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Phase 1/2 Study of Concurrent Decitabine in Combination with G-CSF, Cladribine, Cytarabine, and Mitoxantrone (G-CLAM) in Adults with Newly Diagnosed Acute Myeloid Leukemia (AML) or High-Risk Myelodysplastic Syndromes (MDS)

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FHCRC IRB Approval

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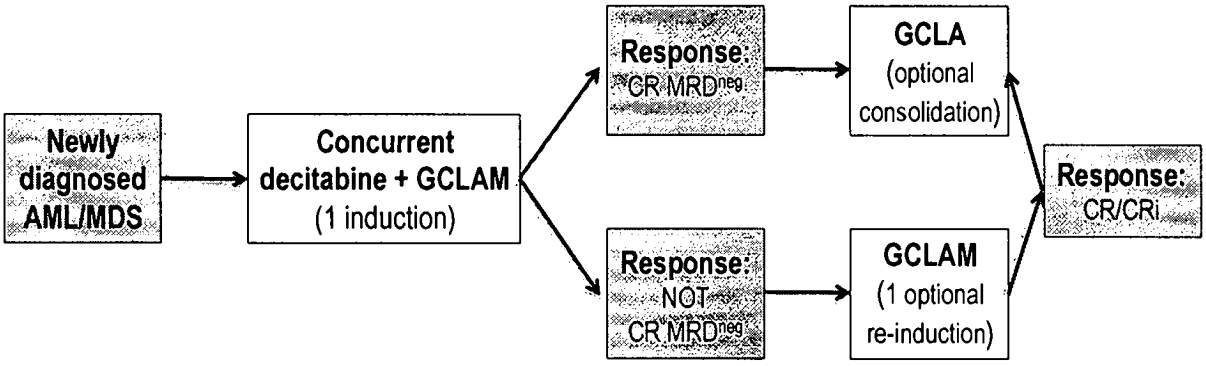
OVERVIEW OF THE TREATMENT PLAN



Abbreviations: CR, complete remission; CRi, CR with incomplete neutrophil or platelet recovery; GCLA, G-CSF, cladribine, cytarabine; GCLAM, GCLA with mitoxantrone; MRD, measurable residual disease

PHASE 1 DOSE ESCALATION SCHEME

Level	Decitabine (20 mg/m^2 IV) [1,2]	G-CSF (SQ, D0 to D5)[3]	Cladribine (IV, D1 to D5) [1]	Cytarabine (IV, D1 to D5) [1,4]	Mitoxantrone (IV, D1 to D3) [1]
1	10 days (D1 to D10)	300 or 480 µg	5 mg/m^2	2 g/m^2	18 mg/m^2
-1	7 days (D1 to D7)	300 or 480 µg	5 mg/m^2	2 g/m^2	18 mg/m^2
-2	5 days (D1 to D5)	300 or 480 µg	5 mg/m^2	2 g/m^2	18 mg/m^2

[1]Dosing based on actual patient weight. [2]Administered immediately prior to other chemotherapy. [3]Dosing based on patient weight: <76 kg vs. ≥76 kg; D0 and D1 dose may be omitted if WBC >20,000/µL. [4]Started 2h after completion of cladribine.

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1.0 BACKGROUND AND RATIONALE

Acute myeloid leukemia (AML) remains a difficult-to-treat malignancy, claiming approximately 10,000 lives each year in the U. S. alone [1-4]. Treatment improvements have been very gradual at best over the last two decades, and even with contemporary, intensive treatment regimens, only few patients will be alive 2-5 years after initial diagnosis [5, 6].

The majority of AML patients will require salvage therapy because of the high rates of failure to respond to initial therapy or relapse after standard remission induction therapy. Probably the most commonly used salvage regimen employs high-dose cytarabine (HiDAC). Patients with relapsed/refractory AML have poor outcomes. In patients who have relapsed within 1 year of initial remission or who have primary refractory disease, the complete remission (CR) rate with HiDAC is only about 15% [7]; such patients represent the great majority presenting for salvage therapy. Given these poor results, the need for new therapies in AML is clear.

Rationale for use of hypomethylating agents in AML

Decitabine 20 mg/m2 has been given as a single agent typically in 5-day cycles; CR rates in older newly diagnosed patients are about 25%.[8] Subsequently, several groups experimented with lengthening to 10-day cycles (also at 20 mg/m2 daily). Ritchie et al described with CR rates of 40% for newly diagnosed and 15.7% for relapsed / refractory patients, and other than hematologic / infectious toxicities, AEs were mild with no grade >2 events reported.[9] In fact, the authors even conclude by suggesting that the safety profile of 10 days of decitabine suggests that this could be combined safely with other agents. Blum et al reported on decitabine 20 mg/m2 daily for 10 days in 53 newly diagnosed patients and demonstrated a 47% CR rate; Non-hematologic / infectious grade >2 toxicities were rare, including fatigue (6%), anorexia (2%), mucositis (6%), hypoxia (15%), arrhythmia (6%), rash (4%). The only patients who died within 2 months of treatment were those who had failed to achieve remission (n=7) / had a severe infection (n=1).[10] Similar trends have been seen with non-randomized data for single-agent azacitadine: 7-day courses were associated with non-significantly improved CR rates (16.7% vs. 12.1%) and survival (14.9 vs. 13.2 months), with similar rates of hematologic toxicity and similar rates of requiring dose modification / treatment interruption.[11] While there is some evidence that longer duration of hypomethylating agents may improve efficacy, and while these treatments appear to be safe, remission rates are disappointingly low, suggesting that we should explore more effective treatments for AML. One such option is combining two treatments with limited efficacy (decitabine and chemotherapy) with the hope of finding synergistic efficacy. However, we currently do not know how best to combine these agents. Concurrent dosing may be the most obvious and most efficient method, but there is some pre-clinical evidence suggesting that giving decitabine prior to chemotherapy may improve efficacy.

