRSF2 (Exon 1), STAG2 (CDS), STAT3 (Exons 20-21), TP53 (CDS), U2AF1 (Exons 2, 6), WT1 (CDS),* and *ZRSR2 (CDS)*

5.1.2 Allowable Prior Therapy

For the purposes of this study intensive chemotherapy will include regimens listed below. Additional regimens may be included at the discretion of the study PI.

- Any regimen including cytarabine at a dose of 100mg/m2/day for at least 7 days *and* an anthracycline at any dose +/- gemtuzumab ozogamicin (GO)

- Any regimen including cytarabine at a dose of at least 100mg/m2/day for at least 5 days *and* a purine analog at any dose (e.g. clofarabine, fludarabine, cladribine) +/- GO

5.2 **Patient Inclusion Criteria**

Each patient must meet all the following criteria within 14 days of study start (unless otherwise noted) to be enrolled in the study:

- Ability to understand and voluntarily sign a written informed consent document (ICF).
- Male or female patients, age ≥ 18 years at the time of consent.
- Absence of a concomitant illness with a likely survival of < 1 year.
- Medically fit, defined as a treatment related mortality score (TRM) of ≤ 13.1 calculated according to Walter et al, JCO 2011.(Walter, Othus, et al., 2011)
- Additionally, subjects should be eligible in the opinion of their treating physician for allogeneic transplantation.
- Patients with normal or acceptable organ and marrow function listing hematologic and blood chemistry parameters.
    - Bilirubin ≤ 2.5 x institutional upper limit of normal, unless elevation is thought to be due to Gilberts syndrome or hemolysis
    - Left ventricular ejection fraction ≥ 40% assessed by Multiple Gated Acquisition Scan (MUGA), echocardiography or other appropriate diagnostic modality within 12 months of enrollment with no clinical evidence of decompensated congestive heart failure.
    - Creatine clearance of ≥30mL/min as measured by Cockcroft Gault equation
- Consent of female patients with a negative serum or urine pregnancy test to use a medically acceptable method of contraception throughout the entire study period and for 6 months following the last dose of CPX-351.
- Male patients must be willing to refrain from sperm donation for 6 months following the last dose of CPX-351 and must use adequate contraception throughout the entire study period and for 6 months following the last dose of CPX-351.
- Patients enrolling in this trial should intend to complete the treatments described and should be eligible in the opinion of the treating physician for allogeneic transplantation.
- Patients must have a caregiver capable of providing post-HCT care, who will be present once conditioning therapy begins.

5.3 **Patient Exclusion Criteria**

- Allogeneic myeloablative hematopoietic cell transplant within 6 months.
- Autologous hematopoietic cell transplant within 6 months.
- Known Hypersensitivity to CPX-351.
    - Patients may not have known hypersensitivity to CPX-351, daunorubicin, cytarabine, or liposomal products.

- Prior treatment with two or more cycles of CPX-351.
- Treatment within the last 30 days of other investigational antineoplastic agents.
- Evidence of organ dysfunction likely to preclude safe transplantation including the following:
    - Symptomatic coronary artery disease or uncontrolled arrhythmia within the prior 3 months and since most recent anthracycline exposure
    - Myocardial impairment of any cause resulting in heart failure as determined by NY Heart Association Criteria (Class III or IV)
    - DLCOc <40% or FEV1 < 50%
    - Need for supplemental oxygen
- Active systemic fungal, bacterial, viral or other infection, unless under treatment with anti-microbials and/or controlled or stable (e.g. if specific, effective therapy is not available/feasible or desired [e.g. chronic viral hepatitis, HIV]).
- Female patients who are pregnant, nursing, or lactating.
- Patients with an inability to accept blood transfusions
- Inability to give informed consent, or unable to comply with the treatment protocol including appropriate supportive care, follow-up and tests.
- Any other condition that would cause a risk to patients if they participate in the trial.

6.0 INFORMED CONSENT OF SUBJECT

Subjects will be referred here treatment, possibly to include consideration of an alloHCT. The subject will be completely evaluated. Before initiating protocol-specific procedures that would not otherwise be done for a subject, the investigator must discuss the protocol thoroughly with subject, including all known risks to the subject and donor. The procedure and alternative forms of therapy will be presented as objectively as possible and the risks and hazards of the procedure explained to the subject or, in the case of minors, to the subject's responsible family members. Consent will be obtained using forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. The ICF must be signed and dated by the subject or by the subject's legally authorized representative if the subject is unable to sign. The case history for each subject will document that informed consent was obtained before the subject's study participation, detailing what was covered. A copy of the ICF must be provided to the subject or the subject's legally authorized representative.

Signed ICFs must remain in each subject's chart and must be available for verification by monitors or regulatory agencies at any time.

7.0 SUBJECT REGISTRATION

Informed consent must be signed prior to the performance of any study related procedures or assessments.

Subjects will be registered into the system by the Clinical Coordinators Office (CCO) (Intake Office) and assigned a UPN (Unique Patient Number). The CCO will register the subject on to the protocol through the Data Management Office.

8.0 TREATMENT PLAN

This is an open label phase II trial to compare the feasibility of using CPX-351 to eliminate MRD in patients with AML and other high-grade myeloid neoplasms following intensive chemotherapy prior to an alloHCT. Following one cycle of chemotherapy, subjects will be randomized to alloHCT (ARM A) or up to two cycles of CPX-351 followed by an alloHCT (ARM B).

8.1 **Treatment Plan Overview**

Patients will be enrolled following at least one cycle of intensive chemotherapy and must have been found to be MRD positive at the time of bone marrow evaluation pre-transplant.

All patients will undergo a bone marrow biopsy, those who are negative for MRD or have > 5% blasts will be excluded from the study. Patients who remain MRD positive will be randomized between two treatment arms: **ARM A** – early alloHCT, and **ARM B** – CPX-351 followed by alloHCT.

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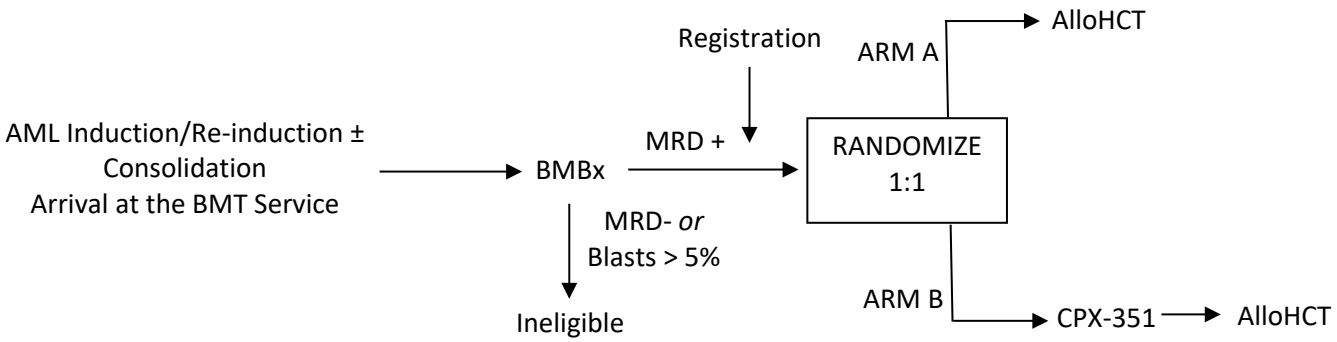


8.2 **Administration of allogeneic transplantation (ARM A)**

Following randomization patients in treatment **ARM A** will be referred for alloHCT. The transplant regimen and the timing of its initiation will be at the discretion of the transplant physician.

