A Single-Center Phase 1/2 Study of Single- or Fractioned-Dose Gemtuzumab Ozogamicin in Combination with G-CSF, Cladribine, Cytarabine and Mitoxantrone (GCLAM) for Previously Untreated Adult Acute Myeloid Leukemia or High-Grade Myeloid Neoplasm

Short title: Gemtuzumab ozogamicin plus GCLAM for AML

NCT03531918

April 17, 2022

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UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

Current version: 11/10/2020
Previous version: 08/27/2019

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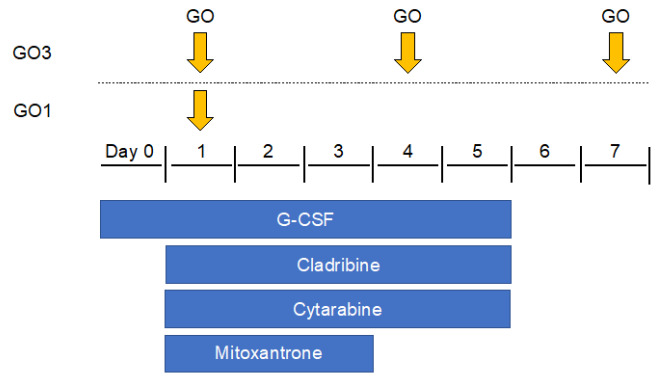
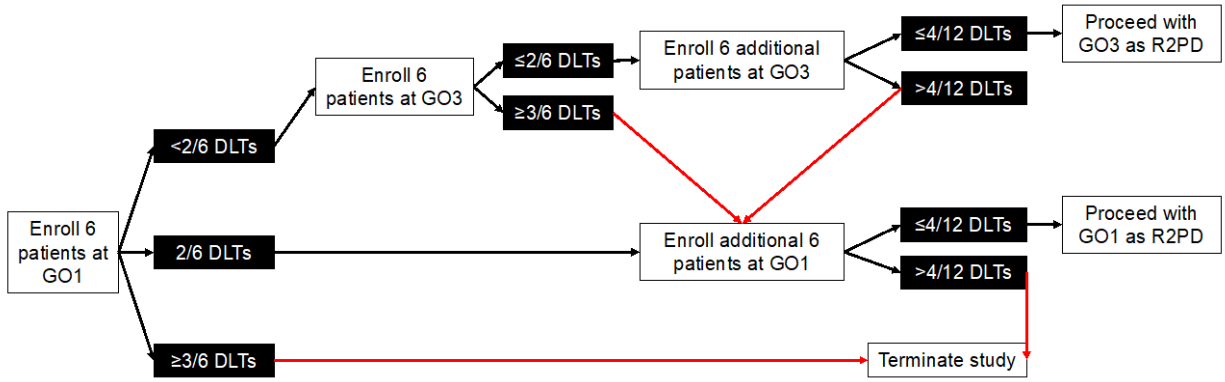
OVERVIEW OF THE TREATMENT PLAN



Abbreviations: G-CSF, granulocyte colony stimulating factor; GO gemtuzumab ozogamicin.

DOSE ESCALATION SCHEME (PHASE 1 PORTION)



Abbreviations: DLTs, dose-limiting toxicities; GO, gemtuzumab ozogamicin; GO1, single dose gemtuzumab ozogamicin; GO3, fractionated-dose ozogamicin; R2PD, recommended phase 2 dose.

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1.0 BACKGROUND AND RATIONALE

Despite some recent improvements in treatment, adult acute myeloid leukemia (AML) remains difficult-to-treat, with only gradual improvements over the last 3-4 decades.[1,2] Although many patients will achieve a complete remission (CR) with 1 or 2 courses of curative-intent, intensive induction chemotherapy, the majority will ultimately relapse, and few patients will be alive 2-5 years after diagnosis. Thus, the need for new, effective first-line therapies which are more effective at preventing later relapse is unquestioned.

1.1 GCLAM as intensive chemotherapy for adults with newly-diagnosed AML

Since 2012, we have used a combination of G-CSF, cladribine, high-dose cytarabine, and mitoxantrone (GCLAM) extensively in patients with newly-diagnosed AML. This use was motivated by data suggesting that GCLAM might be superior to other chemotherapies such as mitoxantrone, etoposide, and high-dose cytarabine (MEC) for relapsed disease.[3] In a large, institutional phase 1/2 trial (FH #2734; NCT02044796), we have established the safety of GCLAM with an escalated dose of mitoxantrone (18 mg/m^2 rather than 10 mg/m^2 as used in the original GCLAM regimen).[4] Between June 2014 and March 2017, we enrolled 121 medically-fit patients with newly-diagnosed AML with a median age of 60 (range: 21-81) years on this trial. Because all dose levels were well tolerated, mitoxantrone at 18mg/m^2, the highest dose, was declared the recommended phase 2 dose (RP2D). Among 88 patients treated at the RP2D, 72 achieved a complete remission (CR; 82%, 66/72 [92%] without measurable residual disease [MRD]) and 6 achieved a CR with incomplete count recovery (CRi; 7%, 5 without MRD) for a CR/CRi rate of 78/88 (89%). 4- and 8-week mortality was 2% and 3%. After multivariable adjustment, the MRD^{neg} CR rate and CR/CRi rate compared favorably to 97 matched historical controls at our center treated with 7+3 (odds ratio [OR]=4.40, p<0.001, and OR=3.86, p<0.01) as well as to 300 patients treated with 7+3 on a cooperative group trial.[4] Together, our trial found GCLAM with mitoxantrone up to 18 mg/m^2/day to be safe and effective in inducing high-quality remissions (MRD^{neg} CR) in adults with newly-diagnosed AML. These results have established GCLAM as our institutional standard for upfront treatment of AML.

1.2 Gemtuzumab ozogamicin (GO) for the treatment of adults with AML

Building on our experience with GCLAM for previously untreated AML, our goal is to further improve outcomes with this chemotherapy backbone by integrating the CD33 antibody-drug conjugate gemtuzumab ozogamicin (GO). As demonstrated in several randomized trials, GO reduces relapse risks and prolongs survival in adults with newly-diagnosed AML when added to intensive chemotherapy (even though remission rates are unchanged), firmly establishing its value in medically fit adults with previously untreated AML.[5,6] An initial meta-analysis published in 2014 has indicated the overall survival benefit is most pronounced in patients with favorable-risk disease but is, to a less degree, also seen in intermediate-risk disease whereas no benefit was found in adverse-risk disease.[6] While confirming these results, extended updated analyses showed a statistically-significant benefit of GO with regard to relapse-risk, event-free survival, and relapse-free survival across all cytogenetic risk groups with essentially identical benefit in intermediate and adverse-risk disease (R.K. Hills; personal communication). Consistent with data from the meta-analysis, a randomized trial found that GO doses of 3 mg/m^2 were less toxic (as estimated by 30- and 60-day mortality and rate of SOS/VOD) than doses of 6 mg/m^2 but were equally effective.[7] While these data suggest that the optimal dose might be 3 mg/m^2, there remains uncertainty as to the optimal dosing schedule of GO, with some data

provided by the meta-analysis suggesting that fractionated dosing, i.e. administration of GO 3 mg/m^2 on days 1, 4, and 7 – a dosing schedule that takes advantage of re-expression of CD33 binding sites 48-72 hours after antibody exposure – may have substantially better anti-leukemia activity than a single dose without increase in toxicity, but no controlled study has so far compared 1 vs. 3 doses of GO at 3 mg/m^2.[6]

1.3 Rationale for combining GCLAM with GO for the treatment of newly-diagnosed AML

Benefit of GO in combination with intensive induction chemotherapy has been demonstrated with several chemotherapy backbones, including 7+3 and FLAG-Ida.[5] However, GO has so far not been combined with GCLAM. We therefore conduct a phase 1/2 trial to determine the feasibility, and estimate the efficacy, of integrating one or three (i.e "fractionated") doses of GO in the GCLAM regimen. This trial is built on the following considerations/hypotheses:

- Data from randomized trials indicate that a single dose of GO of 3 mg/m^2 is tolerated without significant increase in toxicities when added to intensive chemotherapy, whereas a single dose of 6 mg/m^2 adds toxicity without treatment benefit beyond what is obtained with 3 mg/m^2.[5-7] The data further suggest that fractionated dosing with GO 3 mg/m^2 on days 1, 4, and 7 may have superior efficacy than a single dose of 3 mg/m^2 albeit at the expense of greater hematologic (and possibly non-hematologic) toxicities.[6] This trial will therefore determine the safety of adding 1 dose or 3 fractionated doses of GO 3 mg/m^2 to intensive chemotherapy. Since a single dose is likely well tolerated, our study will only enroll 6 patients at single dose GO (GO1) but cohorts of 6+6 patients when 3 doses are given (GO3). Once the recommended phase 2 dose (RP2D) is determined, the treatment cohort will then be expanded to a total of 60 patients. Preference for this approach over the classic 3+3 dose escalation scheme is given to have greater ability to characterize toxicity profiles at different GO dose levels, better estimate the relationship between GO dose and duration of cytopenias and have a higher chance of capturing less common but clinically significant toxicities.
- When added to intensive induction chemotherapy, GO improves outcomes even though the specific chemotherapy backbone varied significantly between individual trials.[5,6] Thus, though the combination of GCLAM and GO is novel, we hypothesize that GO will provide similar incremental benefit over GCLAM alone. The substantial number of newly-diagnosed AML patients treated on protocol #2734 provides a large "historical" cohort of patients treated with GCLAM alone.[4] This control cohort will allow us to accurately estimate the benefit of adding GO to GCLAM chemotherapy.
- In prior trials investigating the use of GO, addition of GO had little to no impact on early endpoints such as remission rates, and changes in remission rates did not consistently translate to changes in survival, arguing against using remission rates as a primary endpoint. Instead, we will use 6-month event-free survival (EFS) to compare the outcomes of patients treated on our trial with patients treated previously at our institution with GCLAM. In protocol #2734, EFS at 6 months (68%) was similar to 1-year EFS (57%), supporting the use of this earlier endpoint for comparison of patients treated with GO + GCLAM and GCLAM alone.[4] A total of 60 patients treated with GO + GCLAM in the current protocol will allow for 80% power to detect an improvement in 6-month EFS to 80%. Subsequent analysis of protocol #2734 with longer follow-up demonstrated a revised 1-year EFS estimate of 60%. Given the late emerging benefits in patients treated

with GO in randomized clinical trials compared to patients not treated with GO,[6] we will also include 1-year EFS as a second primary endpoint.