Over the last several years, new insight into leukemogenesis has led to the exploration of targeting epigenetic mechanisms for treatment of AML. Epigenetic silencing of structurally normal genes by aberrant DNA methylation, mediated by DNA-methyltransferase (DNMT) enzymes, has been shown to contribute to myeloid leukemogenesis by disrupting important cellular pathways such as differentiation, proliferation, and apoptosis [12, 13]. In contrast to structural changes, epigenetic changes can be pharmacologically reversed, resulting in gene re-expression and restoration of normal cellular functions. Decitabine and azacitidine are cytidine analogs that deplete DNA methyltransferases, often referred to as a "hypomethylating cytidine analogs" (HCAs). Pre-clinical studies on the functional effects of HCAs in hematopoietic cell lines demonstrated that decitabine induced a dose-dependent demethylation and apoptotic

response in myeloid leukemia cells [14]. Additionally, Qin *et. al.* found that combinations of decitabine and cytarabine synergistically killed leukemic blasts [15], with exposure to decitabine followed by treatment with cytarabine resulting in a more pronounced synergistic killing effect compared to concomitant use of both agents [14].

Hypomethylating agents *prior* to intensive chemotherapy: evidence on tolerability

Building on the hypothesize that pretreatment (i.e., priming) with a hypomethylating agent such as decitabine might sensitize resistant leukemic cells to cytotoxic chemotherapeutics and increase the efficacy of induction chemotherapy in AML, a few studies have examined combinations of decitabine priming and cytotoxic chemotherapy for the treatment of patients with AML. A good way to determine whether HMAs add to the toxicity profile of standard AML chemotherapy is in the context of a randomized trial, which are rare in acute leukemia. In this case, however, we can draw from the AML-AZA trial, which randomized patients to 7+3 induction chemotherapy and high-dose cytarabine consolidation with or without 5 days of azaciditine priming before cycles. The difference in AEs was not significant, and early death rates did not differ (6% vs. 5%).[16] Several trials have used decitabine priming prior to intensive chemotherapy using a dose escalation strategy starting at 20 mg/m^2 for 5 days, and none has been able to identify an MTD. Scandura et al used decitabine priming for 7+3 chemotherapy with decitabine escalated from 5 to 7 days. Duration of neutropenia and incidence of infection were similar. There was more GI toxicity and mucositis at higher doses, but all toxicities were grade 3 or lower and would not have limited dose escalation had the protocol been written to allow for this.[17] More recently, we have submitted to ASH the final results of our phase 1/2 study of decitabine-primed mitoxantrone, etoposide, and cytarabine (MEC), which uses $1g/m^2$ of Ara-C daily for 6 days and safely administered decitabine for 10 days. Earlier results from this protocol, FH #2652, were included in our prior submission. Updated results with 52 patients with relapsed / refractory AML showed that grade >2 non-hematolgoic / non-infectious toxicities were fatigue, nausea, and mucositis. Among 9 deaths on the study, 5 were related to infection, 1 to disease, 2 to respiratory failure, and 1 to hemorrhage. We have also analyzed unpublished data on patients treated off protocol with this regimen, and in total 71 patients have received decitabine-primed MEC between 2013 and 2015. Many patients treated off protocol were given decitabine prior to MEC without the standard 5 day rest period between decitabine and chemotherapy, and there was no difference in treatment efficacy or survival among these patients vs. those who had the 5 day break, indicating that the pre-clinical models suggesting better efficacy with optimal priming may not transplant perfectly into the clinic. In fact, it is possible that the benefits of hypomethylating agents do not actually require priming, and that they could be seen with concurrent administration.

Hypomethylating agents *concurrent* with intensive chemotherapy: evidence on tolerability

It remains unknown whether priming is indeed the optimal way of combining decitabine with intensive chemotherapy, or whether similar results could be achieved with concurrent administration of decitabine along with chemotherapy. Decitabine at 25-30 mg/m^2 for 4 days has been combined with intermediate doses of ara-C without increase in severe infection rate or early mortality over decitabine alone in MDS (NCT01674985).[18] Our institution has been giving decitabine $20mg/m^2$ for 10 days concurrent with high-dose cytarabine as part of protocol FH #9109 since 2014. While induction uses intermediate doses of cytarabine, patients who fail to achieve remission with this induction are then given 10 days of decitabine concurrent with high-

dose cytarabine (1g/m^2 daily for 5 days). Per their protocol, "Although ara-C has yet to be combined with 10 days of decitabine the absence of toxicity with tosedostat plus... higher doses of ara-C (1g/m^2) suggests excess toxicity will not be an issue..." This protocol was approved for use in older patients at high risk for treatment-related mortality (TRM scores >13.1), compared to those required for this present study (<9.2). Despite the high-risk patient population in 9109, this combination has been well-tolerated, with unpublished results communicated by the study PI showing that only 4 of 12 patients had a *temporary* grade 3-4 non-infectious / non-hematologic adverse event related to treatment (muscle weakness in 2, fatigue in 1, and hyperbilirubinemia in 1).

Rationale for studying concurrent decitabine rather than primed decitabine

While it remains unknown whether priming is the optimal way of combining decitabine with intensive chemotherapy, we found that having a randomized study of concurrent vs. primed decitabine with GCLAM was impractical from an enrollment standpoint. After an initial 10 months of having this study open as a randomized study with low accrual, we found that the major barrier to enrollment was the randomization component. Specifically, patients did not want to be randomized to the sequential arm because this would entail an additional 15 days of treatment. For patients receiving treatment at our center who were lodged away from home, having an extra 15 days in Seattle was considered overly onerous, and therefore patients chose to enroll in different protocols or receive treatment off protocol rather than being randomized on protocol 9713. We did not note any clear difference in toxicity or efficacy between study arms at that point, and so we opted to shut down the sequential arm and enroll on the concurrent arm alone. This is consistent with unpublished data from our institution on 71 patients treated with decitabine-primed MEC both on and off protocol between 11/15/12 and 11/15/16 showing that there is no difference in CR rates or survival outcomes between patients who had a 5-day chemo-free interval after decitabine priming versus those who received MEC immediately following the last dose of decitabine, and so it may be that pre-clinical models of priming effects do not translate perfectly *in vivo*. It is possible that any benefits of hypomethylating agents like decitabine do not require priming, and that these benefits could be achieved with concurrent administration of decitabine and chemotherapy.

