Accelerated dose schedule of cytarabine consolidation therapy for older patients with acute myeloid leukemia (AML) in complete remission

Protocol Number: UF-HEM-009

Protocol Version Number: **4.1**

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Clinical Phase: Phase II

IND Status: N/A

NCT#: NCT04914676

Investigational Agent(s): N/A

Keywords: ***acute myeloid leukemia (AML), high dose cytarabine (HiDAC), consolidation, chemotherapy schedule, bone marrow, elderly***

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ABBREVIATIONS

AE	adverse event
AESI	adverse event of special interest
ALT	alanine transaminase (also SGPT)
AML	Acute myeloid leukemia
ANC	absolute neutrophil count
AST	aspartate transaminase (also SGOT)
BAL	bronchoalveolar lavage
BSA	body surface area
BSI	bloodstream infection
BUN	blood urea nitrogen
CBC	complete blood count
CG	cytogenetics
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
CNS	central nervous system
CR	complete remission
CRF	case report form
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTMS	clinical trials management system
CTO	Clinical Trials Office
DISC	Data Integrity and Safety Committee
DLT	dose-limiting toxicity
DNA	Deoxyribonucleic acid
ECOG	Eastern Cooperative Oncology Group
EOT	End of Treatment
FISH	fluorescence in situ hybridization
FC	flow cytometry
FLT3	FMS-like tyrosine kinase 3
GCP	Good Clinical Practice
HiDAC	High-dose cytarabine
HCT	Hematopoietic stem cell transplant
ICF	informed consent form
ICH	International Conference on Harmonization
IDH	Isocitrate dehydrogenase
IRB	Institutional Review Board
IFI	invasive fungal infection
IV	Intravenous

kg	kilogram(s)
LDH	lactic dehydrogenase
LFS	Leukemia-free survival
LOS	length of stay
LRTD	lower respiratory tract disease
MDS	Myelodysplastic syndrome
MRD	Minimal residual disease
NCI	National Cancer Institute
NGS	Next-generation sequencing
NSAE	non-serious adverse event
ORR	overall response rate
OS	overall survival
PB	Peripheral blood
PCR	Polymerase chain reaction
PD	progressive disease
PFS	progression free survival
PMO	Project Management Office
PI	principal investigator
PK	Pharmacokinetics
PO	By mouth
PR	partial remission
PRN	As needed
PS	performance status
RBC	red blood cells
RECIST	Response Evaluation Criteria In Solid Tumors
RR	Relapse rate
RVI	Respiratory viral infection
SAE	serious adverse event
SNP	Single nucleotide polymorphism
SCR	Serum creatinine
SOC	Standard of care
SQ	Subcutaneous
SD	stable disease
SGOT	serum glutamic oxaloacetic transaminase (also AST)
SGPT	serum glutamic pyruvate transaminase (also ALT)
TNFα	tumor necrosis factor alpha
UF	University of Florida
UFHCC	University of Florida Health Cancer Center
ULN	upper limit of normal
US	United States

USA	United States of America
WBC	white blood cell
WHO	World Health Organization

Protocol Signature Page

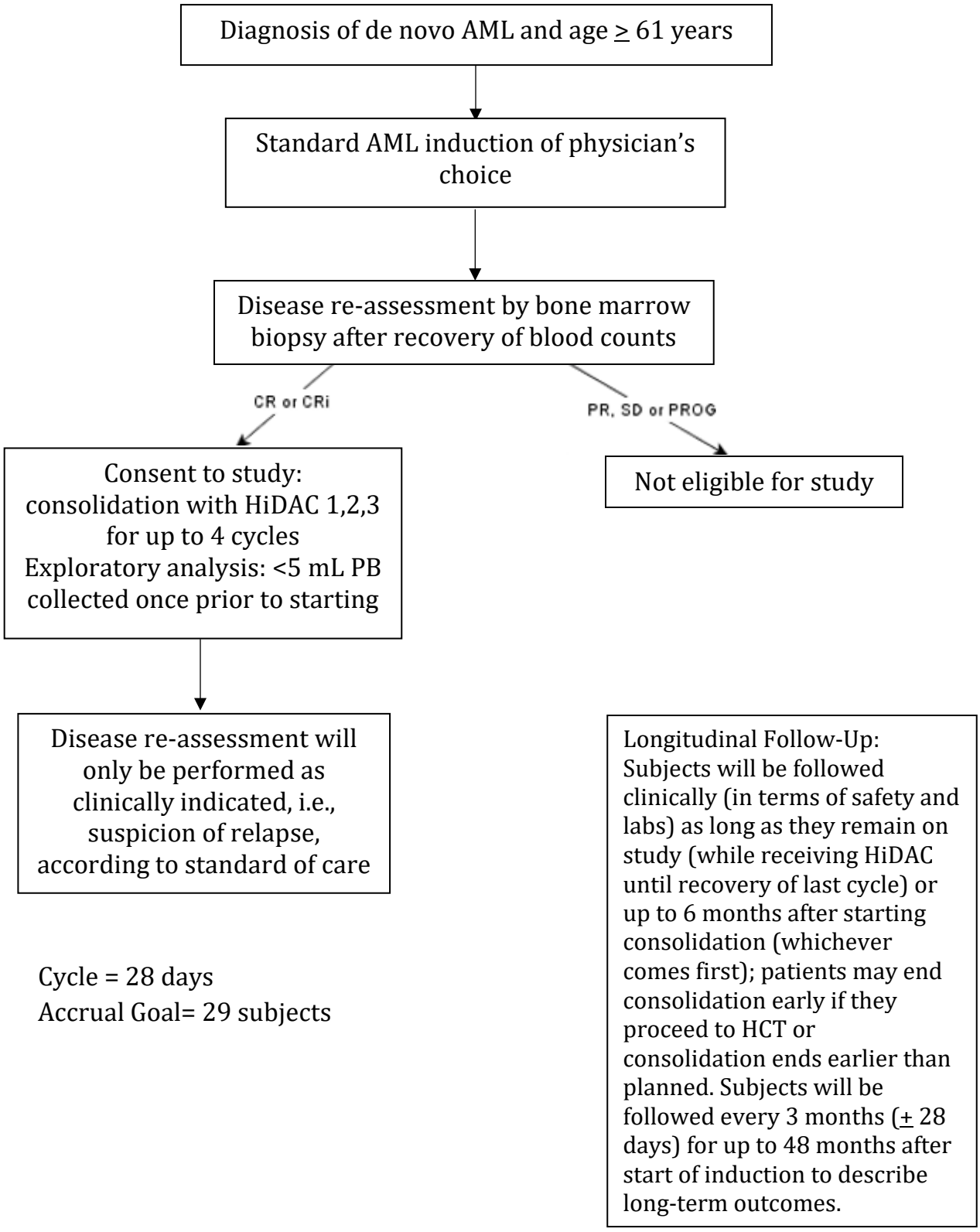
Accelerated dose schedule of cytarabine consolidation therapy for older patients with acute myeloid leukemia (AML) in complete remission

Principal Investigator:

Signature of Investigator	Date
Jack W. Hsu, MD Printed Name of Investigator	
University of Florida Name of Facility	
FL Location of Facility (City/State)	

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.