- Study eligibility will be based on explicit criteria for medical fitness, using a treatment-related mortality (TRM score) that we developed in over 2,200 patients with AML,[8] and now widely use in our clinical trials.
- We will ask patients to agree to collection of peripheral blood and bone marrow specimens to be used in future laboratory assays to study biologic predictors of response to GO therapy. The collection is voluntary, and declining will not affect a patient's participation in this study. Some of these potential biomarker assays may be centered around CD33 expression levels, presence of CD33 splice isoforms that reduce GO efficacy, and other genes which determine response to the calicheamicin toxin component of GO.[5]

1.4 Interim data update: phase 1 experience with GCLAM/GO

Between 9/1/2018 and 5/29/2019, the first 18 patients, median age 66 (range: 28-77) years, median TRM score 3.92 (range: 0.14-10.3) with newly-diagnosed AML (n=14) or other high-grade myeloid neoplasm (n=4) were enrolled. Among these, 7 were "favorable", 4 "intermediate and 7 "adverse" by 2017 European LeukemiaNet (ELN) criteria. The first 6 patients were treated at GO1. Because there was only 1 DLT (left ventricular systolic dysfunction) the subsequent 12 patients were treated at GO3. Three dose-limiting toxicities (DLTs) occurred at this second dose level (aminotransferase level increase, posterior reversible encephalopathy syndrome, treatment delay by >14 days). Since ≤4/12 DLTs were observed, the MTD was not reached and GO3 was declared the recommended phase 2 dose. Among 18 evaluable patients, 13 achieved CR and 2 CRi for a CR/CRi rate of 83% (95% confidence interval: 59-96%). 13 of these 15 CR/CRi patients were negative for MRD by MFC and cytogenetics, for an MRDneg CR/CRi rate of 72% (49-88%). The 3 patients not achieving CR/CRi had marrow aplasia without MFC evidence of AML when they went off study. Two underwent allogeneic hematopoietic cell transplantation in aplasia or MLFS; the other received no further therapy, with disease recurrence 2.5 months following induction therapy. 4/18 patients did not have platelet recovery to 50,000/μL prior to the second cycle of therapy. Among the 14 patients with hematopoietic recovery, median times to an absolute neutrophil count of 500/μL and platelet count of 50,000/μL were 30.5 (range: 22-42) days and 28.5 (range: 24-41) days, respectively. Besides infections and neutropenic fever, hypertension and left ventricular systolic dysfunction were the most common adverse events. One patient had liver toxicity not meeting typical clinical criteria for sinusoidal obstructive syndrome. There were no deaths within 28 or 56 days of starting induction in this study cohort.

2.0 OBJECTIVES

2.1 Primary objectives

2.1.1 Phase 1 objective: to determine the maximum-tolerated dose (MTD) of GO when added to GCLAM in patients with newly-diagnosed AML requiring induction chemotherapy.

2.1.2 Phase 2 objective: to evaluate the 6-month and 1-year event-free survival (EFS) rate with GO + GCLAM treated at the MTD.

2.2 Secondary objectives

2.2.1 Describe, within the limits of a phase 1/2 study, the toxicity profile of the study regimen.

2.2.2 Compare, within the limits of a phase 1/2 study, measurable residual disease (MRD) rates with GO + GCLAM at the MTD to patients treated previously with GCLAM alone.

2.2.3 Estimate, within the limits of a phase 1/2 study, the relationship between MRD status after induction therapy and relapse risk/time to relapse as well as relapse-free and overall survival.

2.2.4 Compare, within the limits of a phase 1/2 study, complete remission rates with GO + GCLAM at the MTD to patients treated previously with GCLAM alone.

2.2.5 Compare, within the limits of a phase 1/2 study, overall survival rates with GO + GCLAM at the MTD to patients treated previously with GCLAM alone

2.2.6 Evaluate, within the limits of a phase 1/2 study, the impact of GO dosing regimens on the duration of cytopenias.

2.2.7 Collect biological specimens for use for the future laboratory investigation of biomarkers for response to GO.

3.0 PATIENT ELIGIBILITY

3.1. Inclusion criteria

3.1.1 Age ≥18 years

3.1.2 Diagnosis of untreated "high-grade" myeloid neoplasm (≥10% blasts in blood or bone marrow) or AML other than acute promyelocytic leukemia (APL) with t(15;17)(q22;q12) or variants according to the 2016 WHO classification.[9] Outside diagnostic material is acceptable to establish diagnosis; submission of peripheral blood specimen for flow cytometry performed at the study institution should be considered. Diagnostic material must have been submitted for cytogenetic and/or molecular testing as clinically appropriate.

3.1.3 Medically fit, as defined by treatment-related mortality (TRM) score ≤13.1 calculated with simplified model.[8] (see **Appendix A**)

3.1.4 The use of hydroxyurea prior to study registration is allowed. Patients with symptoms/signs of hyperleukocytosis, WBC >100,000/μL or with concern for other complications of high tumor burden (e.g. disseminated intravascular coagulation) can be treated with leukapheresis or may receive up to 2 doses of cytarabine (up to 500 mg/m^2/dose) prior to enrollment.

3.1.5 Patients may have received low-intensity treatment (e.g. azacitidine/decitabine, lenalidomide, growth factors) for antecedent low-grade myeloid neoplasm (i.e. <10% blasts in blood and bone marrow).

3.1.6 Adequate organ function.

3.1.6.1 Bilirubin ≤2.5 x Institutional Upper Limit of Normal (IULN) unless elevation is thought to be due to hepatic infiltration by AML, Gilbert's syndrome, or hemolysis (assessed within 14 days prior to study day 0).
3.1.6.2 Serum creatinine ≤2.0 mg/dL (assessed within 14 days prior to study day 0).
3.1.6.3 Left ventricular ejection fraction ≥45%, assessed within 12 months prior to study day 0, e.g. by MUGA scan or echocardiography, or other appropriate diagnostic modality and no clinical evidence of congestive heart failure.

3.1.7 Women of childbearing potential and men must agree to use adequate contraception.

3.1.8 Provide written informed consent.

3.2 Exclusion criteria

3.2.1 Myeloid blast crisis of chronic myeloid leukemia (CML), unless patient is not considered candidate for tyrosine kinase inhibitor treatment.

3.2.2 Concomitant illness associated with a likely survival of <1 year.

3.2.3 Active systemic fungal, bacterial, viral, or other infection, unless disease is under treatment with anti-microbials, and/or controlled or stable (e.g. if specific, effective therapy is not available/feasible or desired [e.g. known chronic viral hepatitis, HIV]). Patient needs to be clinically stable as defined as being afebrile and hemodynamically stable for 24 hours. Patients with fever thought to be likely secondary to leukemia are eligible.

3.2.4 Known hypersensitivity to any study drug.

3.2.5 Confirmed or suspected pregnancy or active breast feeding.

3.2.6 Treatment with any other investigational anti-leukemia agent. In phase 2, treatment with a tyrosine kinase inhibitor for patients with FLT3-mutated AML is permissible.

4.0 EVALUATION AND COUNSELING OF PATIENT

The patient will be completely evaluated with a history, physical examination, diagnostic testing if necessary, and review of outside slides and records if available. The protocol will be discussed thoroughly with the patient and family (if present), with description of all known risks to the patient. Alternative forms of treatment will be presented objectively, and the risks and hazards of the study explained to the patient. Consent will be obtained using forms approved by the Institutional Review Board (IRB).

5.0 PROTOCOL REGISTRATION

To register, the provider(s) involved in the care of the potential study participant must contact a Study Investigator or the Study Coordinator and fax the Research Subject Registration Form (**Appendix B**) to the study team ([redacted]). For registration, a completed Research Subject Registration Form including completed eligibility checklist (**Appendix B**), a

copy of the signed consent form, and a signed HIPAA authorization must be available, and all eligibility requirements according to section 3.0 must be met. To complete the registration process, the Principal Investigator or his designee will assign a patient study number and register the patient on the study.