Rationale for combining decitabine with G-CLAM/Demonstration of safety and tolerability of using escalated doses of mitoxantrone as part of G-CLAM

In 2008, Polish investigators reported their experience with the use of G-CSF, cladribine (5 mg/m^2 on days 1-5), high-dose cytarabine (2,000 mg/m^2 on days 1-5), and mitoxantrone (10 mg/m^2 on days 1-3; "G-CLAM") for the treatment of primary refractory AML or early relapsed disease, with encouraging activity – among 118 treated patients, a complete remission (CR) rate of 58% was obtained – and acceptable tolerability with low rates of grade >2 non-infectious / non-hematologic AEs, the most common ones being nausea (20%), diarrhea (14%), mucositis (10%), and bleeding (10%) [19]. Starting in 2014, we have conducted a phase 1/2 study of dose-escalated G-CLAM in adults with newly diagnosed or relapsed/refractory AML or high-risk MDS (NCT02044796, Fred Hutch #2734.00). Preliminary results from the phase 1 portion of this study were presented in abstract form at the American Society of Hematology in 2015: 18 mg/m^2/day was identified as the MTD of mitoxantrone in newly diagnosed patients, and 16 mg/m^2/day in relapsed/refractory patients. Among 31 newly diagnosed patients with median age 58 (range: 27-77) years, median TRM score 2.83 (range: 0.15-5.54), 1 patient died within 28

days of treatment initiation from thrombocytopenia-related intracranial hemorrhage. Overall, 29/31 patients (93.5%) achieved a CR, including 24 (77.4%) with complete count recovery, with 1-2 cycles of chemotherapy. One DLT occurred at each of dose levels 3 and 4 (respiratory failure in both cases), establishing 18 mg/m^2/day as the MTD of mitoxantrone. Besides infections and neutropenic fever, maculopapular rash, nausea, and hypoxia were the most common grade 3+ adverse events. There were 26 relapsed and refractory patients treated in Phase 1 with median age 57 (37-77) years, median TRM score of 1.73 (range: 0.29-3.92). 2 patients died within 28 days of treatment initiation from sepsis and cardiogenic shock respectively. Overall, 13/36 patients (50%) achieved a CR, including 8 (31%) with complete count recovery. One DLT occurred at dose level 1 (nausea) and 2 at dose level 4 (encephalitis and cardiogenic shock), establishing 16 mg/m^2/day as the MTD of mitoxantrone. Besides infections and neutropenic fever, the most common grade 3+ adverse events were nausea, hypoxia (fluid overload and infection related), and maculopapular rash.[20] The final results from the phase 1/2 study in newly-diagnosed patients were submitted in abstract form to ASH this year; similar rates of response and adverse events were reported.

PROTOCOL UPDATE (2-2018)

Rationale for restricting enrollment to newly diagnosed (ND) patients
Analysis of treatment outcomes for relapsed/refractory (RR) patients enrolled in this protocol revealed an increased rate of delayed blood count recovery (MLFS) over what would be expected based on experience with GCLAM without decitabine. Based on these cases, revisions to the RR arm of this protocol are being discussed. These revisions are beyond the scope of what can be accomplished with a protocol modification, and so we have elected to restrict this protocol to ND patients and to work on a new protocol for RR patients.

Rationale for allowing only 1 cycle of decitabine + GCLAM
This protocol initially called for multiple cycles of decitabine in combination with chemotherapy: patients who failed to achieve CR without measurable residual disease (MRD), or MRDneg CR, were eligible for a 2nd cycle of decitabine + GCLAM, while those who did achieve this outcome were eligible for consolidation with decitabine + GCLA. Review of outcomes from the 4 ND patients who received on-protocol consolidation showed that 2 had delayed or absent recovery of neutrophils and platelets not related to leukemia, or morphological leukemia-free state (MLFS). Although it is difficult to interpret results from so few patients, in light of results from RR patients showing higher rates of MLFS than we would normally expect, we opt to restrict patients to a single cycle of decitabine in combination with GCLAM. Patients achieving MRDneg CR have the option of receiving GCLA consolidation (which is a standard therapy that they could receive on or off protocol), while those who do not achieve MRDneg CR will be taken off study. Because we recognize MLFS as a concern with this regimen, we will add criteria to the stopping rules to include bone marrow aplasia.

Rationale for adding a Phase 2 expansion cohort
Based on the 6 ND patients who have had their responses assessed, the combination of decitabine for 10 days and GCLAM appears to be effective. Within our first cohort of 6 patients, we had 1 DLT (a patient experienced early death), but responses in the other 5 patients showed MRDneg CR. Without an expansion cohort, we only have room to enroll another 4 patients on

the trial to determine the MTD or recommended Phase 2 dose (RP2D) assuming that the remaining patients have only 0 or 1 DLT. Because of these promising early results, we plan to expand our study in ND patients at the MTD/RP2D. The Phase 2 expansion includes stopping rules for both efficacy and safety.

2.0 OBJECTIVES

2.1 Primary Objective

2.1.1 Estimate the maximum tolerated dose (MTD) of decitabine when used concomitantly with G-CLAM in patients with newly diagnosed AML and high-risk MDS.

2.1.2 Compare, within the limits of a phase 1/2 study, the rate of complete remission without measurable residual disease (MRDneg CR) with decitabine + G-CLAM at the MTD compared to similar patients treated previously with G-CLAM alone.

2.2 Secondary Objectives

2.2.1 Evaluate, within the limits of a phase 1/2 study, disease response (complete remission, overall response rate) relapse-free survival (RFS), event-free survival (EFS), and overall survival (OS) in patients with newly-diagnosed AML / high-risk MDS.

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

3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

3.1.1 Age ≥18 years

3.1.2 For patients with *newly diagnosed disease*: diagnosis of "high-grade" MDS (≥10% blasts by morphology) or AML other than acute promyelocytic leukemia (APL) with t(15;17)(q22;q12) or variants according to the 2016 WHO classification. For patients with *relapsed/refractory disease*: prior diagnosis of "high-risk" MDS or non-APL AML, with relapsed/refractory disease according to 2003 recommendations of the International Working Group [21], requiring first or subsequent salvage therapy. Patients with mixed phenotype acute leukemia (MPAL) are eligible.

3.1.3 Outside diagnostic material is acceptable as long as peripheral blood and/or bone marrow slides are reviewed at the study institution. Flow cytometric analysis of peripheral blood and/or bone marrow should be performed according to institutional practice guidelines.

3.1.4 Patients with prior autologous or allogeneic hematopoietic cell transplantation (HCT) are eligible if relapse occurs provided symptoms of graft-versus host disease are well controlled with stable use of immunosuppressive agents.