As soon as potential patients are identified for this protocol, the treating physician or the study staff will initiate the process of identifying an appropriate source of donor stem cells and a suitable transplant regimen. It is the intent of the study that patients randomized to treatment ARM A begin transplant conditioning within 45 days of randomization.

Any transplant regimen is allowed. The treating physician will indicate the donor stem cell type (matched related, matched unrelated, mismatched unrelated, haploidentical, or cord blood) as well as the transplant conditioning regimen and transplant conditioning regimen intensity (myeloablative vs reduced intensity) via questionnaires.

The specific evaluations, medications and procedures conducted during the course of the allogeneic transplant will be managed by the treating physician and may include experimental protocols and/or medications available at the treating institution.

8.3 **Administration of CPX-351 (ARM B)**

Patients randomized to treatment ARM B will be treated with CPX-351 prior to alloHCT. Below is a sample timeline for subjects randomized treatment **ARM B**:

ARM B Treatment Schedule	
Day	***Treatment***
1	*CPX-351 (daunorubicin 44 mg/m2 and cytarabine 100 mg/m2) IV over 90 min*
3	*CPX-351 (daunorubicin 44 mg/m2 and cytarabine 100 mg/m2) IV over 90 min*
5	*CPX-351 (daunorubicin 44 mg/m2 and cytarabine 100 mg/m2) IV over 90 min*
14 - 35	*Bone marrow aspirate and biopsy**
≤60	*CPX-351 cycle 2 **OR** allogeneic HCT*

For patients assigned to **ARM B**, a second cycle of CPX-351 may be given at the discretion of the treating physician. In the event that a patient receives a second cycle of CPX-351, the expected time frame for start of HCT conditioning regimen will be considered within 60 days from CPX-351 Cycle 2, Day 1.

ARM B Treatment Schedule Cycle 2	
Day	***Treatment***
1	*CPX-351 (daunorubicin 44 mg/m2 and cytarabine 100 mg/m2) IV over 90 min*
3	*CPX-351 (daunorubicin 44 mg/m2 and cytarabine 100 mg/m2) IV over 90 min*
14 - 35	*Bone marrow aspirate and biopsy**
≤60	*Allogeneic HCT*

If hypersensitivity or infusion-related reactions occur, infusion of CPX-351 may be prolonged to two hours (120 minutes) or more.

For patients assigned to **ARM B**, allogeneic transplant should be considered following either one or two cycles of CPX-351. The timing of transplantation (defined as start of transplant conditioning regimen) should be scheduled within 60 days of the most recent cycle of chemotherapy, unless delay is judged to be in the patient's interest by the transplant physician.

*Timing of bone marrow aspirate and biopsy procedures: The timing of the marrow evaluation is flexible and should occur not prior to 14 days and not later than 35 days following initiation of treatment, with a preference for the sample to be collected within 1 week of the count recovery (defined as two complete blood counts showing an absolute neutrophil count of ≥1,000/uL and a platelet count of ≥100k/uL). If count recovery has not occurred prior to day 28, a marrow aspirate should be completed prior to day 35.

8.4 **Concomitant Medication and Supportive Care Guidelines**

All patients will be adequately hydrated and received appropriate anti-emetics based on institutional standard of care guidelines.

Additional growth factors may be used according to institutional standard of care guidelines or the preference of the attending physician.

The use of antimicrobial prophylaxis will be used according to institutional standard of care guidelines. In the case of neutropenic fever, standard of care diagnostic testing will be performed, and empiric antibiotic coverage will be utilized as per stand of care institutional practices.

The use of transfusion support (red blood cells [RBCs] and platelets) will be carried out according to institutional standard of care guidelines.

8.5 **Duration of Therapy**

The duration of treatment is not specified by this protocol. Subjects will be evaluated at a frequency determined by the treating physician on their alloHCT protocol and according to standard of care guidelines.

8.6 **Duration of Follow-Up**

Patients will be followed for up to 2 years from the time of enrollment and subsequently for demographic and survival assessment.

9.0 SUBJECT EVALUATION

9.1 **Pre-randomization evaluations**

Patient work-up will be in accordance with Standard Practice Policy Manual Evaluation Guidelines for alloHCT patients with some additional required testing individualized to the treating physician's protocol. The following information will be obtained through patient records to establish trial eligibility and allow patient characterization and disease prognostication. Results of tests and/or procedures conducted as per standard of care may be used to determine study eligibility if conducted within an appropriate window prior to screening. Outside testing and previously collected clinical data may be used if within the appropriate time frame.

- ***Comprehensive History and Physical Examination, including the details of the patient's diagnosis, prior treatment and response, history of cardiac or pulmonary symptoms and pre-existing medical diagnoses.***
- ***Vital Signs***
- ***Bone marrow aspiration for pathology, flow cytometry, and cytogenetic studies within 30 days of planned donor cell infusion.***

9.2 Evaluations for patients on ARM A and ARM B proceeding with transplant

Patient work-up will be in accordance with Standard Practice Policy Manual Evaluation Guidelines for alloHCT patients with some additional required testing individualized to the treating physician's protocol. The following information will be obtained through patient records to establish trial eligibility and allow patient characterization and disease prognostication. Results of tests and/or procedures conducted as per standard of care may be used to determine study eligibility if conducted within an appropriate window prior to screening. Outside testing and previously collected clinical data may be used if within the appropriate time frame.

- ***Comprehensive History and Physical Examination, including the details of the patient's diagnosis, prior treatment and response, history of cardiac or pulmonary symptoms and pre-existing medical diagnoses.***
- ***Neurological Exam***
- ***Vital Signs***
- ***Pulmonary function studies***
- ***EKG***
- ***MUGA or ECHO for EF evaluation if there exists a history of cardiac symptoms or if the patient is aged >60 years***
- ***Labs:***
    - *Hematology: CBC, differential, platelets*
    - *Serum Chemistries: including calcium, SGOT, SGPT, alkaline phosphatase, total bilirubin, BUN, creatinine, electrolytes, and glucose*
- ***Bone marrow aspiration for pathology, flow cytometry, and cytogenetic studies***

9.3 Evaluations for patients on ARM B (CPX-351)

- ***Vital Signs-*** *Collected immediately before initiation of each infusion of CPX-351 on Days 1, 3, and 5 of the course, as well as the end of the study/early termination.*
- ***Physical Examination and Medical History-*** *Weight will be measured prior to study treatment for each course for all patients. Height will be measured only once prior to the initial treatment dose. Review and record concomitant medications.*
- ***Labs:***
    - *Hematology: CBC, differential, platelets*
    - *Serum Chemistries: including calcium, SGOT, SGPT, alkaline phosphatase, total bilirubin, BUN, creatinine, electrolytes, and glucose*
    - *HCG (quantitative pregnancy) [PG] in females past menarche and pre-menopause.*
- ***Record patient's overall response to CPX-351***