6.0 TREATMENT PLAN

This study is an open-label, Phase 1/2 dose escalation trial to attempt to estimate the maximum tolerated dose (MTD) of GO when used concomitantly with GCLAM in patients with newly-diagnosed AML or other high-grade myeloid neoplasm. Bone marrows will be reassessed upon blood count recovery or between day Days +25 to +31 after start of chemotherapy, whichever occurs first. If an MRD-negative CR is not achieved with the first cycle of chemotherapy (a response associated with particularly favorable outcome in a recent institutional study[10,11]), patients are eligible for re-induction with GCLAM without GO provided all non-hematologic toxicities have resolved to grade <2. Patients achieving a CR or CRi with 1-2 courses of induction therapy can receive post-remission ("consolidation") therapy with GCLA (i.e. mitoxantrone omitted) for one cycle and high-dose cytarabine (HiDAC) for 2 additional cycles. No GO will be given during post-remission therapy.

6.1 Baseline/pre-treatment assessment

The following studies should be obtained at baseline before initiation of study therapy to establish trial eligibility and allow patient characterization and disease prognostication:

6.1.1 History and physical examination (assessed within 14 days prior to study day 0).

6.1.2 Bone marrow examination with morphologic and flow cytometric assessment, routine cytogenetic analysis, and molecular testing (*e.g.* FLT3/ITD, NPM1, CEBPA) as appropriate; a bone marrow biopsy should be obtained if spicules are absent from the aspirate sample. Bone marrow examination is not required if there are immunophenotypically malignant blasts comprising ≥10% of total white blood cells in the peripheral blood or pathologically-confirmed extramedullary disease as per International Working Group recommendations[2,12] (assessed up to 2 months prior to study day 0). If diagnosis is based on outside diagnostic material, submission of peripheral blood specimen for flow cytometric assessment at UW/SCCA should be considered to establish leukemia-associated immunophenotype.

6.1.2 Complete blood counts with differential blood count, including immature cells/blasts; platelet count (assessed within 14 days prior to study day 0).

6.1.3 Metabolic panel, including bilirubin, albumin, and creatinine (assessed within 14 days prior to study day 0).

6.1.4 MUGA scan or echocardiography, or other appropriate diagnostic modality to assess left ventricular ejection fraction (LVEF; assessed within 12 months prior to study day 0).

6.2 Pre-treatment

At the discretion of the treating physician, allopurinol may be considered in all patients without known allergies to allopurinol to reduce the risk of tumor lysis. Patients may receive rasburicase, a recombinant urate oxidase, for the prevention and/or treatment of tumor lysis syndrome at the discretion of the treating physician. All patients should be adequately hydrated and receive anti-emetics as necessary.

6.3 Administration of GO

6.3.1 Patients will receive GO at 1 of two schedules: a single dose of 3 mg/m^2 on Day 1 (GO1) or three doses of 3 mg/m^2 on Days 1, 4, 7 (GO3), not to exceed 4.5 mg per dose as per 6.3.2. If GO1 is found to be too toxic as per section 16, addition of GO to GCLAM will be considered not feasible, and the study will be terminated.

6.3.2 The dose of GO is calculated using the patient's actual weight but will be capped at 4.5 mg per dose (equivalent to 1 vial of GO).[13]

6.3.3 The following medications should be used before GO administration as per the package label: acetaminophen, benadryl, and/or solumedrol or dexamethasone. Demerol may be given if the patient experiences severe chills. GO should be infused as per the package label (over approximately 2 hours).

6.3.4 All treatment is given as intent-to-treat; missed doses will not be made up.

6.4 Administration of GCLAM

6.4.1 The doses of the individual elements of GCLAM chemotherapy will be as follows: G-CSF 300 or 480 μg (based on actual weight: <76 kg vs. ≥76 kg) subcutaneously daily on Days 0-5; cladribine 5 mg/m^2 daily IV over 2 hours on Days 1-5; cytarabine 2,000 mg/m^2 daily IV over 2 hours on Days 1-5; and mitoxantrone 18 mg/m^2 daily IV on Days 1-3. Note that we refer to the first day of GCLAM chemotherapy as Day +1 for this regimen. Note that day 0 G-CSF is often given as outpatient and the remainder of therapy is given as inpatient. Thus, a >24 hour delay between day 0 G-CSF and the start of day 1 therapy is allowed.

6.4.2 The doses of G-CSF, cladribine, cytarabine, and mitoxantrone are calculated using the patient's actual weight as recorded in the electronic medical record.

6.4.3 If WBC >20,000/μL, or if the patient has recently required cytoreductive therapy with cytarabine and/or hydroxyurea, Day 0 and Day 1 G-CSF may be omitted at provider discretion.

6.4.4 Administration in the outpatient clinic can be considered but should be discussed with the study investigators.

6.4.5 All treatment is given as intent-to-treat; missed doses will not be made up.

6.4.6 For phase 2 only: for patients with FLT3 internal tandem duplication mutations (FLT3-ITD) or FLT3 tyrosine kinase domain (FLT3-TKD) mutations, use of clinically-available tyrosine kinase inhibitors such as midostaurin or sorafenib on published schedules[14-16] is allowed.

6.4.7 No investigational or commercial agents or therapies other than those described herein may be administered with the intent to treat the patient's malignancy.

6.5 Monitoring during/after induction therapy

For patient monitoring, the following assessments and study intervals are suggested:

6.5.1 Complete blood counts with differential blood count, including immature cells/blasts, and platelet count at least 3 times weekly until ANC >500/µL and self-sustained platelet count >20,000/µL.

6.5.2 Metabolic panel, including electrolytes (Na, K), bilirubin, ALT/AST, and creatinine at least weekly until ANC >500/µL and self-sustained platelet count >20,000/µL.

6.5.3 If patients develop signs or symptoms suggestive of cardiac dysfunction, LVEF should be assessed using the same method to evaluate baseline LVEF status (MUGA scan or echocardiography, or another appropriate diagnostic modality).

6.6 Assessment for response after first induction course

A bone marrow aspirate should be obtained upon blood count recovery (i.e. ANC >1,000/µL and platelet count >100,000/µL) or between Days +25 to +35 after start of GCLAM chemotherapy, whichever occurs first. A bone marrow biopsy should be obtained if spicules are absent from the aspirate sample. In patients with unclear response status, the bone marrow examination should be repeated every 7-10 days until the response can be assessed or until Day +42. Responses are defined in section 8.1.

6.6.1 Achievement of MRD-negative CR: Patients are eligible for consolidation chemotherapy, as described in section 6.8.

6.6.2 Response other than MRD-negative CR: patients are eligible for a second course of induction chemotherapy provided all non-hematologic toxicities have resolved to Grade <2. Treatment will be with GCLAM (and possibly tyrosine kinase inhibitor as per section 6.4.6) at identical doses as given during the first cycle of chemotherapy. No GO will be given for re-induction. For patients who experienced ≥Grade 3 non-hematologic toxicities during the first induction, a dose reduction is recommended as described in section 6.7.

6.6.3 Persistent severe cytopenias (as defined in section 8.1) without evidence of disease after Day +42: Patients will be removed from the treatment portion of the protocol.

6.7 Dose modifications of chemotherapeutic drugs for subsequent treatment cycles

The following dose modifications are suggested for subsequent treatment cycles:

6.7.1 If a patient develops Grade ≥3 non-hematologic toxicity, other than Grade 3 infections, within 21 days from the last dose of GCLAM, subsequent doses of cladribine and cytarabine will be reduced by 25% and dose of mitoxantrone will drop by 2 mg/m^2.

The patient may receive future cycles using this dose-reduced regimen if he/she demonstrated potential response and once the non-hematologic toxicity has resolved to a level of Grade ≤2.

If a patient develops Grade ≥3 non-hematologic toxicity, other than Grade 3 infection, within 21 days from the last dose of the dose-reduced regimen, the dose of the cladribine and cytarabine for subsequent cycles will be reduced by 50% and the dose of mitoxantrone will drop by 4 mg/m^2.

The patient may receive future cycles using the new dose-reduced regimen, if the patient demonstrated potential response and once the non-hematologic toxicity has resolved to a level of < Grade 2. If, however, at any time, a patient displays Grade ≥3 non-hematologic toxicity other than Grade 3 infections while receiving the new dose-reduced regimen, the patient will not be eligible for additional therapy as part of the protocol. If patients require treatment interruption of > 21 days from the planned start of the next cycle, the patient will not be eligible for additional therapy on this study.

6.7.2 Mitoxantrone: Consider dose reduction of mitoxantrone by 50% if the bilirubin concentration is 1.5-4.5 x IULN. The dose of mitoxantrone will be reduced to 25% if the bilirubin concentration is >4.5 x IULN.

6.7.3 Cladribine: If the serum creatinine exceeds 2.0 mg/dL and/or estimated creatinine clearance (calculated by Cockcroft-Gault) decreases to less than 50 mL/min during therapy, we will consider dose reduction in discussion with the Oncology Pharmacist.

6.7.4 Cytarabine: If the serum creatinine exceeds 2.0 mg/dL and/or estimated creatinine clearance (calculated by Cockcroft-Gault) decreases significantly during therapy, dose reduction will be considered in discussion with the Oncology Pharmacist and/or attending physician.

6.8 Post-remission therapy

After a maximum of 2 cycles of induction therapy, patients are eligible for post-remission therapy if CR/CRi is achieved by the end of induction.