3.1.5 Treatment-related mortality (TRM) score $\leq$ 9.2 as calculated with simplified model [22] (see Appendix A).

3.1.6 Should be off any active therapy for AML with the exception of hydroxyurea for at least 14 days prior to study registration unless patient has rapidly progressive disease, and all Grade 2-4 non-hematologic toxicities should have resolved.

3.1.7 May have previously received monotherapy with demethylating agents for MDS or AML or treatment with a mitoxantrone- or cladribine-based regimen for MDS or AML, including G-CLAM, but *not* demethylating agent as priming for or in combination with chemotherapy.

3.1.9 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.

3.1.10 Adequate organ function.

3.1.10.1 Bilirubin $\leq$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 registration).

3.1.10.2 Serum creatinine $\leq$2.0 mg/dL (assessed within 14 days prior to registration).

3.1.10.3 Left ventricular ejection fraction $\geq$45%, assessed within 3 months prior to registration, e.g. by MUGA scan or echocardiography, or other appropriate diagnostic modality and no clinical evidence of congestive heart failure. If the patient had anthracycline-based therapy since the most recent cardiac assessment, cardiac evaluation should be repeated if there is clinical or radiographic suspicion of cardiac dysfunction, or if the previous cardiac assessment was abnormal.

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

3.1.12 Ability to understand and willingness to sign a written 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. chronic viral hepatitis, HIV]). Patient needs to be clinically stable as defined as being afebrile and hemodynamically stable for 24-48 hours.

3.2.4 Known hypersensitivity to any study drug.

3.2.5 Pregnancy or lactation.

3.2.6 Patients may not be receiving any other investigational agents.

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 as objectively as possible, and the risks and hazards of the study explained to the patient. Consent will be obtained using forms approved by the local Institutional Review Board (IRB).

5.0 PROTOCOL REGISTRATION

To register, the attending physician involved in the care of the potential study participant must contact either the Principal Investigator or, in her absence, a PI-designated member of the study team, and fax a copy of the signed consent form and a signed HIPAA authorization form to the study team (FAX: +1-206-667-6519). The study PI will be responsible for ensuring that all eligibility requirements according to section 3.0 are met. To complete the registration process, the Principal Investigator or his or her designee will assign a patient study number and register the patient on the study. A complete, signed study consent and HIPAA consent are required for registration.

6.0 TREATMENT PLAN

This study is a single-center, open-label phase 1/2 study of decitabine given concomitantly with G-CLAM chemotherapy in adults with newly diagnosed AML or high-risk MDS. Since prior studies have suggested that the hypomethylating activity of decitabine plateaus at 20 mg/m^2/d [23] and since data from Fred Hutch #2652.00 discussed above show that decitabine can be given safely for 10 days prior to intensive chemotherapy, we will start our trial with 10 days of decitabine at 20 mg/m^2/d and will de-escalate the dose based on DLTs. We will use G-CLAM dose established based on data from Fred Hutch #2734.00 described above. Bone marrows will be reassessed upon blood count recovery or between day Days +25 to +35 after start of G-CLAM chemotherapy, whichever occurs first. Patients achieving CR or CR with incomplete neutrophil or platelet count recovery (CRi) after one induction course are eligible for consolidation chemotherapy with G-CSF, cladribine, and cytarabine ("G-CLA"), which should begin within 6 weeks of achieving CR/CRi once patients have recovered to $\leq$ Grade 2 toxicities from the previous course of 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 registration).

6.1.2 Peripheral blood and/or bone marrow examination with morphologic and flow cytometric assessment, routine cytogenetic analysis, and molecular testing (FLT3/ITD, NPM1, CEBPA) as needed to establish the diagnosis; a bone marrow biopsy should be obtained if spicules are absent from the aspirate sample

(assessed within 2 months of study day 0 as long as no anti-AML therapy has been given in the interim; if anti-AML therapy has been given, disease status should be reassessed subsequent to this therapy and prior to study day 0).

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

6.1.3 Testing of renal function, bilirubin, and albumin (should be assessed within 14 days prior to registration).

6.1.4 MUGA scan or echocardiography, or other appropriate diagnostic modality, to assess left ventricular ejection fraction (LVEF; assessed within 3 months prior to registration).

6.2 Pre-Treatment

At the discretion of the treating physician, allopurinol 300 mg po daily (or equivalent dose adjusted for renal function) may be considered in all patients without known allergies to allopurinol to reduce the risk of tumor lysis. Higher doses of allopurinol are permitted if patients develop tumor lysis syndrome. Patients may receive rasburicase, a recombinant uric acid 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 Decitabine

Dose Escalation Scheme for Reference

Level	Decitabine (20 mg/m^2 IV) [1,2]	G-CSF (SQ, D0 to D5)[3]	Cladribine (IV, D1 to D5) [1]	Cytarabine (IV, D1 to D5) [1,4]	Mitoxantrone (IV, D1 to D3) [1]
1	10 days (D1 to D10)	300 or 480 µg	5 mg/m^2	2 g/m^2	18 mg/m^2
-1	7 days (D1 to D7)	300 or 480 µg	5 mg/m^2	2 g/m^2	ND: 18 mg/m^2
-2	5 days (D1 to D5)	300 or 480 µg	5 mg/m^2	2 g/m^2	ND: 18 mg/m^2

[1]Dosing based on actual patient weight. [2]Administered immediately prior to other chemotherapy. [3]Dosing based on patient weight: <76 kg vs. ≥76 kg; D0 and D1 dose may be omitted if WBC >20,000/µL.
[4]Started 2h after completion of cladribine.

6.3.1 Patients will receive decitabine at a pre-specified dose level described below. If dose Level 1 is found to be too toxic, therapy will be de-escalated to Level -1. If dose Level -1 is found to be too toxic, therapy will be de-escalated to level -2.

- ***Level 1***: 10 days of decitabine (day 1 to day 10). The total dose of decitabine delivered will be 200 mg/m^2 for Level 1.
- ***Level -1***: 7 days of decitabine (day 1 to day 7). The total dose of decitabine delivered will be 140 mg/m^2 for Level -1.
- ***Level -2***: 5 days of decitabine (day 1 to day 5). The total dose of decitabine delivered will be 100 mg/m^2 for Level -2.