STUDY SCHEMA



PROTOCOL SYNOPSIS

Title:	Accelerated dose schedule of cytarabine consolidation therapy for older patients with acute myeloid leukemia (AML) in complete remission
Funding Source(s):	All services will be provided by University of Florida CRO, if applicable.
Investigational Agent Source:	N/A
Rationale:	Various chemotherapy regimens have been used in the treatment of AML, and common agents used include anthracyclines and pyrimidine/purine analogs. Once complete remission is confirmed after induction chemotherapy, a common regimen for AML patients to receive is standard HiDAC chemotherapy consolidation (cytarabine ≥ 1000 mg/m^2 up to 3000 mg/m^2 IV q12h on Days 1,3,5) x 3-4 cycles in the USA. Doses depend on age, renal function, performance status (PS) and institution/provider. UF Health Shands Hospital routinely uses HiDAC consolidation as above. Recently published results from two European trials showing that a modified HiDAC consolidation regimen (given on Days 1,2,3) reduced the duration of neutropenia and number of blood product transfusions in younger AML patients.[1] Another recent publication of a retrospective study confirmed the result of reduced hematologic recovery and less days of hospitalizations between the two regimens in subjects 60 years or less, however they did not see a reduced number of blood products.[2] Due to differences in induction treatment strategies and differences in patterns of health care systems it is not clear that similar benefits and reductions in health care resource utilization would be seen in patients treated in the US. Also, many patients with AML are diagnosed over the age of 60 years, and currently this abbreviated HiDAC consolidation has not been studied in the elderly population. In older patients cytarabine is given as 1000 mg/m^2 IV q12h for 6 doses. In this trial, we will evaluate the safety of this abbreviated regimen in our AML patient population who are age 61 years or older, and try to reproduce the hematologic toxicity results seen in the younger population using standard practices here in the US. We would like to adopt this practice as a standard regimen if we are able to confirm the results of these aforementioned published trials. This would potentially decrease

	length of stay, infections, transfusions and other potentially-related complications for AML patients.
Objectives:	Primary: ● To determine the duration of neutropenia after consolidation chemotherapy with HiDAC 123 compared to matched historical controls who received HiDAC 135. Neutropenia is defined in days, as the first day of absolute neutrophil count (ANC) < 500 after chemotherapy to day of recovery [ANC > 500 (defined as first day above 500 if consecutive x 2)]. Secondary: ● To define duration of thrombocytopenia after consolidation chemotherapy with HiDAC 123 compared to matched historical controls who received HiDAC 135. Thrombocytopenia is defined as the interval between the first day of platelets <50x103/uL after consolidation chemotherapy to the first day of recovery (defined as first day of platelets >20x103/uL if consecutive x 2 and not transfused). ● To determine the safety of HiDAC 123 compared to standard HiDAC 135 in case controls by evaluating the differences in: o duration of neutropenia and thrombocytopenia o incidence of documented infections (e.g., bloodstream infection (BSI), pneumonia, invasive fungal infection (IFI), *Clostridium difficile* infection (CDI), typhlitis, (see Appendix A) and rates of febrile neutropenia (defined as temperature ≥ 38.3 C° once or ≥ 38 C° for more than 1 hour and ANC less than 500/mm^3 or expected to fall below 500/mm^3 in next 48 hours) o number of RBC and platelet transfusions given per cycle will be recorded in units o readmission rates (incidence and reason for admission) and LOS recorded in days (total, including chemotherapy administration, and readmission days) o non-hematologic toxicity: focus on CNS/cerebellar and ocular as these are specific to high dose cytarabine, but will record incidences of any other grade 3-5 organ systems according to CTCAE v5 criteria.[3]

	○ time to next treatment (next chemotherapy cycle, transplant, etc.; if chemotherapy was discontinued prior to planned date, what was the reason). <u>Exploratory:</u> ● Relapse rate (RR). Relapse rate defined as incidence of confirmed relapse by peripheral blood flow or bone marrow biopsy results. Patients will be monitored up to 48 months after starting induction and will be reported if available. ● Overall survival (OS) and leukemia-free survival (LFS). Overall survival defined as the time from date of complete remission (CR) to death from any cause. Leukemia-free survival defined as time from CR to AML relapse or death, whichever comes first. Cause of death will be recorded. Patients will be monitored up to 48 months after starting induction and will be reported if available. ● Examine minimal residual disease (MRD) positivity after recovery from induction prior to study entry. The following modalities on bone marrow biopsy and aspirate will be used: flow cytometry (FC), karyotyping utilizing cytogenetics (CG), and fluorescence in situ hybridization (FISH). Patients with evidence of disease by any technique above will be classified as being MRDpos, FC will be performed on bone marrow specimens using monoclonal antibodies either as a large panel if the patient was newly evaluated or as a limited but targeted panel based on previously known patient-specific leukemia immune phenotype. MRD will be reported as a percentage of CD45 positive white blood cells (WBCs) and will be labeled MRDpos if leukemic cells account for ≥0.1% of the analyzed total WBCs. CG will be performed using standard G-banding methods on 20 metaphase cells. FISH will be reported as a percentage of abnormal nuclei among the examined 300 interphase nuclei. MRDpos by CG will be defined as abnormal karyotype seen in at least two metaphase cells, or less than two cells if it was a previously known abnormality for the given patient. FISH positivity of a prior known abnormality will be labeled MRDpos.[4] ● Pharmacogenomics: Dr. Lamba's group has developed an Ara-C SNP score (ACSS) consisting of 4 SNPs that is predictive of intracellular leukemic cell ara-CTP levels and thus impacts toxicity and efficacy to cytarabine containing regimens.[5] We will genotype SNPs of relevance (already established in Dr. Lamba's

	lab) and based on the ACSS score classify into two groups having ACSS score ≥ 0 or <0). ACSS score will be evaluated for association with certain endpoints (duration of neutropenia, thrombocytopenia, RR and OS). Peripheral blood samples will be collected and analyzed through Dr. Lamba's lab at the University of Florida and NGS results will be used to calculate the ACSS score.
Study Design:	This is a non-randomized, open-label, single group phase II study for the prospective arm. These subjects will be compared to matched historical controls who received Day 135 consolidation for the primary and secondary objectives. Historical controls will be matched with prospective subjects for age (+/- 3 years), gender (male or female), Karnofsky PS (90-100/70-80/50-60), and number of HiDAC cycles received.
Accrual Goal:	A total of 29 subjects in HiDAC 123 arm. Data collected from these subjects will be compared to data from 29 matched historical controls who received standard HiDAC 135 consolidation.
Inclusion Criteria:	Individuals eligible for study participation must meet the following criteria: A. Both males and females ≥ 61 years of age B. A clinical diagnosis of de novo, non-M3 acute myeloid leukemia (AML) confirmed by greater than 20% blasts in peripheral blood or on diagnostic bone marrow biopsy who have completed intensive induction chemotherapy and are confirmed in complete remission #1 (defined by < 5% myeloblasts on recovery BM biopsy (ANC > 1000/uL and platelets > $100x10^3$/uL) and able to receive HiDAC consolidation #1. C. Patients on the prospective arm must be willing to have labs/clinic visits at UF Health Shands after discharge from chemotherapy approximately every 48 hours +/- 24 hours until neutrophil and platelet count recovery after each chemotherapy cycle per standard of care to be included. - If prospective subjects cannot be followed at the UF site then telephone visits are allowed to follow for toxicity and transfusions. Records can be requested from subject's local physician office.