6.8.1 Patients can receive up to 3 courses of post-remission therapy. No GO will be given during consolidation.

6.8.1.1 The first cycle of post-remission treatment is similar to GCLAM given in induction but without mitoxantrone (i.e. G-CSF, cladribine, and cytarabine, "GCLA"), provided the patient had <Grade 3-4 non-hematologic toxicity during induction. If there was such toxicity, doses should be reduced as described in section 6.7.

6.8.1.2 The second and third cycle of post-remission treatment is given with high-dose cytarabine alone. Cytarabine should be given as 1,000 mg/m^2 every 12 hours on days 1-6 for a total of 12 doses.

6.8.2 For phase 2 only: for patients with FLT3 internal tandem duplication mutations (FLT3-ITD) or FLT3 tyrosine kinase domain (FLT3-TKD) mutations, use of clinically-available tyrosine kinase inhibitors such as midostaurin or sorafenib on published schedules[14-16] is allowed.

6.8.3 Post-remission courses should start within 6 weeks of achieving CR/CRp/CRi once patients have recovered to ≤Grade 2 toxicities from the previous course of therapy.

6.8.4 Patients can proceed to transplantation barring contraindications and if a suitable donor is available.

6.9 Supportive Therapy

6.9.1 All patients will be adequately hydrated and receive appropriate anti-emetics based upon institutional standard of care guidelines.

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

6.9.3 Antimicrobial prophylaxis should be used according to institutional standard of care guidelines. In case of neutropenic fever, standard of care diagnostic testing will be performed, and empiric antibiotic coverage will be utilized as per standard of care institutional practices.

6.9.4 Transfusion support should be carried out according to institutional standard of care guidelines.

6.10 Treatment of CNS Disease

Treatment of CNS disease is done according to institutional practice guidelines or the preference of the attending physician.

6.11 Recommended Follow-up Care

After completion of protocol treatment or early termination of treatment (see section 6.12), patients should be evaluated according to institutional and/or national guidelines or the discretion of the attending physician. These evaluations may include peripheral blood studies and/or bone marrow examinations, as clinically indicated.

6.12 Criteria for removal from treatment prior to completion of protocol treatment

All reasons for discontinuation of treatment must be documented:

6.12.1 Consolidation with HCT after achievement of CR or CRi.

6.12.2 Failure to achieve CR or CRi after up to 2 cycles of induction therapy.

6.12.3 Persistent severe cytopenias (as defined in section 8.1) without evidence of leukemia after Day +42.

6.12.4 Relapse after achievement of CR or CRi during treatment.

6.12.5 Adverse toxicities that prevent continuation with study treatment.

6.12.6 Withdrawal of consent; the patient may withdraw from the study at any time for any reason.

7.0 INFORMATION ON STUDY DRUGS

7.1 Drug information on G-CSF (granulocyte colony-stimulating factor)

7.1.1 Mechanism of action: G-CSF is a growth factor that stimulates the production, maturation, and activation of neutrophils. Further, it promotes premature release of neutrophils from the bone marrow and enhances their phagocytic capacity.

7.1.2 Pharmacokinetics: Peak G-CSF concentrations after sub-cutaneous dosing occur in 2 to 8 hours, though the onset of action is approximately 24 hours, with plateau concentrations in 3-5 days, and elimination over an 11-20 day period. G-CSF is cleared by systemic degradation. Notably, as G-CSF binds neutrophils, plasma levels are controlled in large part by the absolute neutrophil count.[17]

7.1.3 Adverse effects (AEs): *Common drug-related AEs (occurring in >10% of patients)* include fever, petechiae, elevated uric acid, splenomegaly, bone pain, and epistaxis.

Less common drug-related AEs (occurring in 1% -10% of patients) include hyper- or hypotension, arrhythmias, headache, nausea, vomiting, leukocytosis, and transfusion reaction.

Infrequent drug-related AEs (occurring in <1% of patients) include acute respiratory distress syndrome, allergic reactions, alopecia, alveolar hemorrhage, arthralgia, bone density decrease, capillary leak syndrome, cerebral hemorrhage, vasculitis, dyspnea, edema, erythema nodosum, hematuria, hemoptysis, hepatomegaly, hypersensitivity, injection site reaction, pericarditis, proteinuria, psoriasis exacerbation, pulmonary infiltrates, renal insufficiency, sickle cell crisis, splenic rupture, Sweet's syndrome, tachycardia, and thrombophlebitis.

7.1.4 Recommended dose adjustments for organ dysfunction: There is limited or no data examining the toxicity of G-CSF in patients with renal or liver dysfunction. Therefore, administration of G-CSF to patients with liver or kidney disease must be done with caution.

7.2 Drug Information on cladribine (2-chloro-2'-deoxyadenosine, 2-CdA)

7.2.1 Mechanism of action: Cladribine is a prodrug that is converted to an adenosine deaminase-resistant triphosphate derivative (2-CdATP). This molecule is then activated by deoxycytidine kinase to a 5'-triphosphate derivative (2-CdAMP), which is incorporated into DNA where it acts as a transcription regulator. In addition to its cytotoxic properties in dividing cells, cladribine induces death in quiescent cells of lymphoid origin through an unknown mechanism.[18]

7.2.2 Pharmacokinetics: Cladribine is renally excreted, with 18-35% as unchanged drug. It is able to penetrate the CSF, where it achieves 25% of plasma concentrations. It is 20% protein-bound. The half-life for elimination after a 2-hour infusion is 6.7±2.5 hours in patients with normal renal function.

7.2.3 Adverse effects: *Common adverse effects (occurring in >10% of patients)* include fever, fatigue, headache, rash, nausea, anorexia, vomiting, myelosuppression (including grade 3/4 neutropenia/thrombocytopenia), injection site reaction, and infection.

Less common adverse effects (occurring in 1 to 10% of patients) include edema, tachycardia, thrombosis, chills, dizziness, insomnia, malaise, diarrhea or constipation, weakness, myalgias and arthralgias, cough, dyspnea, epistaxis, and diaphoresis.

Rare adverse effects (occurring in <1% of patients) include aplastic anemia, bacteremia, opportunistic infections, lymphocytopenia, altered mental status, hemolytic anemia, hypersensitivity, myelodysplastic syndrome, quadriparesis, and renal dysfunction/failure.

7.2.4 Reconstitution: Cladribine is supplied as a sterile, preservative-free, isotonic solution containing 10 mg of cladribine (1 mg/mL) in 10 mL single-use vials. Cladribine should be passed through a sterile 0.22µm filter prior to introduction into the infusion bag containing 0.9% Sodium Chloride Injection, USP.

7.2.5 Administration and compatibility: The use of 5% dextrose is not recommended as a diluent because of increased degradation of cladribine. The infusion solution is stable for 24 hours at room temperature.

7.2.6 Storage and stability: Store refrigerated 2° to 8°C (36° to 46°F). Protect from light during storage.

7.2.7 Recommended dose adjustments for organ dysfunction: Specific guidelines for cladribine dosing in patients with hepatic/renal dysfunction or hypoalbuminemia are not clearly defined. Because of the potential for compensatory elimination of cladribine in patients with hepatic and/or renal dysfunction, specific guidelines for dosing are difficult to define. Thus, when deciding whether to adjust cladribine doses for renal dysfunction, the risks for potential toxicities (e.g., myelosuppression, neurotoxicity) against the benefits and goals of treatment must be considered.

7.3 Drug information on cytarabine (cytosine arabinoside)

7.3.1 Mechanism of action: Cytarabine is a synthetic pyrimidine analog, in which the sugar moiety (normally a ribose or deoxyribose) has been replaced with arabinose. Although its mechanism of action is not completely understood, the active form of cytarabine is probably incorporated into the DNA and interferes with DNA synthesis. As such, cytarabine has been found to primarily effect dividing cells, blocking their progression from G_1 to S phase.

7.3.2 Pharmacokinetics: Cytarabine is metabolized by deoxycytidine kinase and other kinases into its most active form (aracytidine triphosphate). Aracytidine triphosphate is converted to nontoxic uracil derivatives by pyrimidine nucleoside deaminases. This balance between the levels of kinases and deaminases is critical for regulating the sensitivity/resistance of cells to the drug. The plasma clearance of cytarabine is biphasic, with an initial rapid phase and more prolonged second clearance phase. The rapid clearance phase has a relatively short half-life ($t_{1/2\alpha}$ = 10 minutes), while the half- life of the second clearance phase is slightly longer ($t_{1/2\beta}$ = 1-3 hours). The nontoxic metabolites from the drug are excreted in the urine, and within 24 hours after the infusion, approximately 80% of these nontoxic metabolites can be recovered from the urine.

7.3.3 Adverse effects: The dose-limiting toxicity for cytarabine is myelosuppression. *Adverse Events Associated with Standard Dose Cytarabine: Frequent AEs (not definitely quantified)* include the following: myelosuppression (leucopenia, anemia, neutropenia, thrombocytopenia), pyrexia, rash, anorexia, diarrhea,

nausea, vomiting, mucositis, anal inflammation or ulceration, hepatic dysfunction or increased liver enzymes, and local thrombophlebitis.

Less frequent AEs (not definitely quantified) include chest pain, pericarditis, dyspnea dizziness, headache, neural toxicity, neuritis, alopecia, pruritis, skin freckling, skin ulceration, urticaria, abdominal pain, bowel necrosis, esophageal ulceration, esophagitis, pancreatitis, sore throat, urinary retention, jaundice/hyperbilirubinemia, local site cellulites, renal dysfunction, allergic edema or anaphylaxis, sepsis, and sudden respiratory distress syndrome.