6.3.2 The dose of decitabine is calculated using the patient's actual weight.

6.3.3 Decitabine will be administered IV as per institutional standards.

6.3.4 Administration in the outpatient clinic is permitted.

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

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

6.4 Administration of G-CSF, Cladribine, Cytarabine, and Mitoxantrone

6.4.1 The dose of the G-CLAM 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 g/m^2 daily IV over 2 hours on Days 1-5, and mitoxantrone 18 mg/m^2 daily IV over 60 minutes on days 1-3. Note that we refer to the first day of G-CLAM induction as Day +1.

6.4.2 The doses of G-CLAM are calculated using the patient's actual weight.

6.4.3 If WBC >20,000/μL, 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 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 studies 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 >1,000/μL and then at least weekly until platelet count >100,000/μL.

6.5.2 Metabolic panel, including electrolytes (Na, K), bilirubin, ALT/AST, and creatinine at least weekly until ANC >1,000/μL and platelet count >100,000/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 other 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 G-CLAM 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 +45.

6.6.1 Patients achieving MRDneg CR: Patients are eligible for consolidation chemotherapy, as described in section 6.8.

6.6.2 Patients achieving CR with MRD, CRi, or those with persistent disease: patients are eligible for a second course of induction chemotherapy consisting of G-CLAM without decitabine provided all non-hematologic toxicities have resolved

to Grade <2. For patients who experienced ≥ Grade 3 non-hematologic / non-infectious toxicity during the first induction, a dose reduction is recommended as described in section 6.7. Patients who achieve CR or CRi after a second induction are eligible to receive consolidation chemotherapy, as described in section 6.8.

6.6.3 Patients with persistent aplasia without evidence of disease after Day +45: patients will be removed from protocol.

6.7 Dose Modifications of Chemotherapeutic Drugs for Initial and Subsequent Treatment Cycles

The following dose modifications are suggested both for induction cycle #1 (i.e. if organ function parameters worsen between baseline and start of chemotherapy) and 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 G-CLAM, subsequent doses of cladribine and cytarabine will be reduced by 25% and the dose of mitoxantrone will drop by 2 mg/m^2. The patient may receive future cycles using this dose-reduced regimen, if the patient 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 first dose-reduced regimen, the dose of the cladribine and cytarabine for subsequent cycles will be reduced by 50%. Mitoxantrone will only be given for 1st and 2nd induction and is not part of post-remission chemotherapy, and so further dose reductions will not be required.. 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, unless, at any time, a patient displays Grade ≥3 non-hematologic toxicity other than Grade 3 infections while receiving the 50% dose-reduced regimen, at which point the patient will not be eligible for additional therapy as part of the protocol. Furthermore, if patients require treatment interruption of > 21 days from the planned start of the next cycle, the patient will be not be eligible for additional therapy as part of the protocol.

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 to less than 50 mL/min during therapy, we will consider dose reduction in discussion with the Oncology Pharmacist.

6.8 Consolidation Therapy

After a achieving MRDneg CR after 1 cycle of decitabine + G-CLAM or after achieving CR/CRi after 1 cycle of decitabine + G-CLAM followed by GCLAM without decitabine, patients are eligible for consolidation therapy with G-CLA. Consolidation will not include decitabine.

6.8.1 The treatment is identical to the induction course but without decitabine or mitoxantrone (i.e. G-CSF, cladribine, and cytarabine, "G-CLA"), provided the patient had <Grade 3-4 non-hematologic / non-infectious toxicity during induction. If there was such toxicity, doses should be reduced as described in section 6.7.

6.82 Consolidation courses should start within 6 weeks of response assessment once patients have recovered to Grade ≤2 toxicities from the previous course of therapy.

6.8.3 Patients can receive up to 4 courses of consolidation 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 NCCN guidelines [24].

6.9.1 Growth factors may be used according to institutional practice guidelines or the preference of the attending physician.

6.9.2 Antimicrobial prophylaxis should be used according to institutional practice guidelines. In case of neutropenic fever, standard diagnostic testing will be performed, and empiric antibiotic coverage will be utilized as per usual care and standard institutional practices (SCCA, UWMC, and Fred Hutch).

6.9.3 Transfusional support should be carried out according to institutional practice 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, patients should be evaluated by treating physicians 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

All reasons for discontinuation of treatment must be documented:

6.12.1 Completion of protocol treatment.

6.12.2 Consolidation with HCT after achievement of CR or CRi.

6.12.3 Failure to achieve CR or CRi after at most 2 induction cycles.

6.12.4 Persistent aplasia without evidence of leukemia after Day +45.

6.12.5 Relapse after achievement of CR or CRi during treatment.

6.12.6 Adverse toxicities that prevent continuation with study treatment.

6.12.7 The patient may withdraw from the study at any time for any reason.

6.13 Concomitant Medications

Patients may take concomitant medications other than active anti-AML therapy while receiving study drugs.

7.0 INFORMATION ON STUDY DRUGS

7.1 Drug Information on Decitabine (5-Aza-2'-deoxycytidine)

7.1.1 Mechanism of action: The exact mechanism of action for decitabine is unknown. As a cytosine analog, decitabine is converted to a deoxynucleotide triphosphate form and incorporated into DNA during cell division in lieu of cytosine [25]. At lower concentrations (0.001-1 μM), its incorporation into DNA traps and depletes DNA methyltransferases (MTases), leading to depletion of methylated DNA in cell progeny [14, 25]. At higher concentrations (>1 μM), incorporation of the drug into DNA probably leads to a large number of DNA breaks, initiating pro-apoptotic signals and cell death [14].

7.1.2 Human pharmacokinetics: The peak decitabine concentration after 20-30 mg/m^2 infused over 1 hour is reached in approximately 30 minutes after completion of the infusion and approaches 0.5 μM. Decitabine is rapidly cleared from the plasma in a biphasic manner: an initial rapid phase has a very short half- life ($t_{1/2\alpha}$ = 7 minutes), such that the decitabine concentration falls below 0.01 μM within 1 hour after completion of the infusion; the half- life of the second clearance phase is more prolonged ($t_{1/2\beta}$ = 30 minutes) [26]. Plasma protein binding is negligible (<1%) [27].