	D. Written informed consent obtained from the subject and the subject agrees to comply with all the study-related procedures as stated above. For subjects on the historical arm, there will be a waiver of informed consent (as these patients may be deceased or not be available for retrospective consent).
Exclusion Criteria:	Subjects with any of the following will not be eligible for study participation: A. Age < 61 years B. Patients unable to provide informed consent for prospective arm C. Secondary AML (documented history of antecedent hematological disorder, such as myelodysplastic syndrome or therapy-related AML) or CML in blast crisis D. Patients receiving, received, or who will receive a FLT3 inhibitor E. Patients receiving, received, or who will receive an IDH1 or IDH2 inhibitor F. SCr greater than 2 mg/dL G. Karnofsky PS of 40 or less at study entry H. History of any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of protocol therapy or that might affect the interpretation of the results of the study or that puts the subject at high risk for treatment complications, in the opinion of the treating physician I. Prisoners or subjects who are involuntarily incarcerated, or subjects who are compulsorily detained for treatment of either a psychiatric or physical illness J. For historical arm, subjects will be excluded if adequate data is not available in electronic medical record (e.g., if patient was followed by their local oncologist between chemotherapy cycles and labs/transfusions/clinic notes, etc. are not available)

Efficacy Assessments:	Duration of neutropenia after consolidation chemotherapy is primary efficacy endpoint. Neutropenia is defined in days, as the interval between the first recorded day of ANC < 500 after chemotherapy started to recovery [ANC > 500 (recorded as first day above 500 if consecutive x 2)]. Primary analysis will be with first consolidation cycle; however, data will be collected for all consolidation cycles up to six months after starting consolidation. Duration of thrombocytopenia will be a secondary efficacy endpoint. Thrombocytopenia is defined as interval between the first recorded day of platelets < 50x103/uL after consolidation to recovery (recorded as first day of platelets > 20x103/uL if consecutive x 2 and not transfused within the past 7 days). Analysis will be with first consolidation cycle; however, subjects will be monitored for up to six months after start of consolidation.
Statistical Considerations:	Group sample sizes of 23 subjects from a prospective cohort and 23 subjects from historical baseline group will achieve 81% statistical power to reject the null hypothesis of equal means when the population mean difference in neutropenia duration is three days (16 vs 19 days) with a standard deviation for both groups of four days and statistical significance level (alpha) of 0.05 using a one-sided, two sample, equal-variance t-test. Including a 20% drop out rate, we will accrue 29 subjects per cohort. The primary outcome will be analyzed using one-sided t-test with equal variance or the corresponding non-parametric statistical method, depending on the validation of the assumptions.
Estimated Enrollment Period:	36 months
Estimated Study Duration:	60 months

1. BACKGROUND

Cytarabine is a standard chemotherapy agent used in the treatment of AML. Cytarabine is an antimetabolite, specifically a pyrimidine nucleoside analog that is converted inside cells to an active metabolite that ultimately results in inhibition of DNA synthesis. It is used in various doses and can be used alone or in combination with other chemotherapy agents for the treatment of AML. High doses of cytarabine, known as HiDAC (doses > 1000 mg/m2 IV), are given in many different regimens. A standard AML consolidation regimen given after induction chemotherapy is HiDAC 1000-3000 mg/m2 IV q12 hours on Days 1, 3, and 5. HiDAC can also be given daily as an induction chemotherapy regimen in AML as HiDAC IV q12h x 12 doses (Days 1-6). This dose schedule was not based on a pharmacologic basis but has been widely used. Common side effects of cytarabine are nausea and/or vomiting, bone marrow suppression and associated toxicities (such as infection), rash, and mucositis. Specific to high-dose cytarabine only, ocular and neurologic toxicity can occur and patients are monitored closely for these adverse effects. Patients are provided with eye drops during and after chemotherapy, and given tests to evaluate for cerebellar toxicity (such as signing their name prior to each dose).

1.1 Acute Myeloid Leukemia Cancer Therapy

Acute myeloid leukemia (AML) is a rare type of cancer (1.1% of all new cancer cases); AML affects 4.3 people per 100,000 men and women per year.[6] Patients undergo treatment to induce a complete remission if fit to receive chemotherapy. Once complete remission is confirmed after induction chemotherapy, AML patients receive consolidation chemotherapy. A standard AML consolidation regimen is HiDAC (cytarabine 1000-3000 mg/m^2 IV q12h on Days 1, 3, 5 every 28 days) x 3-4 cycles in USA.[7] Many institutions use HiDAC as consolidation chemotherapy or as a bridge to hematopoietic stem cell transplantation. Dosing of cytarabine varies for treatment of AML; for consolidation, some regimens may include cytarabine 100-200 mg/m^2 continuous IV infusion Days 1-5 given in combination with anthracycline or HiDAC alone for 3-4 cycles. For induction regimens, often HiDAC is incorporated into various regimens, including cytarabine $\geq$1000 mg/m^2 IV q12h x 8-12 doses. Doses depend on age, renal function, performance status and institution/provider. Generally, patients over the age of 60 years receive cytarabine 1000 mg/m^{2}for HiDAC consolidation.[8] UF Health Shands Hospital routinely uses cytarabine 1000 mg/m2 IV q12h on Days 1, 3, and 5 up to 4 cycles (if tolerated) for standard chemotherapy consolidation in older AML patients. Jaramillo et al recently published results from two European trials showing that a modified consolidation regimen of HiDAC on Days 1, 2 and 3 reduced the duration of neutropenia and number of blood product transfusions in younger AML patients compared to standard HiDAC consolidation on Days 1, 3 and 5 with no adverse effects on long-term outcomes.[1] Dumas et al subsequently published a retrospective study of centers in France showing that adult patients age 18 to 60 years old with de novo or secondary AML

treated with the condensed HiDAC regimen showed 3 days less of neutropenia compared to the standard HiDAC regimen without any negative effect on OS, relapse or non-relapse mortality.[2] After these two trials were published, the NCCN guidelines recommended HiDAC 123 for AML patients ages 60 and under as another consolidation option.[9] Since European centers use a different induction strategy often with two induction courses before consolidation and with a different health care system, it is not clear if similar findings of safety and less health care utilization would be seen in US centers. Both published trials included only patients 60 years or younger. However, the median age at diagnosis of AML is 68 years old in the US.[6] We will evaluate this abbreviated regimen and compare it to the standard of care regimen on Days 1, 3, and 5 in our older AML patient population, to try to reproduce the hematologic toxicity results seen in younger individuals using standard practices here in the US. We do not anticipate non-hematologic safety concerns since twice daily high dose cytarabine regimens are commonly used for patients who receive a second induction regimen if leukemia is not cleared by day 14 or at recovery after the first induction chemotherapy regimen and doses are routinely given for 6 continuous days rather than the 3 days of this regimen.[10] Patients older than 50 years received cytarabine 2,000 mg/m2 IV q12h D1-6 for induction chemotherapy, and this study only reported 7% severe neurotoxicity in this population.

In the proposed exploratory analysis, we will genotype patients for SNPs of relevance to cytarabine that can generate Ara-CTP SNP scores (ACSS as described in recent publication[5]). Given small sample size we will consider dividing patients into two groups (ACSS ≥ 0 and <0) and will evaluate for association with toxicity and response to cytarabine that may be able to provide a model to identify patients who could be at higher risk for cytarabine toxicity. These SNPs are used in conjunction with next-generation sequencing (NGS) results to quantify the leukemia-associated variant allele frequencies. We hope to validate the clinical utility of ACSS to apply this score to explore if there is an association with relapse rate and risk of adverse effects in older adult AML patients undergoing HiDAC consolidation chemotherapy. This is an exploratory analysis to see if it is feasible to use this ACSS score and deep genetic sequencing to eventually be used in clinical practice and provide a patient-specific treatment plan. For example, we may be able to identify patients more at risk for adverse effects if he/she has high ACSS score and/or predict treatment failure in patients with low ACSS scores that could help guide different dosing strategies for dose optimization for individualized patients to decrease cytarabine toxicity without compromising cytarabine effectiveness. This approach could lead to the development of clinical research aimed at enhancing the adoption of best practices in the community if it is later evaluated in a larger population if we find this approach feasible.

1.2 Overview of Non-Clinical Studies

Cytarabine is a prodrug requiring activation to ara-CTP for its anti-leukemic effect. Intracellular levels of ara-CTP vary among AML patients and are critical for achieving significant leukemic cell death.[5] One of the mechanisms underlying ara-C resistance is insufficient intracellular levels of ara-CTP, which may be due to: a) inefficient cellular uptake due to transporter levels; b) reduced activation due to alterations in enzymes as DCK, CMPK1; c) increased inactivation due to NT5C2, SAMDH1, CDA, or DCTD; d) increased cellular dCTP pools (regulated by ribonucleotide reductases), that can compete with DNA incorporation of ara-CTP and also inhibit DCK activity.