Infrequent AEs (not definitely quantified) include aseptic meningitis, cardiopulmonary arrest, cerebral dysfunction, cytarabine syndrome (bone pain, chest pain, conjunctivitis, fever, maculopapular rash, malaise, myalgia), exanthematous pustulosis, hyperuricemia, intestinal pneumonitis, increased lipase, paralysis with intrathecal and IV combination therapy, rhabdomyolysis, veno-occlusive disorder, and death.

Adverse Events Associated with High Dose Cytarabine include cardiomegaly and cardiomyopathy, coma, severe neurotoxicity, personality change, somnolence, total body alopecia, severe rash or skin desquamation, gastrointestinal ulceration, peritonitis, intestinal pneumatosis, necrotizing colitis, liver abscess or damage, peripheral neuropathy, corneal toxicity, hemorrhagic conjunctivitis, pulmonary edema, sudden respiratory distress syndrome, and sepsis.

7.3.4 Reconstitution: Cytarabine should be reconstituted in sterile water and can be further diluted using either 5% dextrose or sodium chloride solutions into appropriate concentrations for infusion.

7.3.5 Administration and compatibility: The diluted cytarabine solution should be inspected for particulate matter, discoloration, and haze prior to infusion. If there is evidence of particulate matter, discoloration, or haze the solution should not be infused. Patients should be medicated with standard anti-emetic therapy. Cytarabine is not compatible (1) during Y-site administration with allopurinol, amphotericin B, ganciclovir; (2) in syringe with metoclopropamide; or (3) admixed with fluorouracil, heparin, insulin (regular), nafcillin, oxacillin, penicillin G. Cytarabine may have variable compatibility when admixed with gentamycin, hydrocortisone, and methylpredinsone.

7.3.6 Storage and stability: Vials of non-reconstituted cytarabine should be stored at room temperature 15°C - 30°C (59°F - 86°F). The diluted cytarabine solution may be stable for up to 48 hours if stored at room temperature.

7.3.7 Drug-drug interaction: Reversible decreases in the plasma steady-state concentration for digoxin and cardiac glycosides may occur. Cytarabine may diminish the therapeutic effect of flucytosine. There is *ex vivo* data suggesting that cytarabine may reduce the effectiveness gentamycin for killing *K. pneumoniae*.

7.3.8 Warnings and precautions: *Ex vivo* and *in vivo* studies have found that cytarabine causes extensive chromosomal damage and potential malignant transformation. Although there have been some case reports describing cytarabine use in pregnant humans, these cases reports are few. Thus, cytarabine is considered Pregnancy

Category D. Women should be advised not to become pregnant while receiving cytarabine, and men should be advised not to father a child while receiving cytarabine and for at least 3 months after completing the therapy. It is not known whether cytarabine or its metabolites are excreted in breast milk; thus, it is not recommended for lactating females who are breast-feeding. As with any highly immunosuppressive medication, cytarabine may diminish the effectiveness of dead and live vaccines and enhance the toxic/adverse effect of live vaccines. One should avoid use of live vaccines while receiving it. A small percentage of patients will have a hypersensitivity reaction to cytarabine, and these individuals should not receive the drug again.

7.3.9 Recommended dose adjustments for organ dysfunction: Guidelines for adjusting cytarabine dose due to renal or liver dysfunction are not standardized, but many clinicians will adjust the dose based upon the function of these organs.

7.4 Drug information on mitoxantrone

7.4.1 Mechanism of action: Mitoxantrone (dihydroxyanthracenedione) is an anthracenedione derivative that intercalates with DNA, resulting in inhibition of nucleic acid synthesis.

7.4.2 Pharmacokinetics: Mitoxantrone is 78% bound to plasma proteins. A three-compartment model was described after a single intravenous dose of mitoxantrone. The mean alpha half-life is 6 to 12 minutes, the mean beta half-life is 1.1 to 3.1 hours, and the mean terminal (gamma) or elimination half-life is 23 to 215 hours (median 75 hours). Mitoxantrone has extensive distribution into body tissues and is metabolized in the liver to two main inactive metabolites (monocarboxylic acid derivative and dicarboxylic acid derivative). The major route of excretion for mitoxantrone appears to be biliary into the feces; approximately 11% of the dose is recovered in the urine within 5 days of drug administration, with 65% of this being unchanged drug.

7.4.3 Adverse effects: *Common adverse effects (occurring in >10% of patients)* include edema, fever, fatigue, headache, alopecia, nausea/vomiting, diarrhea, mucositis/stomatitis, myelosuppression, weakness, dyspnea, cough, and infection.

Less common adverse effects (occurring in 1 to 10% of patients) include congestive heart failure, decreased left ventricular ejection fraction (LVEF), hypertension, chills, anxiety, cutaneous mycosis, hypcalcemia, hypokalemia, hyponatremia, menorrhagia, jaundice, myalgia, arthralgia, renal failure, proteinuria, rhinitis, diaphoresis, and infection.

Mitoxantrone may cause cardiac toxicity with prolonged administration and doses exceeding 80 to 100 mg/m^2. When used after doxorubicin, cardiotoxicity is more frequent; an analysis by the Southwest Oncology Group revealed a risk of 6% at 134 mg/m^2 prior doxorubicin and 60 mg/m^2 mitoxantrone, rising to a 15% risk at 120 mg/m^2 mitoxantrone. Cardiac events reported included arrhythmias, decreased left ventricular function, chronic heart failure, tachycardia, ECG changes, and, infrequently, myocardial infarction. Bradycardia has been rarely reported. Patients with prior treatment with anthracyclines, prior mediastinal

radiotherapy, or with preexisting cardiovascular disease may have more frequent occurrences of cardiac toxicity.

7.4.4 Reconstitution: Mitoxantrone must be diluted prior to use. The dose of mitoxantrone should be to at least 50 mL with either 0.9% Sodium Chloride Injection (USP) or 5% Dextrose Injection (USP). Mitoxantrone may be further diluted into Dextrose 5% in Water, Normal Saline or Dextrose 5% with Normal Saline and used immediately.

7.4.5 Administration and compatibility: Care in the administration of mitoxantrone will reduce the chance of extravasation. Mitoxantrone should be administered into the tubing of a freely running intravenous infusion of 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Care should be taken to avoid extravasation at the infusion site and to avoid contact of mitoxantrone with the skin, mucous membranes, or eyes. If any signs or symptoms of extravasation have occurred, including burning, pain, pruritis, erythema, swelling, blue discoloration, or ulceration, the injection or infusion should be immediately terminated and restarted in another vein.
Mitoxantrone should not be mixed in the same infusion as heparin since a precipitate may form.

7.4.6 Storage and stability: Mitoxantrone should be stored between 15°C - 25°C (59°F - 77°F).

7.5 Drug information on GO

7.5.1 Mechanism of action: GO is directed against the CD33 antigen expressed by hematopoietic cells; binding of the CD33 antibody portion with the antigen results in the formation of a complex that is internalized. Upon internalization, the calicheamicin derivative is released inside the lysosomes of the myeloid cell. The released calicheamicin derivative binds to DNA in the minor groove resulting in DNA double strand breaks and cell death.

7.5.2 Human pharmacokinetics: After administration of a first 9 mg/m^2 dose of GO, given as a 2-hour infusion, the elimination half-lives of total and unconjugated calicheamicin were about 41 and 143 hours, respectively. After the second 9 mg/m^2 dose, the half-life of total calicheamicin was increased to about 64 hours and the area under the concentration-time curve (AUC) was about twice that in the first dose period. The AUC for the unconjugated calicheamicin increased 30% after the second dose. Age, gender, body surface area (BSA), and weight did not affect the pharmacokinetics of GO. Patients, especially patients previously treated with stem cell transplantation, have an underlying risk of sinusoidal obstruction syndrome (SOS; previously known as veno-occlusive disease [VOD]). The AUC of total calicheamicin was correlated with additional risk of hepatomegaly and the risk of SOS. No pharmacokinetic studies have been performed after administration of a 3 mg/m^2 dose of GO.

7.5.3 Hypersensitivity reactions: GO administration can result in severe hypersensitivity reactions (including anaphylaxis), and other infusion-related reactions which may include severe pulmonary events. Infrequently, hypersensitivity reactions and pulmonary events have been fatal. In most cases,

infusion-related symptoms occurred during the infusion or within 24 hours of administration of GO and resolved. GO infusion should be interrupted for patients experiencing dyspnea or clinically significant hypotension. Patients should be monitored until signs and symptoms completely resolve. Discontinuation of further GO treatment should be strongly considered for patients who develop anaphylaxis, pulmonary edema, or acute respiratory distress syndrome. Patients with high peripheral blast counts may be at greater risk for such reactions.