7.1.3 Human toxicology and adverse events (AEs): *Frequent drug-related AEs (occurring in 26% -50% of patients)* observed in patients treated with standard doses of decitabine (20 mg/m^2/day times 5 days) include: anemia, neutropenia, thrombocytopenia, constipation, diarrhea, nausea, fatigue, peripheral edema, pyrexia, cough, and dyspnea. *Less frequent drug-related AEs (occurring in 10% - 25% of patients)* include: febrile neutropenia, anorexia, vomiting, dyspepsia, stomatitis, abdominal pain, asthenia, chills, upper respiratory tract infection, pneumonia, hypokalemia, arthralgia, back pain, pain in extremity, headache, dizziness, insomnia, epistaxis, petechiae, rash, and hypotension. *Infrequent drug-related AEs (occurring in 5% -10% of patients)* include: thrombocythemia, cardiac failure, tachycardia, otalgia, dysphagia, gastro-esophageal reflux, oral pain, toothache, tooth abscess, chest pain, generalized edema, mucosal inflammation, generalized pain, cellulites, oral candidiasis, sinusitis, bacteremia, urinary tract infection, elevated liver function tests, weight loss, dehydration,

hyperglycemia, hypomagnesaemia, bone pain, muscle spasm, weakness, musculoskeletal pain, myalgia, anxiety, confusion, depression, pharyngeal pain, pleural effusion, sinus congestion, dry skin, ecchymosis, skin erythema, night sweats, pruritis , other skin lesions, hypertension, and death. *Rarer drug-related AEs (occurring in <5% of patients)* include: splenomegaly, myocardial infarction, cardio-pulmonary arrest, cardiomyopathy, atrial fibrillation, supraventricular tachycardia, cholecystitis, increased bleeding with and without procedures, fungal infections, sepsis, periventricular abscess, mycobacterium avium infection, mental status changes, renal failure, pulmonary embolism, pulmonary mass, and hypersensitivity reaction [28].

7.1.4 Formulation and reconstitution: Decitabine is supplied as a sterile, lyophilized white powder, in single vial. Each vial contains 50 mg of the drug. DAC should be aseptically reconstituted with 10 ml sterile water, providing a concentration of 5.0 mg/ml with a pH 6.7-7.3. Immediately after reconstitution, the reconstituted decitabine solution should be further diluted with 0.9% sodium chloride, 5% dextrose, or lactated Ringers to a final drug concentration of 0.1-1.0 mg/ml.

7.1.5 Administration and compatibility: The diluted decitabine solution should be inspected for particulate matter and discoloration prior to IV infusion. If there is evidence of particulate matter or discoloration, the solution should not be infused. Patients should be pre-medicated with anti-emetic therapy as per standard practice.

7.1.6 Storage and stability: Vials of non-reconstituted decitabine should be stored at room temperature 25°C (77°F). Unless used within 15 minutes, the diluted decitabine solution must be prepared using cold infusion liquids (2-8°C, 36-46°F) and can be stored at 2-8°C for up to 7 hours.

7.1.7 Drug-drug interaction: No formal drug-drug interactions have been studied for decitabine. *Ex vivo* studies in human liver microsomes suggest that decitabine is unlikely to inhibit or induce cytochrome P450 enzymes.

7.1.8 Warnings and precautions: *Ex vivo* and *in vivo* animal models have found that decitabine is mutagenic and may harm the fetus, but the effect of DAC on the human fetus has not been systematically examined. Thus, it is considered pregnancy category D. Women should be advised not to become pregnant while receiving decitabine, and men should be advised not to father a child while receiving decitabine and at least 3 months after completing the therapy. It is not known whether decitabine or its metabolites are excreted in breast milk; thus, it is not recommended for lactating females who are breast-feeding. A small percentage of patients will have hypersensitivity reactions to decitabine, and these individuals should not receive the drug again.

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

7.2 Drug Information on G-CSF (Granulocyte colony-stimulating factor)

7.2.1 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.

7.2.2 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.[29]

7.2.3 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.

7.2.4 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.

7.3 Drug Information on Cladribine (2-chloro-2'-deoxyadenosnie, 2-CdA)

7.3.1 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.[30]

7.3.2 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.

7.3.3 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.

7.3.4 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.

7.3.5 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.

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

7.3.7 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.

7.4 Drug Information on Cytarabine (Cytosine arabinoside)

7.4.1 Mechanism of Action: Cytarabine is a synthetic pyrimidine analog, in which the sugar moiety (normally a ribose or deoxyribose) has been replaced with arabionose. 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 [31, 32].

7.4.2 Human 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. Like decitabine, the plasma clearance of cytarabine is biphasic: 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 [31, 32].

7.4.3 Human Toxicology and AEs Associated with Standard Doses of Cytarabine: The dose-limiting toxicity for cytarabine includes myelosuppression and cardiotoxicity. Cumulative doses beyond 550 mg/m^2 result in increased risk for congestive heart failure. *Frequent AEs (not definitely quantified)* include the following: myelosuppressionleucopenia, anemia, neutropenia, thrombocytopenia and related bleeding, 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 [31, 32].

7.4.4 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 [31, 32].

7.4.5 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 [31, 32].

7.4.6 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 [31, 32].

7.4.7 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*.

7.4.8 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.

7.4.9 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.

7.5 Drug Information on Mitoxantrone

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

7.5.2 Pharmacokinetics: 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.

7.5.3 Adverse Effects: The most common toxicities associated with mitoxantrone are alopecia, nausea/vomiting, Mitoxantrone will 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.

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, aplasia, 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 [21, 33]. Patients are routinely assessed for the presence of

measurable residual disease (MRD) as detected by multiparameter flow cytometry, as per institutional practice.

8.2 Toxicity Criteria

This study will use the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 4.0 for Toxicity and Adverse Event reporting. A copy of the CTCAE v4.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov).

8.3 Definition of Dose-limiting Toxicities (DLTs)

Reported adverse events and potential risks for decitabince, G-CSF, cladribine, cytarabine, and mitoxantrone are described in section 7. Dose-limiting toxicities (DLTs) used for trial monitoring are defined as follows:

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.

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

8.3.3 Lack of recovery of ANC to ≥ 500/μL and lack of self-sustained platelet count ≥ 50,000/μL by treatment day +49 with no evidence of residual leukemia.