Dr. Lamba's group has previously reported association of SNPs in DCK, NT5C2 and RRMs with intracellular levels of ara-CTP in leukemic cells.[11-13] Using the most informative SNPs within the ara-C metabolic pathway, we have recently developed a comprehensive Ara-CTP SNP score (ACSS) that is predictive of intracellular levels of ara-CTP within leukemic cells. See Figure 2. A comprehensive ara-CTP-SNP-score (ACSS) developed from top 4 SNPs classified patients into 3 groups: High-ACSS (score>0), Intermediate-ACSS (score=0) and Low-ACSS (score<0). ACSS designation significantly predicted with intracellular ara-CTP levels (p=0.00012), suggesting a cumulative or synergistic effect of the significant SNPs.[5] More recently a study (unpublished results) with Moffitt Cancer Center, we observed significant correlation between induction 1 response being significantly (p<0.0001) better in patients with high ACSS score (≥0) vs low ACSS score (0). These results open up opportunities for the clinical utility of ACSS score to help identify patients who may be high risk of treatment failure or toxicity based on the ACSS scores.

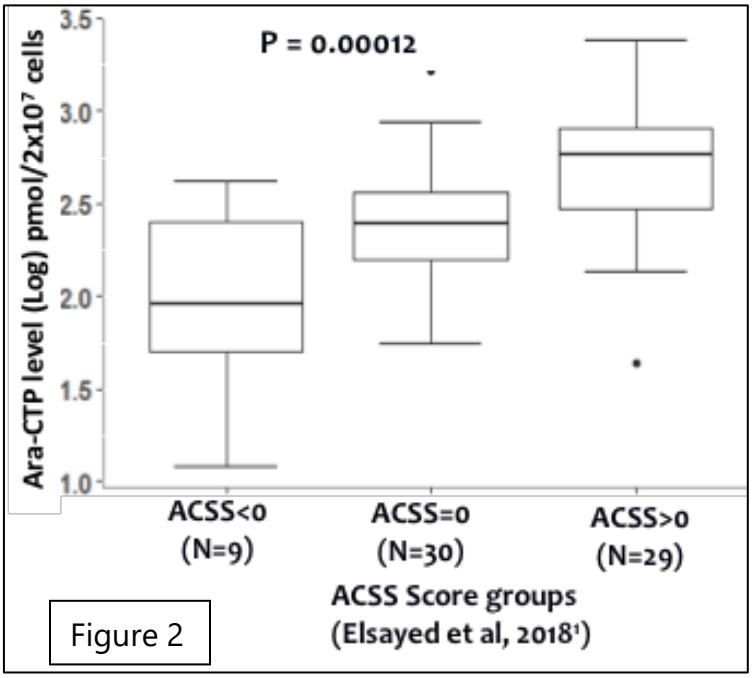


Figure 2

1.3 Overview of Clinical Studies

For more detail refer to the prescribing information for cytarabine.[14]

Historically, poor outcomes were reported when conventional doses of cytarabine were used as post-remission therapy in AML, so groups studied higher doses of cytarabine for post-remission therapy. The Cancer and Leukemia Group B (CALGB) compared various doses of cytarabine consolidation in AML patients in CR after induction therapy.[7] Subjects in this trial were randomized into three groups (each planned to receive 4 cycles of consolidation of the following): 1.) 100 mg/m2 continuous IV x 5 days, 2.) 400 mg/m2 IV daily x 5 days or 3.) 3000 mg/m2 IV q12h x 6 doses on Days 1, 3, and 5. These authors found that subjects who received high dose cytarabine were more likely to remain in CR and lived longer than patients who received lower doses of cytarabine. This trial was pivotal in establishing HiDAC as the standard post-remission/consolidation chemotherapy in the USA, as it was the first to show superior efficacy outcomes compared to anthracycline and conventional dose cytarabine for consolidation. Hematologic toxicity was main adverse effect, and 71% of patients were hospitalized due to infection or febrile neutropenia. Growth factors were not used in the trial. After excluding subjects over the age of 60 years after seeing higher rates of CNS toxicity (32%), severe CNS toxicity was recorded in 12% of subjects aged 60 years or younger.

However more than half of patients are diagnosed over the age of 60 years with lower rates of CR compared to younger patients, so Sperr and colleagues designed a trial evaluating lower doses of cytarabine as consolidation chemotherapy in elderly AML subjects in CR.[8] In this trial, patients received up to four cycles of cytarabine 1000 mg/m2 IV q12h on Days 1, 3, and 5. Prophylactic growth factor was not routinely used. Neutropenic fever was recorded in 44-72% of cycles. No severe CNS adverse effects were reported. Drug fever was seen in 32% and rash occurred in 17% of cycles. The results of this trial demonstrated that elderly AML patients can tolerate attenuated doses of HiDAC, which became the standard consolidation regimen for many institutions in the US.

In 2006, the American Society of Clinical Oncology published guidelines that recommended prophylactic white blood cell growth factors after AML consolidation chemotherapy regimens. Bradley, et al published results of patients who did or did not receive prophylactic growth factor after first cycle of consolidation chemotherapy with at least 1000 mg/m2 cytarabine q12h D1, 3, 5.[15] Patients who received granulocyte colony stimulating factor (GCSF) had less readmissions for febrile neutropenia vs patients who did not receive GCSF (14% vs 37%).

In general, the neutrophil nadir starts around Days 7-10 after HiDAC consolidation, and most patients start to recover neutrophils between Day 21 and 24, but hematologic recovery

depends on various factors, such as age, amount of prior treatments, prior antecedent hematologic disorder, etc. The use of GCSF has decreased the time to hematologic recovery. Sierra, et al showed median time to neutrophil recovery after HiDAC consolidation therapy to be 17 days if given GCSF.[16] In Jaramillo, et al, the median time to neutrophil recovery after 1-3 HiDAC consolidation(s) was 21-22 days in HiDAC 135 cohort compared to 17-18 days for subjects in the HiDAC 123 arm.1 Dumas, et al reported a median time of neutrophil recovery of 14-16 days with HiDAC 123 compared to 17-19 days with HiDAC 135.[2]

1.4 Rationale for Regimen/Doses/Schedule

This condensed schedule described by Jaramillo[1] and Dumas [2] will lessen the duration of chemotherapy inpatient admission by three days if older patients tolerate this new regimen. The authors also showed this modified HiDAC consolidation regimen reduced the duration of neutropenia and number of blood product transfusions in younger AML patients, so the benefits of this abbreviated regimen may be even greater than just three days less hospitalization. Patients were given pegfilgrastim as primary prophylaxis to prevent febrile neutropenia, however in the Jaramillo trial it was given on Day 10 in patients receiving HiDAC 135 and Day 8 in patients receiving HiDAC 123; this is typically later than many institutions in the US, as it is typically given or started approximately 24 hours after chemotherapy ends. The timing of GCSF prophylaxis described by Dumas et al more closely resembles to what we do in our practice, and the median time to recovery of neutrophils was slightly shorter compared to the results recorded by Jaramillo and colleagues. However, even in the trial described by Dumas, they described practices different than at this institution. For example, patients were routinely admitted on Days 10-12 according to blood cell counts and patients were not given bacterial prophylaxis. These trials were performed in Europe, which induction chemotherapy and supportive care measures are different than institutions in the US. Additionally, since AML patients are typically over 60 years with the median age at diagnosis of 68 years old[6,] we would like to study this patient population to potentially reproduce these reported results similar to the above trials. By reproducing these results seen in the aforementioned trials, it would make the benefits more generalizable to the AML population, especially to other institutions in the USA.