9.3.1 Second CPX-351 course (as determined by the investigator)

- ***Vital Signs****- Collected immediately before initiation of each infusion of CPX-351 on Days 1 and 3 of the course, as well as the end of the study/early termination.*
- ***Labs:***
    - *Hematology: CBC, differential, platelets*
    - *Serum Chemistries: including calcium, SGOT, SGPT, alkaline phosphatase, total bilirubin, BUN, creatinine, electrolytes, and glucose*

- *HCG (quantitative pregnancy) [PG] in females past menarche and pre-menopause.*

- ***Record patient's overall response to CPX-351***

9.4 Patient evaluations post-transplant

See Standard Practice Policy Manual for "Evaluation Guidelines for Transplant Patients". Data from these standard laboratory studies, other clinically indicated studies, and clinical assessments by the primary care team will be reviewed regularly to assess for regimen-related toxicity, occurrence of Serious Adverse Events, and other potential complications such as GVHD and infections.

Patients will be followed routinely with the general institutional guidelines and based on the patient's clinical condition. Common follow-up guidelines used when patients are cared for on an outpatient basis until engraftment are:

- CBC: daily when ANC <500/µL or platelets <10,000/µL; weekly when ANC >500/µL and platelets >10,000/µL.
- Electrolytes: weekly.
- Liver function tests: weekly.
- Clinical assessments: weekly.

10.0 SAFETY PLAN

10.1 Dosing Delays and Modifications

Doses of CPX-351 may be delayed due to adverse events (AEs) in individual patients. Any doses missed or delayed due to and AE may be administered as soon as the patient has recovered per the investigator's judgement. Details of any infusion interruptions (e.g. rate, time, and outcome) will be recorded. Investigators may request to delay or discontinue CPX-351 if it is in the best interest of the patient.

10.2 DRUGS, IRRADIATION AND MARROW/STEM CELL ADMINISTRATION TOXICITIES AND COMPLICATIONS

The specific evaluations, medications and procedures conducted during the course of the allogeneic transplant will be managed by the treating physician and may include experimental protocols and/or medications available at the treating institution. Refer to Standard Practice Manual for administration guidelines.

11.0 TOXICITY MONITORING

11.1 Toxicity criteria

This protocol will conduct safety monitoring throughout the study for the CPX arm. The immediate alloHCT arm will not have monitoring specific to this trial, as these patients will be treated either on a HCT protocol which will monitor toxicity or on an HCT treatment plan. It is not expected that CPX will

lead to excessive toxicity as it has been studied in hundreds of patients. However, it is possible that use of CPX could lead to an unacceptable fraction of patients who do not proceed to transplant or in whom transplant is delayed (defined as start of transplant conditioning regimen more than 60 days following start of CPX) due to toxicity attributable to CPX. Therefore, the CPX arm will be monitored carefully for toxicity and inability for or delay in individual patients to proceed to HCT. Details are provided in Section 17. In addition, safety concerns associated with CPX will be identified by continuous review of the data by the Principal Investigator. The Principal Investigator will review the outcome of the data for each individual patient on an ongoing basis, and the Principal Investigator will have primary responsibility for ensuring that the protocol is conducted as approved by the Fred Hutchinson Cancer Research Center's Scientific Review Committee and Institutional Review Board. The Principal Investigator will also comply with the Fred Hutch Data and Safety Monitoring Plan as described beneath the Data and Safety Monitoring Plan section. The PI will personally review with the Research Nurse the clinical course of all the enrolled patients at least weekly.

All patients who receive CPX are evaluable for toxicity. Patients are evaluated from first day receiving study treatment until death, start of conditioning for HCT, or 56 days following the conclusion of treatment.

12.0 SUBJECT DISCONTINUATION OF ACTIVE TREATMENT

Subjects may be removed from this study at any time at their discretion. Subjects may also be removed from this protocol if they develop any untoward side effects from the study treatment. In addition, there are suspension rules in place for excessive toxicity as detailed in the statistical section.

An explanation for discontinuing treatment is recorded for each subject discontinuing treatment. All subjects, irrespective of treatment status, will continue to be followed for survival. Treatment in this study must be discontinued for any of the following reasons:

- At Investigator's discretion.
- Adverse toxicities that prevent continuation with study treatment.
- Progressive AML requiring off-protocol salvage (chemo/immune) therapy.
- Withdrawal of consent; the patient may withdraw from the study at any time for any reason.

13.0 ASSESSMENT OF DISEASE RESPONSE

13.1 **Treatment response and outcome**

In patients receiving either additional chemotherapy or CPX-351 treatment response (e.g. CR without MRD, CR, CRi, morphologic leukemia-free state [MLFS], partial remission [PR]) or treatment failure (e.g. refractory disease, death in aplasia, death from indeterminate cause, hematologic relapse, molecular relapse) as well as treatment outcome (e.g. overall survival, relapse-free survival, event-free survival, and remission duration) will be determined by peripheral blood count and bone marrow evaluation and categorized according to the 2017 European LeukemiaNet criteria.(Döhner et al., 2017) Patients are routinely assessed for the presence of MRD as detected by multi-parameter flow cytometry and cytogenetic/molecular assessment, as per institutional practice.

14.0 ADVERSE EVENTS AND REPORTING REQUIREMENTS

14.1 **Adverse event**

An adverse event (AE) is any untoward medical occurrence in a clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Events not considered AEs in this study:

- **Disease progression or relapse.** However, clinical events associated with progression/relapse may be reportable as AEs
- **Medical conditions present at screening** (i.e., before the study treatment is administered) will not be considered as adverse events. However, medical conditions present at baseline that worsen in intensity or frequency during the treatment or post-treatment periods will be reported and recorded as adverse events.
- **Abnormal laboratory values outside of normal laboratory parameters** should not be recorded as AEs unless clinical intervention is required or deemed clinically significant by the investigator.
- **Diagnostic tests and medical or surgical procedures** should not be captured as an AE or an SAE in and of themselves. However in some cases the event or condition requiring the test or procedure may be considered and captured as an AE or SAE.
- **Preplanned hospitalizations.** Hospitalizations for elective or protocol-scheduled medical or surgical procedures, treatments planned before enrollment in the treatment plan, or routine check-ups are not SAEs by this criterion. However any AE requiring an unepexpected hospitalization or prolongation of a preplanned hospitalization will be captured as an SAE and subject to SAE reporting requirements.

14.2 **Serious adverse event**

An adverse event should be classified as a serious adverse event (SAE) if it meets one of the following criteria:

Fatal	Adverse event results in death.
Life threatening	The adverse events placed the subject at immediate risk of death. This classification did not apply to an adverse event that hypothetically might cause death if it were more severe.
Hospitalization	It required or prolonged inpatient hospitalization. Hospitalizations for elective medical or surgical procedures or treatments planned before enrollment in the treatment plan or routine check-ups are not SAEs by this criterion. Admission to a palliative unit or hospice care facility is not considered to be a hospitalization.
Disabling/incapacitating	Resulted in a substantial and permanent disruption of the subject's

	ability to carry out normal life functions.
Congenital anomaly or birth defect	An adverse outcome in a child or fetus of a subject exposed to the molecule or treatment plan regimen before conception or during pregnancy.
Medically significant	The adverse event did not meet any of the above criteria but could have jeopardized the subject and might have required medical or surgical intervention to prevent one of the outcomes listed above.