8.4 Duration of Follow-up

After removal from protocol, patients will be monitored for late toxicities/complications for 1 month or until additional anti-AML therapy is given. Patients will be followed after completion of study treatment to determine disease-free survival (for patients achieving CR or CRi) as well as event-free and 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, if absolutely necessary, direct contact of the study participant.

8.5 Study Endpoints

The endpoints of the phase 1 portion of this study are the determination of the MTD of decitabine in newly-diagnosed patients. Provided that the MTD is successfully determined, enrollment in the phase 2 portion of this trial at the MTD will be conducted in this population. The endpoints for the phase 2 portion of this study are (primary) MRDneg CR compared to historical controls of G-CLAM alone and (secondary) disease responses, relapse-free, and overall survival. For the primary outcome comparison, response rates will use the best response after up to 2 cycles of induction (1 with decitabine G-CLAM and 1 with G-CLAM alone). .

9.0 RECORDS

Research data will be recorded in an electronic database using a unique study ID for each patient to assure patient 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 medical records department affiliated with our institution maintains all original inpatient and outpatient

chart documents. Data from source documents are used to compile the study's electronic 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, and imaging. The licensed medical records department, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents. Patient research files are maintained by a designated research coordinator and kept in a locked room with access restricted to personnel authorized by the Clinical Research Division. Access to the database will be restricted by electronic password protection and restricted access to computers (i.e., locked offices).

10.0 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 [2], 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 41 patients treated at the MTD to compare with patients treated historically at our institution with GCLAM. The number of patients to be included in this trial depends on the number of dosing levels tested in Phase 1. The following accrual guidelines reflect the assumption that dosing Level 1 will be safe, and thus n=41 is assumed. If further dosing levels are required and n=12 patients enroll at dose level 1, dose level -1, and dose level -2, enrollment may occur up to 41+12+12 = 65..

Projected Target Accrual
ETHNIC AND GENDER DISTRIBUTION CHART

TARGETED / PLANNED ENROLLMENT: Number of Subjects = 41			
Ethnic Category	**Sex / Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	1	2	3
Not Hispanic or Latino	18	20	38
Ethnic Category Total of All	19	22	41
Racial Categories			
American Indian / Alaska Native	1	2	3
Asian	1	1	2
Native Hawaiian or Other Pacific	0	0	0
Black or African American	1	1	2
White	16	18	34
Racial Categories: Total of All	19	22	41

11.0 GUIDELINES FOR SERIOUS ADVERSE EVENT REPORTING

11.1 Expedited Reporting Requirements and Reporting Period

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 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. AEs will be monitored from the time of initial drug delivery. Upon completion of induction chemotherapy (1-2 cycles), patients will be monitored for late toxicities for 1 month or until additional anti-AML therapy is given off of this protocol.

11.2 Definitions: according to ICH guidelines and 21 CFR 312.32, IND Safety Reports, and ICH E2A,Definitions and Standards for Expedited Reporting, and adverse event is defined as follows:

11.2.1 Adverse Event (AE): 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 AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal product, whether or not considered related to the medicinal product. Abnormal laboratory values should not be recorded as an AE unless an intervention is required (repeating testing to confirm the abnormality is not considered an intervention), the laboratory abnormality results in a serious adverse event, or the AE results in study termination or interruption / discontinuation of study treatment. Otherwise, grade $\geq$3 AEs other than hematologic toxicities will be recorded, graded, and reported as appropriate. While grade $\geq$3 hematolgoic AEs are anticipated and are thus not recorded, we will record duration of cytopenias as well as episodes of febrile neutropenia. If the degree and duration of cytopenias is consistent with significant bone marrow toxicity as defined in 8.3.3, this will be reported as an AE (and as a DLT). Medical conditions present at screening before treatment is administered are not AEs and should not be recorded. On the other hand, pre-existing medical conditions that worsen during treatment or in the post-treatment period will be recorded.

11.2.2 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.

11.2.3 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
- Requirement of 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.

11.2.4 Unexpected AE: An AE is "unexpected" when its nature, severity, or frequency are not consistent with (a) the known of 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 are also 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 prolife for the adverse event.

11.3 Monitoring Adverse Events and Recording Period:

All non-hematologic grade 3-4 AEs and all grade 5 AEs starting at the time of initial drug delivery will be recorded. While grade 3-4 hematologic toxicities are anticipated, we will record duration of cytopenias as well as episodes of febrile neutropenia. Upon completion of induction chemotherapy (1-2 cycles), patients will be monitored for late toxicities/complications for 1 month or until additional anti-AML therapy is given off of this protocol.

All AEs will be assessed by the investigator or qualified designee and recorded in the electronic database. When applicable AEs should be recorded as diagnoses rather than as the individual signs and symptoms. AEs 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 simple 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. For example, 1) simple febrile neutropenia with hospitalization is not an expedited reportable event to the IRB but will be included in the annual report to the IRB, whereas febrile neutropenia complicated by Gram-negative bacteremia with shock or its complications is to be reported expeditiously; 2) thrombocytopenic epistaxis or bleeding from mucosal surfaces easily managed with platelet support is not an expedited reportable event to the IRB but will be included in the annual report to the IRB, whereas major gastrointestinal bleed will be reported expeditiously.

11.4 Recording Adverse Events:

The following information should be recorded: description of the AE, whether the AE is serious, the start and stop date of the event, the severity (grade), a description of potential relatedness of the event to a study drug or other cause, action taken due to the AE, and outcome

of the AE. Subjects who terminate the study will be followed for AEs for 1 month or until additional anti-AML therapy is given, as per section 8.4.

11.5 Grading Adverse Event Severity

All AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. as per section 8.2. 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 AE.

11.6 Attribution of an Adverse Event

Association or relatedness to the study agents 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 AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated.

12.0. 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 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). This trial will be monitored twice yearly. The

DSMC will monitor the trial yearly, and a Data and Safety Monitoring Board (DSMB) will be established to review the trial yearly as well. DSMB reviews will be offset from DSMC reviews such that the trial will be monitored twice annually. The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable DSMP for studies actively enrolling or treating patients. 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.

13.0 ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Institutional Review Board

In accordance with federal regulations (21 CFR 312.66), an Institutional Review Board (IRB) that complies with regulations in 21 CFR 56 must review and approve this protocol and the informed consent form prior to initiation of the study.