2. OBJECTIVES

2.1 Primary

2.1.1 Primary objective

- To determine the duration of neutropenia in older patients with de novo AML after consolidation chemotherapy with HiDAC 123 compared to historical controls with HiDAC 135. We expect to see a shorter duration of neutropenia in subjects who receive HiDAC 123 compared to subjects who received HiDAC 135.

2.1.2 Primary endpoint

- To determine duration of neutropenia after HiDAC123 compared to HiDAC 135. Duration of neutropenia is defined as the number of days between first absolute neutrophil count (ANC) < 500 after chemotherapy to day of recovery, defined as first day when ANC > 500 for two consecutive days.

2.2 Secondary

2.2.1 Secondary objectives

- To define duration of thrombocytopenia in older patients with de novo AML after consolidation chemotherapy with HiDAC 123 compared to historical controls with HiDAC 135.
- To determine the safety of HiDAC 123 compared to standard HiDAC 135 in case controls

2.2.2 Secondary endpoint(s)

- To determine the safety of HiDAC 123 compared to standard HiDAC 135 in case controls by evaluating the differences in:
    - duration of neutropenia and thrombocytopenia (see neutropenia definition above). Thrombocytopenia is defined as the interval between the first day of platelets < $50x10^3$/uL after consolidation chemotherapy to the first day of recovery (defined as first day of platelets > $20x10^3$/uL if consecutive x 2 and not transfused)
    - incidence of documented infections (bloodstream infection (BSI), pneumonia, invasive fungal infection (IFI), Clostridium difficile infection (CDI), typhlitis, (see Appendix A) and rates of febrile neutropenia (defined as temperature ≥ 38.3 C° once or ≥ 38 C° for more than 1 hour and ANC less than 500/mm3 or expected to fall below 500/mm3 in next 48 hours)
    - number of RBC and platelet transfusions given per cycle will be recorded in units
    - readmission rates (incidence and reason for admission) and LOS recorded in days (total, including chemotherapy administration, and readmission days)
    - non-hematologic toxicity: focus on CNS/cerebellar and ocular as these are specific to high dose cytarabine, but will record incidences of any other grade 3-5 organ systems according to CTCAE v5 criteria
    - time to next treatment (next chemotherapy cycle, transplant, etc.; if chemotherapy was discontinued prior to planned date, what was the reason)

2.3 Exploratory

2.3.1 Exploratory objectives

- Pharmacogenomics: to evaluate impact of ACSS score for association with toxicity and efficacy in HiDAC 123 arm
- Describe RR, LFS and OS in each cohort

2.3.2 Exploratory endpoints

- Record pharmacogenomic profiles if results available from next generation sequencing (NGS)/GatorSeq on bone marrow (BM) biopsies for both arms (collected at diagnosis as standard of care).
- Compute ACSS SNP score using the genotype data for subjects in HiDAC 123 arm. We will genotype SNPs of relevance and based on the ACSS score classify into two groups having ACSS score ≥ 0 or <0). ACSS score will be evaluated for association with certain endpoints (duration of neutropenia, thrombocytopenia, RR and OS). Peripheral blood samples will be collected and analyzed through Dr. Lamba's lab at the University of Florida and NGS results will be used to calculate the ACSS score.
- Describe relapse rate (RR). Relapse rate defined as incidence of confirmed relapse by peripheral blood flow or bone marrow biopsy results. Patients will be monitored up to 48 months after starting induction and will be reported if available.
- Describe overall survival (OS) and leukemia-free survival (LFS). Overall survival defined as the time from date of complete remission (CR) to death from any cause. Leukemia-free survival defined as time from CR to AML relapse or death, whichever comes first. Cause of death will be recorded. Patients will be monitored up to 48 months after starting induction and will be reported if available. These are not expected to be different between the two cohorts.[1,2]
- Examine minimal residual disease (MRD) positivity after recovery from induction prior to study entry. The following modalities on bone marrow biopsy and aspirate will be used: flow cytometry (FC), karyotyping utilizing cytogenetics (CG), and fluorescence in situ hybridization (FISH). Patients with evidence of disease by any technique above will be classified as being MRD_{pos}, FC will be performed on bone marrow specimens using monoclonal antibodies either as a large panel if the patient was newly evaluated or as a limited but targeted panel based on previously known patient-specific leukemia immune phenotype. MRD will be reported as a percentage of CD45 positive white blood cells (WBCs) and will be labeled MRD_{pos} if leukemic cells account for ≥0.1% of the analyzed total WBCs. CG will be performed using standard G-banding methods on 20 metaphase cells. FISH will be reported as a percentage of abnormal nuclei among the examined 300 interphase nuclei. MRD_{pos} by CG will be defined as abnormal karyotype seen in at least

two metaphase cells, or less than two cells if it was a previously known abnormality for the given patient. FISH positivity of a prior known abnormality will be labeled MRD_{pos}.

3. STUDY DESIGN

3.1 Study Overview

This is a single center, open-label, non-randomized phase II study. Twenty nine (29) subjects are planned to be enrolled in this study. Each subject will be administered cytarabine 1000 mg/m^2 IV q12h x 6 doses on Days 1-3 in the hospital during the consolidation treatment phase. Subject may receive up to four consolidation cycles. Subjects will have laboratory and toxicity assessments as clinically indicated (at baseline, then daily while inpatient and ideally every 48 hours after hospital discharge +/- 24 hours until count recovery from each cycle). These subjects will be compared to 29 historical controls [matched by age (within 3 years), gender (male or female), PS (Karnofsky: 90-100, 70-80, or 50-60) and number of HiDAC cycles received] who received HiDAC 135 from the time period of 2/1/2017-2/1/2019.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria and who have consented will be entered into the study. The following treatment regimen will be used for prospective arm: cytarabine 1000mg/m2 IV q12h x 6 doses (Days 1-3) for up to four cycles.

Historical arm subjects will have received cytarabine 1000 mg/m2 IV q12h x 6 doses (Days 1, 3, and 5) for up to four cycles from the time period of 2/1/2017-2/1/2019.

Total duration of subject participation will be up to six months from start of first consolidation cycle (each cycle is 28 days, but more time may be required allowing extra time for delayed count recovery in some patients); study participation will end when subject recovers neutrophils and platelets after last administered cycle of chemotherapy, is admitted for conditioning chemotherapy for HCT, dies, or withdraws consent (whichever comes first). However, subjects will be followed every 3 months ($\pm$ 28 days) for up to 48 months after start of induction to determine long-term outcomes. Total duration of the study is expected to be 60 months.

4. SELECTION OF SUBJECTS

Subjects with a clinical diagnosis of de novo acute myeloid leukemia in CR1 who meet the following inclusion and exclusion criteria will be eligible for participation in this study.

4.1 Number of Subjects

Twenty nine (29) subjects are expected to participate in the prospective arm of the study.

4.2 Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for study participation:

A. Both males and females ≥ 61 years of age
B. A clinical diagnosis of de novo, non-M3 acute myeloid leukemia (AML) confirmed by greater than 20% blasts in peripheral blood or on diagnostic bone marrow biopsy who have completed intensive induction chemotherapy and are confirmed in complete remission #1 (defined by < 5% myeloblasts on recovery BM biopsy, ANC > 1000/uL and platelets > $100x10^3$/uL) and able to receive HiDAC consolidation #1
C. Patients on the prospective arm must be willing to have labs/clinic visits at UF Health Shands approximately every 48 hours +/- 24 hours after discharge from chemotherapy admission to be included
   - If prospective subjects cannot be followed at the UF site then telephone visits are allowed to follow for toxicity and transfusions. Records can be requested from subject's local physician office.
D. Written informed consent obtained from the subject and the subject agrees to comply with all the study-related procedures. For subjects on the historical arm, there will be a waiver of informed consent (as these patients may be deceased or not be available for retrospective consent).