7.5.4 Human toxicology: The following toxicities have been observed in patients aged ≥60 years (n=157) treated with GO at 9 mg/m^2, generally given as two intravenous infusions separated by 14 days (the side effect profile of GO given at 3 mg/m^2 has not been studied in detail): abdominal pain 26%, asthenia 36%, back pain 12%, chills 64% (NCI grade 3 or 4 toxicity: 11%), fever 78% (13%), headache 27%, infection 10%, neutropenic fever 19%, pain 18%, sepsis 25% (15%), hemorrhage 9%, hypertension 17%, hypotension 18%, tachycardia 11%, anorexia 27%, constipation 23%, diarrhea 30%, dyspepsia 8%, gum hemorrhage 5%, liver function test abnormalities 20% (7%), nausea 63%, stomatitis 22%, vomiting 53%, anemia 22% (12%), ecchymosis 11%, leukopenia 43% (43%), petechiae 19%, thrombocytopenia 49% (48%), alkaline phosphatase elevation 10%, bilirubinemia 11%, hyperglycemia 11%, hypocalcemia 10%, hypokalemia 24%, hypomagnesemia 3%, hypophosphatemia 6%, LDH elevation 18%, peripheral edema 19%, myalgia 3%, anxiety 10%, depression 10%, dizziness 10%, insomnia 11%, increased cough 18%, dypsnea 26% (10%), epistaxis 24%, pharyngitis 10%, pneumonia 13%, abnormal pulmonary physical findings 8%, rhinitis 7%, herpes simplex skin infection 18%, pruritus 4%, rash 18%, metrorrhagia 2%, vaginal hemorrhage 5%, local reaction 17%.

7.5.5 Formulation: Powder for injection: 4.5 mg.

7.5.6 Storage and stability: The drug product is light sensitive and must be protected from direct and indirect sunlight and unshielded fluorescent light during the preparation and administration of the infusion. Vials are stored under refrigeration 2° to 8°C or 36° to 46°F.

7.5.7 Reconstitution: All preparation should take place in a biologic safety hood with shielded fluorescent light. Contents of each vial are reconstituted with 5 mL Sterile Water for Injection, USP, using sterile syringes, by gently swirling each vial. Each vial should be inspected for complete dissolution of the drug. The final concentration of the reconstituted drug solution is 1 mg/mL. Reconstituted vials may be stored under refrigeration for up to 2 hours.

7.5.8 Dilution and compatibility: An admixture corresponding to 3 mg/m2 dose of GO is prepared by injecting the reconstituted solution into a 100 mL 0.9% sodium chloride injection solution in either a polyvinyl chloride (PVC) or ethylene/polypropylene copolymer (non-PVC) IV bag covered by an ultraviolet (UV) light protector. GO should only be diluted with 0.9% sodium chloride solution, and must not be diluted with any other electrolyte solutions or 5% dextrose or mixed with other drugs. Diluted GO may be stored under refrigeration for up to 16 hours.

7.5.9 Administration: Once the reconstituted GO is diluted into the IV bag containing normal saline, the resulting solution should be infused over a 2-hour period. GO

may be given peripherally or through a central line. During the infusion, only the IV bag needs to be protected from light. An in-line, low protein binding filter must be used for the infusion of GO. The following filter membranes are qualified: 0.22 μm or 1.2 μm polyether sulfone (PES); 1.2 μm acrylic copolymer hydrophilic filter; 0.8 μm cellulose mixed ester (acetate and nitrate) membrane; 0.2 μm cellulose acetate membrane. GO must not be co-administered with other drugs through the same infusion line. Premedication, consisting of acetaminophen and diphenhydramine, should be given before each infusion to reduce the incidence of a post-infusion symptom complex. The infusion must be completed within 20 hours of initial reconstitution of the drug.

8.0 EVALUATION AND END POINT DEFINITIONS

8.1 Treatment response and outcome

Treatment response (e.g. morphologic/cytogenetic/molecular complete remission, partial remission) or treatment failure (e.g. resistant disease, morphologic leukemia-free state, morphological or molecular/cytogenetic relapse) as well as treatment outcome (e.g. overall survival, relapse-free survival, event-free survival, and remission duration) are categorized according to criteria recommended by International Working Groups.[2,12] Since persistent severe cytopenias are not defined per International Working Groups, here we define persistent severe cytopenias as ANC<200/μL AND platelets <20,000/μL (self-sustained without transfusion) without evidence of leukemia on bone marrow aspirate beyond day +42 after therapy.

8.2 Toxicity criteria

This study will use the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 5.0 for Toxicity and Adverse Event reporting. A copy of the CTCAE v5.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov). Only grade ≥3 adverse events other than hematologic toxicities will be graded and recorded.

8.3 Definition of dose-limiting toxicities (DLTs)

Reported adverse events and potential risks for G-CSF, cladribine, cytarabine, mitoxantrone, and GO are described in section 7. Dose-limiting toxicities (DLTs) used for trial monitoring are defined as follows and apply only to cycle 1 of therapy:

8.3.1 Any Grade 3 non-hematologic toxicity lasting >48 hours that results in >7-day delay of the subsequent treatment cycle, with the exception of febrile neutropenia or infection or toxicities secondary to febrile neutropenia or infection.

8.3.2 Any Grade ≥4 non-hematologic toxicity except febrile neutropenia/infection (or toxicities secondary to febrile neutropenia or infection) unless felt to be a direct consequence of treatment-related toxicity (e.g. intestinal infection following mucosal barrier breakdown), and with the exception of constitutional symptoms if recovery to Grade ≤2 within 14 days.

8.4 Multi-parameter flow cytometric detection of MRD

Patients are routinely assessed for the presence of MRD as detected by multi-parameter flow cytometry,[19-22] as per institutional practice and any measurable level of MRD will be considered positive.[19-22]

8.5 Quantification of CD33 expression on AML blasts and normal myeloid cells

In patients with pretreatment bone marrow and/or peripheral blood specimens analyzed in the UW/SCCA Hematopathology laboratory, CD33 expression on AML blasts as well as on normal myeloid cells (monocytes, granulocytes) will be quantified by multi-parameter flow cytometry both as percentage of CD33+ cells as well as median CD33 fluorescence intensity.

8.6 Duration of cytopenias

The duration of neutropenia and thrombocytopenia will be determined as time from day 1 of treatment until an absolute neutrophil count of 500 or a self-sustained platelet count of 50,000 is reached, respectively. Time to achievement of absolute neutrophil count >1,000/μL and platelet count >100,000/μL will also be recorded.

8.7 Duration of follow-up

After removal from protocol, patients will be followed to determine event-free survival and disease-free survival (for patients achieving CR or CRi) as well as overall survival (for all patients) for a maximum of 5 years. Follow-up may include periodic (e.g. every 3 months) review of medical records, and, only if absolutely necessary, direct contact of the study participant.

9.0 RECORDS

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 Principal Investigator and/or study team and kept in a locked office or file room within a secure building. Access to the study database will be restricted by electronic password protection and restricted access to computers (i.e., locked offices).

10.0 CORRELATIVE STUDIES

Trial participants will be encouraged to participate in correlative studies. From patients who agree to providing biospecimens for correlative research (opt-in/out option in consent form), extra bone marrow and/or peripheral blood specimens will be collected at baseline before initiation of study therapy and at various time points throughout the study when marrow/blood specimens are obtained for routine clinical reasons and then stored in a study-specific sample repository located at Fred Hutchinson Cancer Research Center. See **Appendix C** for instructions on specimen preparation.

11.0 PROTOCOL ENROLLMENT AND SPECIAL CONSIDERATIONS

All eligible patients will be included in this study without regard to gender or ethnicity. The incidence of AML is slightly higher in men, so it is expected that the distribution of these patients will reflect a slight male predominance of the disease as well as the general demographic distribution of AML patients seen at our institution. Based on our statistical approach, we will require no more than 60 patients treated at the MTD to compare with patients treated historically at our institution with GCLAM. Depending on DLTs observed in the phase 1 portion, up to an additional 12 patients may be treated for a maximum possible patient accrual of 72 patients.

Projected Target Accrual
ETHNIC AND GENDER DISTRIBUTION CHART

TARGETED / PLANNED ENROLLMENT: Number of Subjects = 72			
Ethnic Category	**Sex / Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	2	4	6
Not Hispanic or Latino	27	39	66
Ethnic Category Total of All Subjects*	29	43	72
Racial Categories			
American Indian/Alaska Native	0	1	1
Asian	2	2	4
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	3	4
White	25	37	62
Other/More Than One Race/Unknown	0	1	1
Racial Categories: Total of All Subjects*	28	44	72

12.0 GUIDELINES FOR SERIOUS ADVERSE EVENT REPORTING

12.1 Expedited reporting requirements

In accordance with Fred Hutch/UW Cancer Consortium IRB policy, all adverse events (AEs; whether occurring on-site or off-site), which in the opinion of the principal investigator (PI) are (1) unexpected, and (2) related or possibly related to the research, and (3) serious or suggests that the research places research participants or others at a greater risk of physical or psychological harm than was previously known or recognized, will be submitted to the IRB within ten (10) calendar days of learning of the problem. Both the "Expedited Reporting Form for Unanticipated Problems or Noncompliance" and the "Adverse Event Reporting Form", or equivalent forms, will be completed for this reporting.

12.2 Definitions

12.2.1 Adverse event (AE): Any harm or untoward medical occurrence in a research participant administered a medical product, medical treatment or procedure even if it does not necessarily have a causal relationship with the product, treatment, or procedure. An adverse event can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medical product, medical treatment, or procedure whether or not considered to be related. Mechanisms of obtaining information on AE include monthly transcripts, assessment forms obtained after each clinic visit, and hospital progress and discharge notes. Grade ≥3 adverse events other than hematologic toxicities will be recorded, graded, and reported as appropriate. While grade >=3 hematologic AEs are anticipated and are thus not recorded, we will record duration of cytopenias as well as episodes of febrile neutropenia. Medical conditions present before treatment are not AEs and should not be recorded. On the other hand, pre-existing conditions that worsen during treatment will be recorded.