13.2 Consent

The Principal Investigator or his designee must explain verbally and in writing the nature, duration, and purpose of the study and possible consequences of treatment. Patients must also be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment. In accordance with federal regulations (21 CFR 50), all patients enrolled in the study must sign the IRB-approved consent form.

13.3 Confidentiality

Patient medical information obtained for the purposes of this study is confidential, and disclosure to third parties, other than those noted below, is prohibited. Upon the patient's request and written permission, medical information may be given to his/her personal physician or other appropriate medical personnel responsible for the patient's welfare. Data generated for this study must be available for inspection on request to representatives of the national or local health authorities, and the associated IRB/IEC. Release of research results or data that reveal patient names or other identifiers, such as photographs, audio or videotapes, must be carried out in accordance with Department of Health and Human Services Final Standards for Privacy of Individual Health Information, 45 CFR 164.508. Written authorization must be obtained from the patient and IRB/IEC prior to the release of such information. Identifiable patient data may not be used for purposes of promoting any drugs used in this trial.

13.4 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.

14.0 STATISTICAL CONSIDERATIONS

14.1 General Considerations:

14.1.1 DLTs are defined in Section 8.3. Only DLTs occurring during Cycle 1 will be used to guide dose escalation.

14.1.2 Patients will be considered evaluable for DLT if they received at least 75% of the assigned decitabine and G-CLAM doses 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.

14.1.3 Maximum tolerated dose (MTD) is defined as the highest dose studied in which the incidence of DLT is <33%.

14.2 Phase 1 Dose De-Escalation Scheme

Within each study arm (newly diagnosed and relapsed/refractory) and within each randomization group (sequential and concurrent decitabine) start with dose Level 1 and evaluate 6 patients:

a) If 0 or 1 patients have DLT, expand cohort to 12 patients; if ≤3 DLTs are observed in 12 patients declare Level 1 = Recommended phase 2 dose (RP2D) for that study arm and randomization group.
b) If more than 1 of 6 or 3 of 12 patients have DLT with dose Level 1, enroll a cohort of 6 patients at dose Level -1.
c) If 0 or 1 patients have DLT, expand cohort to 12 patients; if ≤3 DLTs are observed in 12 patients declare Level -1 = MTD for that study arm and randomization group.
d) If more than 1 of 6 or 3 of 12 patients have DLT with dose Level -1, enroll a cohort of 6 patients at dose Level -2.
e) If 0 or 1 patients have DLT, expand cohort to 12 patients; if ≤3 DLTs are observed in 12 patients declare Level -2 = MTD for that study arm and randomization group.
f) If dose level -2 is found too toxic, the study will terminate for that study arm and randomization group.

Observed versus true DLT rates are summarized here, with two-sided confidence intervals (CIs) for probability of DLT with n=12 patients:

Observed DLTs	**80% CI**	**90% CI**
0 of 12 (0%)	(0%, 17%)	(0%, 22%)
1 of 12 (8%)	(0%, 29%)	(0%, 34%)
2 of 12 (17%)	(5%, 39%)	(3%, 44%)
3 of 12 (25%)	(10%, 48%)	(7%, 53%)
4 of 12 (33%)	(15%, 56%)	(13%, 61%)
5 of 12 (42%)	(22%, 64%)	(18%, 68%)

Note that 3 DLTs / 12 patients are permitted to declare MTD; if >3 DLTs are seen, dose reduction must occur.

In Phase 2, we use the term Recommended phase 2 dose (RP2D) to denote the dose level determined in Phase 1 to be used in Phase 2.

14.3 Phase 2

The primary objective of Phase 2 is to evaluate the rate of MRDneg CR after up to 2 courses of induction chemotherapy. We will also continue to evaluate toxicity during Phase 2.

A Simon Minimax two-stage design will be used. The historic rate of MRDneg CR with G-CLAM alone is 71%. Our regimen will be considered of no further interest if the true rate of MRDneg CR is 65% or less (null), while an MRDneg CR rate of 80% will be of considerable interest (alternative). The design has a one-sided type-1 error rate of 10% and a power of 80% for the primary outcome. Patients treated at the RP2D in Phase 1 will be included for assessment in Phase 2.

The first stage will evaluate 19 patients. If 13 or more patents achieve MRDneg CR, the trial will move to stage 2. Additionally, as we are targeting DLT <33% in Phase 1, if 7 or more of the first 19 patients (36%) have what would qualify as a DLT in cycle 1, we would suspend the study and consider closure or dose reduction. Under the null hypothesis of an MRDneg CR rate of 65%, the probability we stop accrual after the first stage is 51%.

The second stage would evaluate an additional 22 patients (total n=41). If 31 or more of these 41 patients achieve MRDneg CR and if 13 or fewer (<31%) have what would qualify as a DLT in cycle 1, we would consider the regimen worthy of further investigation.

15.0 ADMINISTRATIVE CONSIDERATIONS

16.1 Investigator obligations

The PI is responsible for the conduct of the clinical trial and is responsible for overseeing the treatment of all study subjects. The PI must assure that all study personnel adhere to the study protocol and guidelines regarding clinical trials both during and after study completion. The PI assumes the responsibility of ensuring that all patients have given their informed consent prior to study enrollment.

16.2 Documentation

The PI and study staff are responsible for maintaining a comprehensive filing system containing study related documentation, including signed informed consents for each subject. The files must be suitable for inspection by regulatory agencies at any time.

16.3 Ethical considerations

The Investigator agrees to conduct this study in accordance with applicable United States FDA clinical trial regulations and guidelines, applicable United States FDA clinical trial regulations and guidelines, the ICH (E6) GCP guidelines, the European Union Directive 2001/20/EC for clinical trials conducted in the European Union, 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.

16.3 Informed consent

Subjects meeting the criteria set forth in the protocol may be offered the opportunity to participate in the study. 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. 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. The date and time of time of the Informed Consent must be recorded in the source documents.

16.0 STUDY TERMINATION

The study will terminate as described in section 14.0. The Principal Investigator reserves the right to terminate this study at any time.

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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.

FAX COVER LETTER

DATE:_____

TO: Sarah A. Buckley, MD

FAX (206) 667-6519

RE: RESEARCH SUBJECT REGISTRATION FORM PROTOCOL 9713

FROM:_____

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PHONE:_____

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