4.3 Exclusion Criteria

Subjects with any of the following will not be eligible for study participation:

A. Age < 61 years
B. Patients unable to provide informed consent for prospective arm
C. Secondary AML (*documented* history of antecedent hematological disorder, e.g., myelodysplastic syndrome or therapy-related AML) or CML in blast crisis
D. Patients receiving, received, or who will receive a FLT3 inhibitor
E. Patients receiving, received, or who will receive an IDH1 or IDH2 inhibitor
F. SCr greater than 2 mg/dL
G. Karnofsky PS of 40 or less at study entry
H. History of any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of protocol therapy or that might affect the interpretation of the results of the study or that puts the subject at high risk for treatment complications, in the opinion of the treating physician
I. Prisoners or subjects who are involuntarily incarcerated, or subjects who are compulsorily detained for treatment of either a psychiatric or physical illness

J. For historical arm, subjects will be excluded if adequate data is not available in electronic medical record (e.g., if patient was followed by their local oncologist between chemotherapy cycles and labs/transfusions/clinic notes, etc. are not available)

4.4 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

5. REGISTRATION PROCEDURES

All subjects must be registered with the UF Health Cancer Center prior to participation in this trial and must meet all subject eligibility requirements and complete informed consent. All consented subjects must be entered into a secured electronic data base that is encrypted and password protected.

All eligible and enrolled subjects will receive a unique subject number. This number will be used to identify the subject throughout the study. Subjects withdrawn from the study will retain their subject number.

6. STUDY PROCEDURES

Written informed consent must be obtained prior to performing any study-specific evaluations or tests. Tests or evaluations performed as standard of care within the specified screening period, but prior to informed consent, may be accepted for this study and need not be repeated. Screening measurements below will be obtained within 60 days prior to registration into the study. **After registering a subject into this study, study treatment must begin within 30 days or subject will need to be re-screened**:

- Demographic data, including date of birth, gender, race, ethnicity and zip code
- Medical history, including h/o MDS or other conditions that could cause secondary or treatment-related AML or other conditions that may have major psychiatric or medical illness(es) that may impact if subject able to consent or be monitored for cerebellar toxicity
- Previous diagnostic assessments and treatment(s) for AML including regimens and dates
- Medications that may exclude subject (such as FLT3 inhibitors, e.g. midostaurin, sorafenib, gilteritinib, or any investigational FLT3 inhibitor as well as IDH1 and IDH2 inhibitors, e.g., ivosidenib, enasidenib or any investigational IDH inhibitor
- Bone marrow aspirate and/or biopsy for clinical classification, flow cytometry, cytogenetic classification, molecular markers, NGS for identification of commonly tested mutations in AML at diagnosis and at hematologic recovery after induction chemotherapy

- Hematology laboratory evaluations:
  - Complete blood count (CBC) including hemoglobin, hematocrit, white blood cell (WBC) absolute with differential, and platelet count
- Blood chemistry laboratory evaluations: serum creatinine
- Blood transfusions, growth factors, anti-infective medications, eye drops

6.1 Schedule of Events

Visit: / **Procedure:**	**SCREENING (≤ 60 days prior to Day 1)**	**CYCLE 1**		**CYCLES 2 TO 4 IF SUBJECT CONTINUES**		**END OF TREATMENT[8] (+/- 14 DAYS)**	**FOLLOW UP (UP TO 48 MONTHS AFTER START OF INDUCTION)[9] EVERY 3 MONTHS (+/- 28 DAYS)**
		Day 1	**At each clinic visit per SOC (as clinically indicated ideally q48h+/- 24h)**	**Day 1**	**At each clinic visit per SOC (as clinically indicated ideally q48h+/- 24h)**		
Informed Consent	**X**						
Demographic Information	**X**						
Medical and Cancer Treatment History[1]	**X**						
Medication History[2]	**X**						
Bone Marrow Biopsy and/or Aspirate[3]	**X**					As clinically indicated	
Interval History/Physical Exam[10]	**X**	**X**	PRN SOC	**X**	PRN SOC	PRN SOC	
Karnofsky Performance Status[4]		**X**					
BSA Used for Chemotherapy		**X**		**X**			
Vital Signs	**X**	**X**	PRN SOC	**X**	PRN SOC	**X**	
Pharmacogenomic Sample Collection		**X**					
Blood Chemistry Evaluations[5]	**X**	**X** (+/- 72 HOURS AS SOC)	PRN SOC	**X** (+/- 72 HOURS AS SOC)	PRN SOC	PRN SOC	
CBC w/Diff	**X**	**X** (+/- 72 HOURS AS SOC)	PRN SOC	**X** (+/- 72 HOURS AS SOC)	PRN SOC	PRN SOC	
Administration of Blood and Platelet Transfusions		PRN SOC	PRN SOC	PRN SOC	PRN SOC	PRN SOC	
Administer Cytarabine Inpatient (q12h Days 1-3)		**X**		**X**			
Concomitant Medication Review[6]		PRN SOC	PRN SOC	PRN SOC	PRN SOC	PRN SOC	

Adverse Event Review/Toxicity Assessment[7]		PRN SOC	PRN SOC	PRN SOC	PRN SOC	PRN SOC	
RR and Survival Status (LFS and OS)						**X**	**X**

1) The bone marrow assessment, cytogenetics NGS, and/or molecular markers at diagnosis will be documented.
2) Document specific medications that may exclude patient that have been taken during the 30 days prior to screening.
3) The screening bone marrow assessment must be performed within 30 days after start of induction therapy, and must be reviewed at UF. This must confirm a diagnosis of AML, non-M3 in CR1 (less than 5% blasts on BM biopsy when ANC >1000 and platelets greater than 100). Results will be recorded, including cytogenetics and NGS and/or molecular markers.
4) Performance status will be collected on Day 1 of Cycle 1; however, if this is not documented on this admission progress note, the last documented value within the screening periods will be used for baseline Karnofsky performance status.
5) Blood chemistries including blood urea nitrogen, creatinine, alkaline phosphatase, AST, ALT, total bilirubin
6) Document supportive care medications (pegfilgrastim or equivalent 48 hours +/- 24 hours after chemo ends; antimicrobial prophylaxis when neutropenic and other anti-infectives needed, eye drops until 48 hours after cytarabine ends, etc.) Supportive care medications and blood transfusions should be documented throughout and for 28 days after last treatment.
7) Monitoring for SAEs at the time of consent and AEs collection will start the day patient starts cycle 1 of consolidation chemotherapy and continuing throughout the study. Subjects who have received at least one dose of study drug and discontinue prematurely (regardless of reason) will be monitored for any adverse events that occur during study until 28 days following the most recent dose of study treatment or initiation of a new chemotherapy regimen (whichever comes first). If a SAE or AE that is deemed related to the study intervention and ongoing at the end of the monitoring period the event will be monitored until; resolution, event returns to baseline or in the investigator's opinion, deems the event is unlikely to resolve due to the patients underlying disease.
8) End of treatment visit will occur in subjects who complete 3-4 cycles of consolidation or at study withdrawal (for example, at HCT conditioning, relapsed disease or if ended earlier than planned due to toxicity or physician discretion). Other assessments will be performed as necessary by the treating physician. Outside lab results may be collected if subject unavailable to travel to the institution.
9) All subjects will be followed for long term outcomes until: relapse, death, or until closure of study.
10) This data may be collected via telephone interview.