14.3 **Unexpected adverse event**

An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the applicable investigator brochure; or, for the purposes of IRB reporting, not consistent with the prior medical condition of the subject or other treatment given to the subject. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed and reported in preclinical or clinical studies rather than an experience that has not been anticipated based on the pharmacological properties of the study drug.

14.4 **Monitoring and recording adverse events**

Grade 3 and above adverse events (or highly unusual grade 2 adverse events) and all SAEs that occur during the AE monitoring period (see section 14.7) will be followed and captured in the database. When a grade 3 adverse event increases in severity to grade 4 or above, the event will be captured at its highest grade. Clinically significant AEs beyond the AE monitoring period may also be captured at the discretion of the investigator. When a subject goes on to further treatment off protocol, adverse events will no longer be collected with the exception of death. The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event and/or serious adverse event and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the adverse event using concise medical terminology.
- Description as to whether or not the adverse event is serious, noting all criteria that apply.
- The start date (date of adverse event onset).
- The stop date (date of adverse event resolution).
- The maximum severity (grade) of the adverse event.
- A description of the potential relatedness of the adverse event to study drug, a study procedure, or other causality.
- The action taken due to the adverse event.
- The outcome of the adverse event.

14.5 **Grading adverse event severity**

All adverse events will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. A copy of the CTCAE v5.0 can be downloaded from the CTEP home page (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50). If a

CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event.

14.6 **Attribution of an adverse event**

Association or relatedness to the study agent will be assessed by the investigator as follows:

- Definite: The event follows a reasonable temporal sequence from exposure to the investigational agent, has been previously described in association with the investigational agent, and cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications; AND the event disappears or improves with withdrawal of the investigational agent and/or re-appears on re-exposure (e.g., in the event of an infusion reaction).
- Probable: The event follows a reasonable temporal sequence from exposure to the investigational agent and has been previously been described in association with the investigational agent OR cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Possible: The event follows a reasonable temporal sequence from exposure to the investigational agent but could be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Unlikely: Toxicity is doubtfully related to the investigational agent(s). The event may be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Unrelated: The event is clearly related to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.

For general adverse event assessment, an adverse event is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated.

14.7 **Adverse event reporting period**

Adverse events will be tracked for up to 56 days after the last dose of CPX-351 or until resolution. Collection of AEs should cease with the start of any new therapy or conditioning for transplant.

14.8 **Adverse event reporting requirements**

The Principal Investigator will report adverse events to the FHCRC IRB in accordance with IRB policies, including expedited reporting of serious adverse events that are related or possibly related to the research and unexpected as defined by the IRB.

The Sponsor-Investigator assumes responsibility for IND safety reporting to the FDA in accordance with regulations under 21 CFR 312.32, including notifying FDA of any serious, unexpected, suspected adverse reaction (SUSAR) within 15 calendar days (or 7 days in the event of a fatal or life-threatening SUSAR) after determining that the information qualifies for reporting. Serious adverse events not meeting criteria for reporting as SUSARs will be reported in the annual report to the IND as required by 21 CFR 312.33.

The PI (or designee) must send all SAEs (initial and follow-up) that require collection and reporting per protocol, and which occur in a subject who received a Jazz Pharmaceuticals product as study drug, to the JP Drug Safety Department within 1 business day of their awareness of the SAE (AEreporting@jazzpharma.com). The PI (or designee) will report the SAEs using a US-FDA Form 3500A (MedWatch form). [Note: the MedWatch form, is available at http://www.fda.gov/downloads/Safety/MedWatch/HowToReport/DownloadForms/UCM082728.pdf]. The PI (or designee) must also provide JP Drug Safety Department (PVcomms@jazzpharma.com) with a copy of all submissions made to the FDA at the time the submission is made. In addition, all other adverse events will be reported to the JP Drug Safety Department in summary or line-item form upon JP's request and at the conclusion of the study.

15.0 DATA AND SAFETY MONITORING PLAN

Institutional support of trial monitoring will be in accordance with the FHCRC/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, FHCRC Clinical Research Support (CRS) coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FHCRC employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), FHCRC Scientific Review Committee (SRC) and the FHCRC/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating subjects. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

16.0 DATA MANAGEMENT/CONFIDENTIALITY

Research data will be recorded in a study-specific, password protected database using a unique study ID for each patient to assure patient confidentiality. Data from source documents will be transcribed into this database. Source documents are documents where patient data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, quality of life assessments, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, X-rays, patient files, and records kept at the pharmacy, laboratories, and medico-technical departments involved in the clinical trial. There will be no case reports forms (CRFs) used for this trial.

The Principal Investigator will ensure that data collected conform to all established guidelines. Each subject is assigned a unique subject number to protect subject confidentiality. Subjects will not be

referred to by this number, by name, or by any other individual identifier in any publication or external presentation.

The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents. Patient research files will be maintained under control of the local site Principal Investigator and/or study team as designated by institutional policies.

Access to the study database will be restricted by electronic password protection and restricted access to computers per institutional policies and/or guidelines.

17.0 STATISTICAL CONSIDERATIONS

17.1 **Study Design**

Randomized, open-label interventional trial.

17.2 **Primary/Secondary Endpoints, Objectives and Analytical Methods**

The primary endpoint of the study is overall survival from the time of randomization. The survival (in days) of subjects in the two arms will be compared using the stratified log-rank test (stratified on number of chemotherapy cycles and disease risk, factors that randomization is stratified on). Patients still alive at last contact will be censored. End of study will be defined as 2 years after the last patient is randomized. The primary endpoint will be compared between groups using the intent-to-treat principle.

A secondary endpoint of the study is **relapse-free survival** (RFS) from the time of randomization, to be compared between groups with the stratified log-rank test. For descriptive purposes, we shall also report survival and RFS estimates from time of transplant among patients who receive a transplant. Patients alive without relapse at last contact will be censored for RFS.

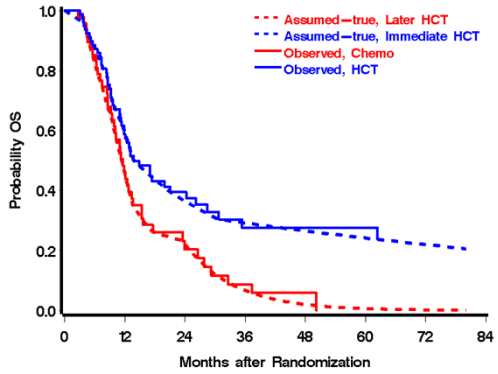
A secondary objective of the study is to compare the **rate of transplantation** among patients with MRD following intensive chemotherapy. The proportion of patients who were transplanted will be compared between arms using the chi-square test

A secondary objective of the study is to examine the **types of transplant** received following treatment with CPX-351 as compared to patients receiving immediate transplantation. Among patients receiving transplantation, the proportion of patients in each arm receiving myeloablative transplant conditioning will be compared using the chi-square or Fisher's exact test. Among patients receiving transplantation, the proportion of receiving donor stem cells from a haploidentical donor, an unrelated donor, or from cord blood unit will be compared across arms using the chi-square or Fisher's exact test.