12.2.2 Related or possibly related AE: An AE is "related or possibly related to the research procedures" if in the opinion of the principal investigator, it was more likely than not caused by the research procedures. AEs that are solely caused by an underlying disease, disorder or condition of the subject or by other circumstances unrelated to either the research or any underlying disease, disorder or condition of the subject are not "related or possibly related". If there is any question whether or not an AE is related or possibly related, the AE should be reported.

12.2.3 Serious AE (SAE): An adverse event that results in any of the following outcomes:

- Death
- Life-threatening adverse event (real risk of dying)
- Prolongation of hospitalization*
- Persistent or significant disability/incapacity/or change in psychosocial status
- Congenital anomaly
- Requires intervention to prevent permanent impairment of damage

*Hospitalization itself will not be considered a serious adverse event if required for complications of AML or comorbid conditions. Hospitalization will be considered a SAE if it fulfills the criteria for a serious and unexpected adverse event as otherwise described.

12.2.4 Unexpected AE: An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent (a) the known or foreseeable risk of adverse events associated with the research procedures described in the protocol-related documents, such as the IRB-approved research protocol, informed consent document and other relevant sources of information such as product labeling and package inserts; and also are not consistent with (b) the characteristics of the subject population being studied including the expected natural progression of any underlying disease, disorder or condition or any predisposing risk factor profile. with the applicable investigator brochure, or 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.

12.3 Grading adverse event severity

All AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 (http://ctep.cancer.gov). 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.

12.4 Monitoring, recording, and standard reporting of adverse events

Only grade ≥3 adverse events (AEs) other than hematologic toxicities will be recorded, graded, and reported as appropriate per 12.1. AEs will be collected only for the first cycle treatment as detailed in 12.5. If a subject decides to terminate the study early the medical record will continue to be followed for AEs for up to 4 weeks after initial response assessment or a new anti-leukemia therapy is started, whichever occurs first.

Adverse events that do not meet the requirement for expedited reporting will be reported to the IRB as part of the annual renewal of the protocol. Myelosuppression and associated complications are expected events during leukemia therapy; therefore, myelosuppression and associated complications such as fever, infections, bleeding, and related hospitalizations will not be reported as individual AE but will be summarized in the annual report to the IRB.

12.5 Adverse event recording period

AEs will be monitored and recorded in the study database only for the first cycle of therapy since this represents the only experimental therapy. Patients will be monitored from the time of first exposure to the therapy in this study (i.e., the start of the first drug administration infusion on day 0 or day 1) until the start of the second cycle of therapy is initiated, or until 1 month following the initial response assessment, whichever is sooner. AEs with an onset date prior to the first exposure to an investigational product will not be recorded. However, in the case of clinically significant worsening of the AE during the specified AE monitoring time frame, the AE will be recorded.

13.0. DATA AND SAFETY MONITORING PLAN

Institutional support of trial monitoring will be in accordance with the Fred Hutch/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan (DSMP). Under the provisions of this plan, Fred Hutch Clinical Research Support (CRS) coordinates data and compliance monitoring conducted by consultants, contract research organizations, or Fred Hutch 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), Fred Hutch Scientific Review Committee (SRC) and the Fred Hutch /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.

14.0 INVESTIGATOR OBLIGATIONS

The Principal Investigator (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.

15.0 ADMINISTRATIVE AND REGULATORY CONSIDERATIONS

15.1 Protocol interpretation and compliance

The procedures defined in the protocol are carefully reviewed by the PI and his/her 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.

15.2 Ethical considerations

The Investigator agrees to conduct this study in accordance with applicable United States FDA 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.

15.3 Informed consent

The PI 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 parents/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 or subject's legally authorized representative and by the person who administers the consent process. A copy of the signed form will be given to the person who signed it, the original signed Consent Form will be filed with the subject's medical records, and copy maintained with the subject's study records. 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. Any amended Informed Consent must be approved by the IRB/EC prior to use. 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, and must be used to document consent from any new subjects enrolled after the approval date of the amendment.

15.4 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.

15.5 Publication statement

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.

16.0 STATISTICAL CONSIDERATIONS

16.1 Phase 1 Statistical Considerations

DLTs are defined in Section 8.3. Only DLTs occurring during Cycle 1 will be used to guide dose escalation. However, before the phase 2 portion is begun, all grade 3 and 4 adverse events of all patients treated in the phase 1 portion will be reviewed to assess for late and cumulative toxicities that were not captured during the DLT observation period. The Principal Investigator reserves the right to select a recommended phase 2 dose lower than the MTD if the drug doses identified in the phase 1 portion of this assessment suggest a significant risk of late/cumulative toxicities. Patients will be considered evaluable for DLT if they received at least 75% of the assigned doses of each chemotherapeutic during Cycle 1 or if they developed a DLT.

If a patient does not develop a DLT but does not receive at least 75% of treatment during Cycle 1, the patient will be considered not evaluable for DLT and will be replaced. The dose escalation scheme to define the maximum tolerated dose (MTD).

The dose escalation scheme is as follows:

A. Evaluate 6 patients at GO1
   - If 0/6 or 1/6 have DLT, change to GO3 and move to (B)
   - If 2 have DLT, declare GO1=MTD and expand cohort to 12 patients and reassess: if ≤4/12 have DLT, proceed with GO1 as MTD, otherwise terminate study, concluding that GO cannot be added to GCLAM chemotherapy.
   - If ≥3/6 have DLT, terminate study, concluding that GO cannot be added to GCLAM chemotherapy.

B. Evaluate 6 patients at GO3
   - If ≤2/6 patients have DLT, expand cohort to 12 patients and reassess: if ≤4/12 have DLT, move forward with GO3 as MTD; if >4/12 have DLT, decrease schedule to GO1, expand GO1 cohort to 12 patients, and reassess: if ≤4/12 have DLT, declare GO1 R2PD. If >4/12 have DLT, terminate study.
   - If ≥3/6 have DLT, decrease schedule to GO1, expand GO1 cohort to 12 patients, and reassess: if ≤4/12 have DLT, declare GO1 MTD. If >4/12 have DLT, terminate study.

Once the MTD has been determined, all toxicities for all dose levels will be reviewed. If the regimen is considered safe, additional patients be treated at the MTD for the Phase 2 portion of the trial.

16.2 Phase 2 Statistical Considerations

The primary Phase 2 objective will be to evaluate 6-month event-free survival (EFS) at the MTD. EFS will be measured from the date of study registration to the first of: off protocol therapy without a response (morphologic complete remission [CR] with or without measurable residual disease [MRD]; best response of CR without complete count recovery will be considered an event), relapse from a response, or death from any cause with patients last known to be alive without event censored at the date of last contact. The primary endpoints will be 6-month and 1-year EFS (both binary). For the primary endpoints, patients censored before 6 months or 1-year (as appropriate) will be considered a failure. If censoring occurs, secondary analyses analyzing 6-month or 1-year EFS accounting for censoring will be done, including estimating 6-month and 1-year EFS using the Kaplan-Meier method.

Patients treated at the MTD from the Phase 1 portion of the trial will be used in the Phase 2 analysis. Patients with newly-diagnosed AML previously treated at our institution with GCLAM had a 6-month EFS of 68% and 1-year EFS of 60% (null hypothesis). We are interested in whether the proposed regimen can improve 6-month EFS to 81% and/or improve 1-year EFS to 73% (alternative hypothesis).

A two-stage design will be used to evaluate the Phase 2 six-month EFS endpoint. The first stage of the 2 design will evaluate 30 patients. If 20 or more of the first 30 patients are alive without

event at 6-months after study registration, an additional 30 patients will be enrolled. If 46 or more of the 60 patients treated at the MTD are alive and without event at 6-months after study registration, we will consider the regimen of interest for further investigation. If 45 or fewer patients treated that the MTD have an EFS event before 6-months after study registration, we will additionally evaluate 1-year EFS motivated by prior research with GO showing late-emerging benefits of the addition of GO.[6] If 42 or more patients are alive without event at 1 year, we will consider the regimen of interest for further investigation. Accrual will not be held while waiting for outcome data for the first-stage analysis. This design has a one-sided type-1 error rate of 13% and a power of 95%. Under the null hypothesis of 6-month EFS = 68%, the probability we stop the trial based the first stage evaluation is 35%. Under the alternative hypothesis of 6-month EFS = 81%, the probability we stop accrual after the first stage is 2%. Under the alternative hypothesis, the probability of considering the regimen of interest for further investigation based on 6-month EFS is 84% and under the null hypothesis is 9%.

16.3 Secondary objectives

Remission rates, MRD rates, and toxicity rates will be estimated and 95% confidence intervals will be calculated. Event-free survival, relapse-free survival, and overall survival will be estimated using the Kaplan-Meier method. Time to relapse will be estimated using non-parametric estimates of the cumulative incidence curve with death analyzed as a competing event. Regression models (logistic regression for binary endpoints, Cox regression for time-to-event endpoints [Cox models for the hazard of the subdistribution for events with competing risks]) will be used to compare outcomes with patients who have received GCLAM without GO at our institution, controlling for measured prognostic factors.

17.0 STUDY TERMINATION

The study will terminate as described in section 16.0. The Principal Investigator reserves the right to terminate this study at any time.