Abbreviations:

SOC= standard of care; PRN = as needed; BSA = OS = overall survival; SAE = severe adverse effects, AEs = adverse effects; HCT = hematopoietic stem cell transplant

6.2 End of Treatment Evaluations

The End of Treatment date will be the date the subject has met one of the following:
- 3-4 cycles of planned consolidation treatment
- Study withdrawal
- HCT conditioning
- Treatment ended earlier than planned (due to toxicity or physician discretion)
- Relapsed disease

All subjects will have the following procedures documented:

- Reason for end of treatment (relapse or transplant, etc.)
- Supportive care medications and transfusions if needed
- AE assessment
- Hematology laboratory evaluations (unless recovery was documented in a previous visit): complete blood count (CBC) including hemoglobin, hematocrit, white blood cell (WBC) including differential and platelet count
- Assessed for complete hematologic recovery
    - Reference Appendix C. ELN Guidelines

6.3 Follow up Evaluations

Subjects will have follow-up visits for the evaluation of adverse events, labs, etc. per standard of care (after discharge from chemotherapy administration, generally every 48 hours +/- 24 hours until neutrophil and platelet recovery after each consolidation chemotherapy cycle). Patients will be evaluated prior to starting next chemotherapy cycle, then admitted for chemotherapy and monitored while inpatient.

If the subject is unavailable to travel to the institution (if situation changes from study inclusion criteria after subject starts first consolidation cycle), the visit may be made via telephone interview to review for adverse event resolution or the occurrence of any new adverse events, and outside labs reviewed if collected. Additionally, medical records may be requested with permission of the subject to complete remote visit reporting e.g., if admitted to an outside institution due to complications of chemotherapy.

If the subject is at the treating facility and the physician decides to hold, reduce, or omit the dose completely, the following procedures should be performed:

- Document reason for delay, decreased or missed doses
- AE assessment
- Hematology laboratory evaluations: complete blood count (CBC) including hemoglobin, hematocrit, white blood cell (WBC) with differential and platelet count

If study therapy is discontinued before planned, disease assessments will be performed as clinically indicated, such as suspicion of relapse. Reason for discontinuation should be documented, e.g., relapse, proceeding to HCT, unexpected toxicity/poor tolerance of chemotherapy, etc. Subjects will continue to be followed every 3 months (± 28 days) for survival data up to 48 months after induction chemotherapy is started, or until relapse or death (whichever comes first). Following discontinuation of study therapy for any reason, report the date as appropriate (date of confirmed relapse, date of death, date of start of HCT conditioning, or recovery from last planned or unplanned cycle).

If a subject becomes unreachable during the course of the study, the investigator or study team will make a reasonable effort to contact the subject and document each attempt. If these attempts are not successful, the subject may be declared "lost to follow up." For subjects lost to follow-up, the termination date will be the date of last contact with the subject.

7. STUDY TREATMENT

7.1 Treatment Schedule/Administration

Cytarabine administration will be recorded including, but not limited to, date of administration, dose (total and mg/m2) and any changes in dose administration (e.g., interruption or reduction in dose due to an adverse event). Cytarabine will be scheduled to be given IV q12h on the first three days of each 28-day treatment cycle, unless there has been a scheduled modification of administration due to not meeting treatment parameters, such as hematologic recovery or toxicity. The product will be administered per institutional standard in terms of nursing administration and pharmacy preparation. Antiemetic medication(s) should be given 30-60 minutes prior to administration of cytarabine per institutional guidelines, and agents may be modified for individual patients based on risk factors and previous antiemetic responses. Eye drops (steroid or artificial tears) should be given prior to cytarabine and continued for 48 hours after last dose of cytarabine given. Prophylactic antimicrobials should be given as standard of care. Pegfilgrastim 6 mg SQ (or equivalent) should be given once 48 hours +/- 24 hours after completion of chemotherapy. Filgrastim (or equivalent) may be given as an alternative (5 mcg/kg rounded to vial size daily until WBC recovery). If patient is unable to receive these aforementioned medications, the reason should be documented.

Patients will be monitored for cerebellar toxicity using NCI CTCAE v5 toxicity grades while admitted for high dose cytarabine. Patients will be asked to sign his/her name prior to each dose of chemotherapy. If there is suspicion of neurotoxicity, a provider should assess the patient as clinically indicated.

7.2 Dose Calculations

Chemotherapy will be dosed according to institutional standard, unless otherwise specified. Generally, cytarabine will be dosed on actual/total body weight, and body surface area (BSA) will be capped at 2.4 m^2.

BSA will be documented per Mosteller formula.
BSA (m^2) = √height (cm) x weight (kg)/3600

7.3 Supportive Care

7.3.1 Supportive Care Guidelines

Subjects should receive full supportive care, including transfusions of blood products, antimicrobials, antiemetics, antidiarrheals, eye drops, analgesics, etc., when appropriate. Supportive care will be administered to all study subjects per institutional guidelines. Subjects will be counseled on the expected side effects of cytarabine and what to do if/when these occur.

Growth factors (granulocyte-colony stimulating factor [G-CSF]) should be given if able, according to the institutional standard of care.[17] Pegfilgrastim (or biosimilar product) 6 mg SQ once 48 hours +/- 24 hours (or on-body injector on the last day of chemotherapy) is generally used for primary prophylaxis of febrile neutropenia. If patient is unable to obtain pegfilgrastim, then filgrastim (or biosimilar) 5 mcg/kg rounded to vial size shall be administered SQ daily until ANC recovery.

Antiviral, antifungal, and antibacterial prophylaxis and treatment should follow conventional guidelines and institutional guidelines.[18] In general, levofloxacin, fluconazole (or mold-active azole if patient has history of IFI), and valacyclovir (or acyclovir) are started when ANC is approximately 500/mm3 or below and continued until count recovery. See Appendix B for UF Health Shands Hospital transfusion guidelines.

7.3.2 Concomitant Therapy

Relevant medical history should be obtained at screening and include prior AML treatment history. Medications will be reviewed at screening for FLT3 and IDH 1/2 inhibitors to assess exclusion criteria.

The use of concomitant therapy will be collected, such as supportive care (e.g., growth factors, antimicrobials, eye drops) throughout consolidation therapy and will be administered per institutional guidelines or patient-specific treatments per the treating physician.

7.3.3 Infections

Chemotherapy puts the patient at higher risk for bacterial, viral, or fungal infections, which are potentially life-threatening. Prophylactic antimicrobial medications and prophylactic granulocyte colony stimulating factor will be initiated per institutional guidelines if able, and patients will be closely monitored for signs of infections and will receive early and appropriate treatment per standard of care. Infections will be scored according to infections listed in Appendix A.

7.3.4 Prohibited Concomitant Therapy

Supportive care measures consistent with optimal subject care will be permitted throughout the study, as long as the therapy is not included in this section as prohibited.

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- FLT3 inhibitors, e.g., midostaurin, sorafenib, gilteritinib, or any investigational FLT3 inhibitor)
- IDH 1 and IDH2 inhibitors, e.g., ivosidenib, enasidenib or any investigational IDH inhibitor

7.3.5 Hematopoietic Stem Cell Transplantation Treatment

Subjects may undergo allogeneic stem cell transplant after study entry; at the start time of conditioning therapy, the subject will be removed from the study. The time to next treatment (date of conditioning) will be recorded. The conditioning regimen, donor type and graft source during the allogeneic HCT period will be recorded.

7.4 Dose Modifications

The National Cancer Institute (NCI) Common Toxicity Criteria (***v5.0***) for Adverse Events (CTCAE) will be used to grade toxicity (http://evs.nci.nih.gov/ftp1/CTCAE/About.html).[3]

Subjects will generally receive cytarabine 1000 mg/m2 IV q12h x 6 doses. Dose modifications may occur per physician discretion. If dose or duration is different than stated above, the reason should be recorded.

7.4.1 Non- Hematologic Toxicity

Dose modifications or interruptions may occur per standard of care or per the treating physician based on patient tolerability.

7.4.2 Hematologic Toxicity

Dose modifications or interruptions may occur per standard of care or per the treating physician based on patient tolerability.

8. TREATMENT DISCONTINUATION

8.1 Screen Failures

Subjects who sign informed consent, but do not meet eligibility criteria, or withdraw prior to eligibility being verified, and undergo at least some of the screening procedures will be considered screening failures. Subjects who sign informed consent and are verified as eligible, but do not proceed to formal registration will be considered "unregistered." A record of screen failures and unregistered subjects will be maintained by the study site.