17.3 **Sample Size and Power**

Outcomes for patients with MRD following intensive chemotherapy at FHCRC since 2008 were reviewed. From these observed data, we fit piecewise exponential survival distributions and used these as the assumed-true distributions for the two arms. These assumed-true distributions are shown

in the figure below. From the observed data, the hazard of death was estimated to be 0.58 (transplant vs chemotherapy).



Estimates of power for specified sample sizes were obtained by randomly drawing survival times from each assumed-true survival distribution for the number of patients assumed for each group, resulting in "simulated" clinical trials under the assumptions depicted in the curves above. The data from this simulated trial were then compared using the log-rank test and a p-value was generated. This process was repeated 500 times, and the proportion of simulated trials that led to a p-value less than the significance level dictated by the trial provides an estimate of power to observe a statistically significant (at a two-sided significance level) difference in survival between the two groups.

Power estimates further assumed uniform accrual over 3 years with minimum follow-up of 2 years (so the longest potential follow-up would be 5 years and the minimum potential follow-up would be 2 years).

Based on the assumptions above, the estimated power for this design is summarized below for various significance levels and 65 patients/group. Power estimated from 500 simulations. The number of events required is estimated to be 107.

Alpha=.05, power ~ 89.4%
Alpha=.10, power ~ 93.8%
Alpha=.15, power ~ 94.8%
Alpha=.20, power ~ 96.4%

17.4 **Randomization**

Subject randomization will be 1:1 and will be done using a permuted-block routine with random block sizes. Randomization will be stratified on two factors: number of chemotherapy cycles (1 vs. 2 courses pre-HCT) and disease risk (standard risk vs. high risk, where high-risk disease is defined based on ELN or being in CRi).

17.5 **Study-suspension Rules and Interim Futility Analysis**

As noted above in section 11, the CPX arm will be followed carefully for toxicity and the inability for patients treated with CPX to proceed to HCT or be delayed to HCT (defined as start of transplant conditioning regimen more than 60 days following the start of CPX; inability to receive HCT or a delayed HCT is defined below as "failure") due to toxicity attributable to CPX. Toxicity attributable to CPX will be defined as any adverse event that is possibly, probably, or definitely related to CPX. Events that do not meet this definition but result in the delay of HCT or inability to proceed to HCT (e.g.

treating clinic logistics, scheduling, donor availability, unrelated adverse events) will not count toward study-suspension rules but will be captured as protocol deviations in the study records. In situations where HCT is delayed or patient is unable to proceed due to toxicity and attribution to CPX is unclear, the DSMB will be consulted.

If toxicity from CPX leads to an observed failure rate of 20% or more, the study will be suspended pending review by the DSMB. This assessment will be made after every 5th enrolled patient, so any of the following would trigger this suspension rule: 1 of 5 or fewer, 2 of 10 or fewer, 3 of 15 or fewer, 4 of 20 or fewer, 5 of 25 or fewer, 6 of 30 or fewer, 7 of 35 or fewer, 8 of 40 or fewer, 9 of 45 or fewer, 10 of 50 or fewer, 11 of 55 or fewer, 12 of 60 or fewer, or 13 of 65 or fewer patients experience a failure.

After 65 patients have been enrolled and minimum follow-up reaches 6 months, the conditional power (given results up to this point) to reach a statistically significant difference at the end of the study will be calculated under the assumptions (for patients to be enrolled in the future) used in the initial power calculations. If this conditional power is less than 20%, consideration will be given to terminating the study due to futility. Other considerations will go into this decision (not just the conditional power) and presented to the DSMB, but this will be a large driver of the ultimate decision. Accrual to the study will not be suspended while the interim anlaysis is being conducted.

Projected Target Accrual
ETHNIC AND GENDER DISTRIBUTION CHART

TARGETED / PLANNED ENROLLMENT: 130			
Ethnic Category	Sex / Gender		
	Females	Males	Total
Hispanic or Latino	1	3	4
Not Hispanic or Latino	55	71	126
Ethnic Category Total of All Subjects*	56	74	130
Racial Categories			
American Indian / Alaska Native	1	3	4
Asian	5	3	8
Native Hawaiian or Other Pacific	0	0	0
Black or African American	1	2	3
White	42	58	100
More Than One Race	7	8	15
Racial Categories: Total of All Subjects*	56	74	130

18.0 INVESTIGATOR OBLIGATIONS

The PI is responsible for the conduct of the clinical trial at the site and is responsible for personally overseeing the treatment of all study subjects. The PI must assure that all study site personnel, including sub-Investigators and other study staff members, adhere to the study protocol and to all applicable regulations and guidelines regarding clinical trials both during and after study completion.

All subjects are informed of the nature of the program, its possible hazards, and their right to withdraw at any time, and each subject signs a form indicating their consent to participate prior to receiving any study-related procedures.

19.0 ADMINISTRATIVE AND REGULATORY CONSIDERATIONS

19.1 **Documentation**

The documentation of clinical data must be stored by the Sponsor according to legal requirements. The PI and study staff has responsibility for maintaining a comprehensive and centralized filing system containing all study-related documentation. These files must be suitable for inspection by the Sponsor, the FDA, and/or other applicable regulatory agencies/competent authorities at any time, and should consist of the following elements: subject files (complete medical records, laboratory data, supporting source documentation, and the Informed Consent); study files (the protocol with all amendments, copies of all pre-study documentation, and all correspondence between the Competent Authorities, IRB/EC, site, and Sponsor); and drug accountability files, containing a complete account of the receipt and disposition of the study drug.

19.2 **Data Collection**

Research data will be recorded in a study-specific, password protected database using a unique study ID for each patient to assure patient confidentiality. Data from source documents will be transcribed into this database. Source documents are documents where patient data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, quality of life assessments, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, X-rays, patient files, and records kept at the pharmacy, laboratories, and medico-technical departments involved in the clinical trial. There will be no case reports forms (CRFs) used for this trial.

19.3 **Protocol Interpretation and Compliance**

The procedures defined in the protocol are carefully reviewed by the PI, co-investigators and study staff prior to the time of study initiation to ensure accurate representation and implementation. Protocol amendments, if any, are reviewed and implemented promptly following IRB/EC and relevant Competent Authorities approval.

19.4 **Disclosure of Data/Publication**

The results of this clinical trial may be used for public dissemination in the form of papers, abstracts, posters, or other informational materials to be presented at scientific meetings, or published in professional journals, or as a part of an academic thesis by an investigator. Identifiable patient data may not be used for any of these presentations, manuscripts, or reports unless directed by law.