18.0 REFERENCES

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APPENDIX A: TREATMENT-RELATED MORTALITY (TRM) SCORE

Calculation of Simplified Treatment-Related Mortality (TRM) Score

Includes covariates: performance status (PS), age, platelet count, albumin, secondary AML, white blood cell count (WBC), peripheral blood blast percentage, and creatinine

Score = $100/(1+e^{(-x)})$, with x = -4.08 + 0.89*PS + 0.03*age - 0.008*platelet count - 0.48*albumin + 0.47*(have secondary AML) + 0.007*WBC - 0.007*(peripheral blood blast percentage) + 0.34*creatinine

Probability of TRM Above and Below Various Simplified TRM Score Cut-offs

TRM Score Interval	Patients below/ within/above TRM Score Interval (%)	TRM Probability if below TRM Score Interval (%)	TRM Probability if within TRM Score Interval (%)	TRM Probability if above TRM Score Interval (%)
0 – 1.9	0/20/80	-	1	12
1.91 – 3.9	20/20/60	1	2	16
3.91 – 6.9	40/20/40	1	7	20
6.91 – 9.2	60/10/30	3	7	24
9.21 – 13.1	70/10/20	4	12	31
13.11 – 22.8	80/10/10	5	20	41
22.81 – 100	90/10/0	6	41	-

From: Walter RB, Othus M, Borthakur G, Ravandi F, Cortes JE, Pierce SA, Appelbaum FR, Kantarjian HM, Estey EH. 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*. 2011;29(33):4417-4424.

APPENDIX B: RESEARCH SUBJECT REGISTRATION FORM

Protocol 10000 Patient Demographics and Eligibility Form
Please fax this completed form to [redacted] for patient registration.
Questions regarding eligibility should go to Roland B. Walter, pager: [redacted] or Colin D. Godwin, pager: [redacted]

UPN#:_____

Patient Name: _____ _____ ___
Last First MI

Date of Birth: ___ ___/___ ___/ ___ ___ ___ ___
Month Day Year

Planned starting day of treatment: ___ ___/___ ___/ ___ ___ ___ ___
Month Day Year

Ethnicity *(<u>choose one</u>)*: instruct the patient to <u>select one</u> of the following:

☐ **Hispanic or Latino** (A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race). Term "Spanish Origin" can also be used in addition to "Hispanic" or "Latino"
☐ **Not Hispanic or Latino**
☐ **Declined to Report**

Race *(<u>check all that apply</u>)*: instruct the patient to <u>select one or more</u> of the following:

☐ **American Indian/Alaska Native** (A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment)
☐ **Asian** (A person having origins in any of the original peoples of the Far East, Southeast, Asia, or the Indian subcontinent including, for example, Cambodia, China, India Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand and Vietnam)
☐ **Native Hawaiian/Pacific Islander** (A person having origins in any of the original peoples of Hawaii, Guam, Samoa or other Pacific Islands)
☐ **Black/African American** (A person having origins in any of the black racial groups of Africa.
☐ **White** (A person having origins in any of the original peoples of Europe, the Middle East or North Africa)
☐ **Research Subject does not know race**
☐ **Declined to Report**

Gender:
☐ Male
☐ Female
☐ Unknown

<u>ATTACH SIGNED CONSENT AND HIPAA AUTHORIZATION FORMS, AND SEND TO PRINCIPAL INVESTIGATOR FOR REGISTRATION.</u>

APPENDIX B cont'd
Protocol FH #10,000 Eligibility

I) Inclusion Criteria:
Each of the following questions (1-10) must be marked "Yes" for the patient to enroll on Protocol FH #10,000

1) Yes ☐ No ☐ Patient signed and dated consent form
Date: _____

2) Yes ☐ No ☐ Patient signed and dated HIPAA authorization

3) Yes ☐ No ☐ Age ≥18 years

4) Yes ☐ No ☐ Diagnosis of untreated "high-risk" myeloid neoplasm (≥10% blasts in blood or bone marrow) or AML other than acute promyelocytic leukemia (APL).

5) Yes ☐ No ☐ Treatment-related mortality (TRM) score ≤13.1 as calculated by simplified model available at https://cstaging.fhcrc-research.org/TRM

Age:	WBC: Date:
Secondary AML ☐ YES ☐ NO	Creatinine: Date:
Platelets: Date:	% PB Blasts: Date:
Albumin: Date:	Performance Status: Date:
TRM score:	

6) Yes ☐ No ☐ No prior therapy for AML other than hydroxyurea or prior low-intensity therapy for low-grade hematologic disorder. Patients with symptoms/signs of hyperleukocytosis or WBC>100,000/μL can be treated with leukapheresis or may receive up to 2 doses of cytarabine (up to 500 mg/m^2/dose) prior to enrollment

7) Yes ☐ No ☐ Bilirubin ≤2.5 x Institutional Upper Limit of Normal (IULN) unless elevation is thought to be due to hepatic infiltration by AML, Gilbert's syndrome, or hemolysis (assessed within 14 days prior to study day 0)

8) Yes ☐ No ☐ Serum creatinine ≤2.0 mg/dL (assessed within 14 days prior to study day 0)

9) Yes ☐ No ☐ Left ventricular ejection fraction ≥45%, assessed within 12 months prior to study day 0, e.g. by MUGA scan or echocardiography, or other appropriate diagnostic modality and no clinical evidence of congestive heart failure.

10) Yes ☐ No ☐ N/A ☐ Women of childbearing potential and men must agree to use adequate contraception.

II) Exclusion Criteria:

The following question (11-16) must be marked "No" for the patient to enroll on Protocol FH #10,000

11) Yes ☐ No ☐ Myeloid blast crisis of chronic myeloid leukemia (CML), unless patient is not considered candidate for tyrosine kinase inhibitor treatment

12) Yes ☐ No ☐ Concomitant illness associated with a likely survival of <1 year

13) Yes ☐ No ☐ Active systemic fungal, bacterial, viral, or other infection, unless disease is 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]).

14) Yes ☐ No ☐ Known hypersensitivity to any study drug

15) Yes ☐ No ☐ Confirmed or suspected pregnancy or active breast feeding.

16) Yes ☐ No ☐ Treatment with any other investigational anti-leukemia agent. In phase 2, treatment with a tyrosine kinase inhibitor for patients with FLT3-mutated AML is permissible.

Name of person completing form:_____

Signature of Study Investigator:_____Date:_____

FAX COVER LETTER

DATE:_____

TO: Roland B. Walter or Colin D. Godwin

[redacted]

RE: RESEARCH SUBJECT REGISTRATION FORM
PROTOCOL FH #10,000

FROM:_____

FAX:_____

PHONE:_____

THE INFORMATION CONTAINED IN THIS TRANSMISSION IS INTENDED ONLY FOR THE ADDRESSEE OR THE ADDRESSEE'S AUTHORIZED AGENT. THE FAX CONTAINS INFORMATION THAT MAY BE PRIVILEGED, CONFIDENTIAL AND EXEMPT FROM DISCLOSURE. IF THE READER OF THE MESSAGE IS NOT THE INTENDED RECIPIENT OR RECIPIENT'S AUTHORIZED AGENT THEN YOU ARE NOTIFIED THAT ANY DISSEMINATION, DISTRIBUTION OR COPYING OF THIS INFORMATION IS PROHIBITED.

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APPENDIX C: GENERAL SPECIMEN SUBMISSION INSTRUCTIONS

As secondary objectives of this study (see sections 2 and 10), *in vitro* correlative studies will be performed. For this purpose, pre-treatment peripheral blood and bone marrow specimens will be collected and stored in a study-specific repository at Fred Hutch.

D.1. Labeling

All submitted specimens must be labeled with the protocol number (FH #10000), source of material (i.e. bone marrow or peripheral blood), study-specific patient number, patient's initials, and date/time of specimen collection.

D.2. Guidelines for transport

D.2.1 The specimen must be wrapped in an absorbable material.

D.2.2 The specimen must be placed in an airtight container (e.g., ziplock bag), which must be marked as "BIOHAZARD".

D.2.3 Store and send samples at room temperature. Send fresh to the Walter laboratory:

[redacted]

Contact person: Colin D. Godwin, MD, pager [redacted]
colindg@uw.edu

D.2.5 Saturday or Sunday deliveries are not permissible.

D.2.6 Send each specimen individually on the day of its collection; maximum time from sample collection to shipment should be no greater than 24 hours. Exceptions:

- Samples collected on weekends or holidays should be shipped the first working day following collection

D.3. Specimens for laboratory studies and banking

D.3.1 Submission of a pre-treatment peripheral blood sample is strongly requested if the patient has agreed to participate in correlative studies.

D.3.1.1 10 mL of PERIPHERAL BLOOD collected into preservative-free heparin (green top tubes) or EDTA (purple top tubes).

D.3.2 Submission of a pre-treatment bone marrow sample is strongly requested if a bone marrow aspirate/biopsy is obtained for routine clinical purposes and if the patient has agreed to participate in correlative studies

D.3.2.1 4-5 mL of BONE MARROW; use approximately 0.2 mL preservative-free heparin (green top tube) or EDTA (purple top tubes) for 4-5 mL of bone marrow per tube.

D.3.2.2 If marrow is not aspirable ("dry tap"), submit 10 mL of peripheral blood only.

If the amounts specified cannot be obtained on any individual patient, then please send the actual sample obtained and as many studies as possible will be done.