19.5 **Ethical Considerations**

Each named Investigator agrees to conduct this study in accordance with applicable United States clinical trial regulations and guidelines, the ICH (E6) GCP guidelines, the IRB/EC and local legal requirements and with the Declaration of Helsinki (1989). The Investigator will conduct all aspects of this study in accordance with all national, state, and local laws of the applicable regulatory agencies.

19.6 **Informed Consent**

Per local institutional policies/guidelines, the PI, sub-Investigators and qualified designees assume the responsibility of obtaining written Informed Consent for each subject or the subject's legally authorized representative before any study-specific procedures are performed. Subjects meeting the criteria set forth in the protocol will be offered the opportunity to participate in the study. To avoid introduction of bias, the Investigator must exercise no selectivity with regard to offering eligible subjects the opportunity to participate in the study.

Subjects or legal guardians of all candidate subjects will receive a comprehensive explanation of the proposed treatment, including the nature of the therapy, alternative therapies available, any known previously experienced adverse reactions, the investigational status of the study drug, and other factors that are part of obtaining a proper Informed Consent. Subjects will be given the opportunity to ask questions concerning the study, and adequate time to consider their decision to or not to participate. Informed Consent will be documented by the use of a written Consent Form that includes all the elements required by FDA regulations and ICH guidelines. The form is to be signed and dated by the subject and by the person who administers the consent process. The original signed Consent Form will be filed in the research record. One copy of the signed form will be given to the patient; another copy will be filed in the patient's medical record.

If an amendment to the protocol changes the subject participation schedule in scope or activity, or increases the potential risk to the subject, the Informed Consent Form must be amended. The revised Informed Consent Form must be used to obtain re-consent from any subjects currently enrolled in the study if the subject is affected by the amendment (as determined by the PI or designee) and must be used to document consent from any new subjects enrolled after the approval date of the amendment.

19.7 **Institutional Review Board/Ethics Committee**

The PI will assure that an appropriately constituted IRB/EC that complies with the requirements of 21 CFR Section 56 or written assurance of compliance with ICH (E6) guidelines will be responsible for the initial and continuing review and approval of the clinical study. Before initiation of the study, the PI or designee will forward copies of the protocol and Consent Form to be used for the study to the IRB/EC for its review and approval. The PI or designee will also assure that all changes in the research activity and all unanticipated problems involving risks to human subjects or others will be reported promptly to the IRB/EC, and that no changes will be made to the protocol without prior IRB/EC approval, except where necessary to eliminate apparent immediate hazards to human subjects. The Investigator or designee will be responsible for submitting periodic progress reports to the IRB/EC at intervals appropriate to the degree of subject risk involved in the study, but not less than once per year and at the completion or termination of the study.

19.8 **Subject Privacy**

The Sponsor and the Investigator affirm and uphold the principle of the subject's right to privacy. The Sponsor, it's designates, and the Investigator shall comply with applicable national and local privacy laws.

To verify compliance with this protocol, the Sponsor, or its designee, will require that the Investigator permit the Sponsor, or its designee's monitor to review the subject's original medical records. Should access to such medical records require a waiver or authorization separate from the statement of Informed Consent, the Investigator will obtain such permission in writing from the subject before the subject is entered into the study.

20.0 STOPPING THE STUDY

The Principal Investigator reserves the right to terminate this study at any time.

21.0 REFERENCES

Appelbaum, J., Loeb, A., Gardner, K., Shaw, C. M., Hendrie, P. C., Godwin, C. D., . . . Estey, E. (2019). *Additional Cytotoxic Chemotherapy Is Unlikely to Eliminate Measurable Residual Acute Myeloid Leukemia (AML)*. Paper presented at the 61st Annual American Society of Hematology Meeting, Orlando, FL.

Araki, D., Wood, B. L., Othus, M., Radich, J. P., Halpern, A. B., Zhou, Y., . . . Walter, R. B. (2016). Allogeneic Hematopoietic Cell Transplantation for Acute Myeloid Leukemia: Time to Move Toward a Minimal Residual Disease-Based Definition of Complete Remission? *J Clin Oncol, 34*(4), 329-336. doi:10.1200/JCO.2015.63.3826

Chen, X., Xie, H., Wood, B. L., Walter, R. B., Pagel, J. M., Becker, P. S., . . . Estey, E. H. (2015). Relation of clinical response and minimal residual disease and their prognostic impact on outcome in acute myeloid leukemia. *J Clin Oncol, 33*(11), 1258-1264. doi:10.1200/JCO.2014.58.3518

Chen, Y., Cortes, J., Estrov, Z., Faderl, S., Qiao, W., Abruzzo, L., . . . Ravandi, F. (2011). Persistence of cytogenetic abnormalities at complete remission after induction in patients with acute myeloid leukemia: prognostic significance and the potential role of allogeneic stem-cell transplantation. *J Clin Oncol, 29*(18), 2507-2513. doi:10.1200/JCO.2010.34.2873

Döhner, H., Estey, E., Grimwade, D., Amadori, S., Appelbaum, F. R., Büchner, T., . . . Bloomfield, C. D. (2017). Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood, 129*(4), 424-447. doi:10.1182/blood-2016-08-733196

Fang, M., Storer, B., Wood, B., Gyurkocza, B., Sandmaier, B. M., & Appelbaum, F. R. (2012). Prognostic impact of discordant results from cytogenetics and flow cytometry in patients with acute myeloid leukemia undergoing hematopoietic cell transplantation. *Cancer, 118*(9), 2411-2419. doi:10.1002/cncr.26539

Freeman, S. D., Virgo, P., Couzens, S., Grimwade, D., Russell, N., Hills, R. K., & Burnett, A. K. (2013). Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. *J Clin Oncol, 31*(32), 4123-4131. doi:10.1200/JCO.2013.49.1753

Grimwade, D., & Freeman, S. D. (2014). Defining minimal residual disease in acute myeloid leukemia: which platforms are ready for "prime time"? *Blood, 124*(23), 3345-3355. doi:10.1182/blood-2014-05-577593

Jongen-Lavrencic, M., Grob, T., Hanekamp, D., Kavelaars, F. G., Al Hinai, A., Zeilemaker, A., . . . Valk, P. J. M. (2018). Molecular Minimal Residual Disease in Acute Myeloid Leukemia. *N Engl J Med, 378*(13), 1189-1199. doi:10.1056/NEJMoa1716863

Lancet, J. E., Uy, G. L., Cortes, J. E., Newell, L. F., Lin, T. L., Ritchie, E. K., . . . Medeiros, B. C. (2018). CPX-351 (cytarabine and daunorubicin) Liposome for Injection Versus Conventional Cytarabine Plus Daunorubicin in Older Patients With Newly Diagnosed Secondary Acute Myeloid Leukemia. *J Clin Oncol, 36*(26), 2684-2692. doi:10.1200/JCO.2017.77.6112

Lin, T. L., Cortes, J. E., Newell, L. F., Ritchie, E. K., Stuart, R. K., Strickland, S. A., . . . Uy, G. L. (2018). *The Impact of Hematopoietic Cell Transplantation on Survival: An Exploratory Analysis of a Phase 3 Study of CPX-351 Versus 7+3 in Older Patients with Newly Diagnosed, High-Risk/Secondary AML*. Paper presented at the 60th Annual American Society of Hematology Meeting, San Diego, CA.

Marcucci, G., Mrozek, K., Ruppert, A. S., Archer, K. J., Pettenati, M. J., Heerema, N. A., . . . Bloomfield, C. D. (2004). Abnormal cytogenetics at date of morphologic complete remission predicts short overall and disease-free survival, and higher relapse rate in adult acute myeloid leukemia: results from cancer and leukemia group B study 8461. *J Clin Oncol, 22*(12), 2410-2418. doi:10.1200/JCO.2004.03.023

Mortland, L., Alonzo, T. A., Walter, R. B., Gerbing, R. B., Mitra, A. K., Pollard, J. A., . . . Lamba, J. K. (2013). Clinical significance of CD33 nonsynonymous single-nucleotide polymorphisms in pediatric patients with acute myeloid leukemia

treated with gemtuzumab-ozogamicin-containing chemotherapy. *Clin Cancer Res, 19*(6), 1620-1627. doi:10.1158/1078-0432.CCR-12-3115

Othus, M., Wood, B. L., Stirewalt, D. L., Estey, E. H., Petersdorf, S. H., Appelbaum, F. R., . . . Walter, R. B. (2016). Effect of measurable ('minimal') residual disease (MRD) information on prediction of relapse and survival in adult acute myeloid leukemia. *Leukemia, 30*(10), 2080-2083. doi:10.1038/leu.2016.120

Percival, M. M., & Estey, E. H. (2019). Current treatment strategies for measurable residual disease in patients with acute myeloid leukemia. *Cancer, 125*(18), 3121-3130. doi:10.1002/cncr.32354

Rubnitz, J. E., Inaba, H., Dahl, G., Ribeiro, R. C., Bowman, W. P., Taub, J., . . . Campana, D. (2010). Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. *Lancet Oncol, 11*(6), 543-552. doi:10.1016/S1470-2045(10)70090-5

Schuurhuis, G. J., Heuser, M., Freeman, S., Bene, M. C., Buccisano, F., Cloos, J., . . . Ossenkoppele, G. J. (2018). Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood, 131*(12), 1275-1291. doi:10.1182/blood-2017-09-801498

Terwijn, M., van Putten, W. L., Kelder, A., van der Velden, V. H., Brooimans, R. A., Pabst, T., . . . Schuurhuis, G. J. (2013). High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from the HOVON/SAKK AML 42A study. *J Clin Oncol, 31*(31), 3889-3897. doi:10.1200/JCO.2012.45.9628

Walter, R. B., Gooley, T. A., Wood, B. L., Milano, F., Fang, M., Sorror, M. L., . . . Pagel, J. M. (2011). Impact of pretransplantation minimal residual disease, as detected by multiparametric flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. *J Clin Oncol, 29*(9), 1190-1197. doi:10.1200/JCO.2010.31.8121

Walter, R. B., Gyurkocza, B., Storer, B. E., Godwin, C. D., Pagel, J. M., Buckley, S. A., . . . Sandmaier, B. M. (2015). Comparison of minimal residual disease as outcome predictor for AML patients in first complete remission undergoing myeloablative or nonmyeloablative allogeneic hematopoietic cell transplantation. *Leukemia, 29*(1), 137-144. doi:10.1038/leu.2014.173

Walter, R. B., Othus, M., Borthakur, G., Ravandi, F., Cortes, J. E., Pierce, S. A., . . . Estey, E. H. (2011). Prediction of early death after induction therapy for newly diagnosed acute myeloid leukemia with pretreatment risk scores: a novel paradigm for treatment assignment. *J Clin Oncol, 29*(33), 4417-4423. doi:10.1200/JCO.2011.35.7525

22.0 APPENDICES

Appendix A: Karnofsky Performance Status Scale
Appendix B: Acute GVHD Grading Scale
Appendix C: Chronic Graft-Versus-Host Disease Grading
Appendix D: Potential adverse events associated or expected with hematopoietic cell transplantation

22.1 APPENDIX A: Karnofsky Performance Status Scale

General	Index	Specific Criteria
Able to carry on normal activity; no special care needed	100	Normal, no complaints, no evidence of disease
	90	Able to carry on normal activity, minor signs or symptoms of disease
	80	Normal activity with effort, some signs or symptoms of disease
Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed	70	Care for self, unable to carry on normal activity or to do work
	60	Requires occasional assistance from others but able to care for most needs
	50	Requires considerable assistance from others and frequent medical care
Unable to care for self, requires institutional or hospital care or equivalent; disease may be rapidly progressing	40	Disabled; requires special care and assistance
	30	Severely disabled, hospitalization indicated, death not imminent
	20	Very sick, hospitalization necessary, active supportive treatment necessary
	10	Moribund
	0	Dead

22.2 APPENDIX B: Acute GVHD Assessment

ACUTE GVHD ASSESSMENT

Staging by Individual Organ Involvement

SKIN: measured by rash first appearing generally between 10 and 70 days after transplant (excludes rashes of known viral or other origin)

Stage	Description
1	Maculopapular rash <25% BSA
2	Maculopapular rash 25 – 50% BSA
3	Generalized erythroderma
4	Generalized erythroderma with bullous formation and desquamation

LIVER*: measured by total serum bilirubin

Stage	Description
1	2.0 – 2.9 mg/dL
2	3.0 – 5.9 mg/dL
3	6.0 – 14.9 mg/dL
4	≥ 15.0 mg/dL

GUT:** includes only diarrhea occurring after Day +21

Score	Adult	Pediatric***
1	upper GI (anorexia, nausea, vomiting) with diarrhea of <1000 mL/day	upper GI (anorexia, nausea, vomiting) with diarrhea of <555 mL/m^2/day
2	1000 – 1499 mL/day diarrhea	556-833 mL/m^2/day diarrhea
3	≥ 1500 mL/day diarrhea	>833 mL/m^2/day diarrhea
4	severe abdominal cramping, bleeding or ileus caused by GVHD	

* In cases where another cause of hyperbilirubinemia antedated the onset of rash, the liver score should be decreased by one stage.

** In cases where peak GI symptoms are exacerbated by a cause other than GVHD, the gut score should be decreased by one stage.

*** Pediatric patients <17 years of age

APPENDIX B: Acute GVHD Assessment, continued

ACUTE GVHD ASSESSMENT

Overall Grade

The determination of an overall GVHD grade should be based on the organ stage, response to treatment and whether GVHD was a major cause of death.

Overall Grade	Organ Stage	Qualifying Conditions	Additional Qualifying Conditions
I	Stage 1 -2 skin	No liver or gut	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD.
II	Stage 3 skin or Stage 1 liver or Stage 1 gut	N/A	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD, but glucocorticoid treatment after the onset of GVHD was generally sufficient to control the disease.
III	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**without** GVHD as a major contributing cause of death	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD and that additional treatment after the onset of GVHD did not readily control the disease.
IV	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**with** GVHD as a major contributing cause of death	GVHD was resistant to both the prophylactic immunosuppressive regimen and any additional treatment after the onset of the disease.

References:

1. Leisenring, WM, Martin, PJ, Petersdorf, EW, et al. An acute graft-versus-host-disease activity index to predict survival after hematopoietic cell transplantation with myeloablative conditioning regimens, Blood, 2006:108: 749-755.
2. Przepiorka D, Weisdorf D, Martin PJ, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995:15: 825-828.

22.3 APPENDIX C: Chronic Graft-Versus-Host Disease Grading

Chronic graft-versus-host disease grading*

In all cases, concomitant processes (i.e. infections or drug reactions) must be ruled out. Karnofsky or Lansky Clinical Performance scores, 60%, > 15% weight loss, and recurrent infections are usually signs of clinical extensive chronic GVHD. Abnormalities that could indicate chronic GVHD are categorized by organ systems as listed below.

Skin	Erythema, dryness, pruritus, pigmentary changes (i.e. hyperpigmentation, vitiligo), mottling, papulosquamous plaques, nodules, exfoliation, macular-papular or urticarial rash, scleroderma, morphea (one or several circumscribed, indurated and shiny lesions)
Nails	Ridging, onychodystrophy, onycholysis
Hair	Premature graying, (scalp hair, eyelashes, eyebrows), thinning scalp hair, alopecia, decreased body hair
Mouth	Dryness, burning, gingivitis, mucositis, striae, atrophy, erythema, lichenoid changes, ulcers, labial atrophy or pigmentary changes, tooth decay, tightness around the mouth
Eyes	Dryness, burning, blurring, gritty eyes, photophobia, pain
Vagina/vulva	Dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not included
Liver	Elevated liver function tests not due to other causes (alkaline phosphatase $\geq$ 3x upper limit of normal, AST or ALT $\geq$4x upper limit of normal or total serum bilirubin $\geq$ 2.5; in the absence of chronic GVHD involving other organs, liver biopsy is required to confirm diagnosis)
Lung	Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis
GI	Anorexia, nausea, vomiting, weight loss, dysphasia, odynophagia, malabsorption
Fasciitis	Stiffness and tightness with restriction of movement, occasionally with swelling pain, cramping, erythema and induration, most commonly affecting forearms, wrists and hands, ankles, legs, and feet, inability to extend wrists without flexing the fingers or the elbows, contractures
Serositis	Chest pain or cardiopulmonary comprise due to pericarditis or pleuritis
Muscle	Proximal muscle weakness, cramping
Skeletal	Arthralgia of large proximal girdle joints and sometimes smaller joints

APPENDIX C: Chronic Graft-Versus-Host Disease Grading, continued

Laboratory testing and diagnostic indicators of chronic GVHD*

Eye	Schirmer's test with a mean value ≤ 5mm at 5 minutes, or symptomatic with values of 6-10mm or keratitis detected by slit lamp examination
Liver	Elevated liver function tests not due to other causes (see definition of clinical limited and extensive chronic GVHD)
Lung	New obstructive lung defect defined as FEV1 < 80% of predicted with either an FEF 25-75 <65% of predicted or RV >120% of predicted, or a decrease of FEV1/FVC by > 12% within a period of less than 1 year. A diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage and evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scans o the chest. A thoracoscopic lung biopsy may be necessary in order to confirm the diagnosis of bronchiolitis obliterans in patients who have obstructive lung disease without air trapping when chronic GVHD involving other organs is absent
Esophagus	Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry
Muscle	Elevated CPK or aldolase, EMG findings consistent with myositis
Blood	Thrombocytopenia (usually 20,000-100,000/μl), eosinophilia, hypogammaglobulinemia, hypergammaglobulinemia, and autoantibodies occur in some cases

* From Standard Practice Guidelines for "Chronic Graft-versus-Host Disease Classification at the time of presentation" developed by Long Term Follow-Up at the FHCRC

22.4 APPENDIX D: Potential adverse events associated or expected with hematopoietic cell transplantation

1. GVHD: GVHD is a major toxicity associated with the infusion of allogeneic donor stem cells. GVHD may be acute or chronic and may affect multiple organ systems, including the skin, liver, and GI tract.

2. Infections: Opportunistic infections, including viral and fungal infections, can result in severe pulmonary, neurologic, hepatic and other organ dysfunction, and possible death.

3. Gastrointestinal toxicity: Nausea and vomiting can be anticipated during the entire course of ablative therapy. Mucositis and diarrhea should be expected. Prednisone can cause GI bleeding.

4. Cardiac toxicity: Cardiotoxicity (congestive heart failure, pericardial effusion, EKG changes) is uncommonly associated with the chemotherapy agents and TBI used in the regimen and these sequelae may prove lethal.

5. Pulmonary toxicity: Diffuse interstitial pneumonitis of unknown etiology and diffuse alveolar hemorrhage occurs with some regularity after BMT and interstitial fibrosis occurs much more rarely. Both are well-described complications of intensive chemotherapy and TBI regimens and may prove lethal.

6. Hepatic toxicity: Veno-occlusive disease of the liver is a common toxicity of high-dose chemoradiotherapy and may result in death. Calcineurin inhibitors may cause elevation of ALT/AST.

7. Renal dysfunction: Chemoradiotherapy may uncommonly cause renal dysfunction. More commonly, nephrotoxicity results from calcineurin inhibitors and generally responds to dose reduction. Rarely, idiopathic or calcineurin inhibitor-associated hemolytic-uremic syndrome may occur and may be progressive and fatal. A syndrome of moderate renal insufficiency and hemolysis has been seen 5-7 months post HSCT after intensive multi-agent conditioning plus TBI.

8. Hemorrhagic cystitis: Hemorrhagic cystitis, manifested either as gross or microscopic hematuria, is a common toxicity after high-dose chemoradiotherapy, but usually associated with regimens that include cyclophosphamide. Hemorrhagic cystitis may predispose to a long-term increased risk of bladder cancer.

9. Central nervous system toxicity: Radiation and chemotherapy can cause CNS toxicity, including seizures, depressed mental status, or leukoencephalopathy. Calcineurin inhibitors can cause seizures or other CNS toxicity.

10. Marrow aplasia: Severe neutropenia, thrombocytopenia, and anemia, is expected to occur for a period of 7 to 42 days after the pre-transplant conditioning regimen. Transfusion of platelets and red blood cells is expected as supportive care. Transfusion of blood products may be associated with acquisition of HIV or a hepatitis virus. Neutropenia may increase the risk for acquiring serious infection. Thrombocytopenia may increase the risk of life-threatening hemorrhage. Hemorrhagic or infectious complications during the expected period of aplasia may result in death.

11. Miscellaneous: Alopecia and sterility are expected complications of the program as a whole. Cataract development is possible after TBI and/or steroids. Deficiencies of growth hormone, thyroid hormone, and sex hormones are possible after TBI. Calcineurin inhibitors can cause transient gingival hyperplasia, tremor, seizure, hypertension, headache, dysesthesia and hirsutism. Steroid therapy can also contribute to fluid retention, easy bruising, hypertension, aseptic necrosis of bone and increased susceptibility to infection. Hospitalization during conditioning and recovery period is expected to be 5-9 weeks in duration.