8.2 Criteria for Study Treatment Discontinuation

Subjects who discontinue participation in the clinical study on their own or subjects who are withdrawn by the investigator, for reasons other than completion of treatment, proceeding to HCT, relapse or toxicity, will be defined as premature withdrawals. The end of treatment data will be collected if available at last visit if confirmed relapse, death, or proceeding to HCT. For those with toxicity that end before planned last cycle, data will be collected up to the end of treatment date and AE data will be collected until 28 days following the most recent dose of study treatment or initiation of a new chemotherapy regimen (whichever comes first).

Subjects will be followed for long term data every 3 months (± 28 days) until relapse, death or until 48 months after induction therapy (whichever comes first).

A subject will be discontinued from protocol therapy under the following circumstances:

- Any adverse event which, in the Investigator's opinion, requires termination of the chemotherapy
- Disease relapse
- If subject proceeds to allogeneic HCT
- The subject uses illicit drugs or other substances, or takes part in activities that may, in the opinion of the Investigator, have a reasonable chance of contributing to toxicity or otherwise interfering with results
- The subject is lost to follow-up
- Death
- Development of an intercurrent illness or situation which would, in the judgment of the investigator, affect assessments of clinical status and study endpoints to a significant degree

The Investigator will make every reasonable effort to keep each subject in the study unless it is in the subject's best interests to discontinue participation. If a subject is removed from the study or declines further participation, all EOT evaluations should be performed if the subject is willing and able to be assessed. A description of the reason(s) for withdrawal from the study must be recorded on the case report form (CRF). The Investigator should also ensure that all subjects are followed up for survival status after the Final Visit.

Subjects who discontinue following entry will have relevant information completed and recorded on the CRF. All subjects who discontinue because of adverse events or clinically significant laboratory abnormalities should be followed up until they recover or stabilize, and the subsequent outcome will be recorded. If any subject should experience an unexpected serious adverse event during the trial or within 28 days of stopping study treatment, the Investigator will inform the UF Health Data Integrity and Safety Committee.

8.3 Replacement of Subjects

Subjects will be replaced if they have not received any doses of study drug prior to their withdrawal.

9. BIOLOGICAL SPECIMENS AND CORRELATIVES

9.1 Source of Specimens

Peripheral blood (<5 mL) will be collected in subjects on the prospective arm for exploratory analysis. Only one sample is needed, and this will be during the pre-admission labs prior to first consolidation chemotherapy cycle.

All correlative studies will be performed in Dr. Lamba's laboratory at University of Florida. Peripheral blood samples will be collected and analyzed in Dr. Lamba's laboratory. We will use genotype patient samples for the 4 SNPs that are part of ACSS score and compute ACSS score for each patient and patients will be classified into having a low (<0) or high ACSS (>=0).

9.2 Correlative Studies

We will compare the ACSS score for association with clinical endpoints using appropriate statistical tests as used in our previous work. Statistical analysis will be performed to compare overall-survival (OS), between ACSS groups using Cox regression and Fisher's exact test for toxicity endpoints and Wilcoxon test for association of ACSS score with duration or neutropenia/thrombocytopenia.

9.3 Preparation, Shipment and Storage of Specimens

Three to five mL of peripheral blood will be collected in EDTA tube on pre-admission or admission day of C1D1 of HiDAC consolidation. Storage in -80 deg C is preferred but sample can be kept at 40 deg C to send to the laboratory. Specimens will be processed after funding is obtained.

10. STUDY DRUG INFORMATION[14]

10.1 Study Drug Name

Cytarabine (Cytosar®); multiple manufacturers; aka cytosine arabinoside, Ara-C.

10.2 Identification

Cytarabine, initially developed by Pfizer, is a standard of care chemotherapy used for intravenous administration in the management of AML patients. Cytarabine is a colorless solution that is diluted in 0.9% sodium chloride. At high doses, it is usually diluted in 250 mL IV and given over 2-3 hours. Preparation and administration is per institutional guidelines and policies as applicable.

10.3 Packaging and Labeling

Vials are supplied from institution's wholesaler via commercial supply. Preparation and labeling of IV infusion will follow institutional policies and procedures.

10.4 Drug Supply

Vials are supplied from institution's wholesaler via commercial supply.

10.5 Storage, Handling and Dispensing

Storage, handling and dispensing of cytarabine and other supportive care medications will follow institution's policies and procedures. This product will be given per SOC except for the interval/days for the HiDAC 123 arm. The pharmacist will verify and dispense the medication(s) and nursing will administer medications as above per institutional policies and procedures.

10.6 Drug Ordering and Accountability

Drug product ordering and accountability will follow institutional policies and procedures.

10.7 Infusion Reactions

Severe allergic reactions are very rare with cytarabine (<1%). Nurses should monitor the patient for any signs/symptoms of anaphylaxis and notify provider if these occur. Patient should be treated per conventional and institutional guidelines for treatment of anaphylaxis. Two adverse effects that patients should be monitored for while receiving cytarabine and shortly after are ocular and neurotoxicity as stated previously in protocol.

10.8 Contraindications

Allergy to cytarabine, patients unable to monitor for ocular or neurotoxicity or any other condition that the treating physician feels is a contraindication to receiving high doses of cytarabine.

10.9 Special Warnings and Precautions for Use

Bone marrow suppression and associated complications (e.g., bleeding and infection), chemical conjunctivitis, and cerebellar toxicity are the most significant complications of high-dose cytarabine. Cytarabine is a chemotherapy agent, so standard universal precautions should be followed in terms of handling and administering the product and handling any bodily secretions and blood of patients receiving cytarabine per SOC and institutional policies and procedures.

There are no clinically significant drug interactions with single agent cytarabine regimens.

10.10 Adverse Event Profile

Most Frequent Adverse Reactions

Anorexia	fever	thrombocytopenia
Nausea	rash	anemia
Vomiting	thrombophlebitis	neutropenia
Diarrhea	oral mucositis	infections

Less Frequent Adverse Reactions

Sepsis	esophageal ulceration	shortness of breath
Bleeding	esophagitis	alopecia
conjunctivitis	chest pain	anaphylaxis
urinary retention	pericarditis	allergic edema
renal dysfunction	bowel necrosis	pruritis

Neuritis	abdominal pain	urticaria
neurotoxicity	pancreatitis	hand-foot syndrome
Headache	hepatic dysfunction	skin ulceration
Dizziness	jaundice	

11. ADVERSE EVENTS

11.1 Definitions

11.1.1 Adverse Event

The term "adverse event" (AE) includes any sign, symptom, syndrome, or illness that appears or worsens in a subject during the period of observation in the clinical study and that may impair the wellbeing of the subject. The term also covers laboratory findings or results of other diagnostic procedures that are considered to be clinically significant (e.g., that requires unscheduled diagnostic procedures or treatment measures, or result in withdrawal from the study). An AE is therefore any unfavorable and unintended symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product.

The adverse event may be:

- A new illness/condition;
- Worsening of a sign or symptom of the condition under treatment, or of a concomitant illness/condition;
- An effect of the study drug; or
- A combination of 2 or more of these factors.

No causal relationship with the study drug or with the clinical study itself is implied by the use of the term "adverse event."

The Investigator or his/her designee will assess for occurrence of AEs during hospitalization and each outpatient visit (or via telephone survey if patient is unable to return to clinic at the planned time). AEs related to cytarabine will be recorded in the subject CRF. AEs will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

Surgical procedures themselves are not adverse events; they are therapeutic measures for conditions that require surgery. The condition(s) for which the surgery is required may be an adverse event. Planned surgical measures permitted by the clinical study protocol and the condition(s) leading to these measures are not adverse events.

When a clear diagnosis is available that explains the abnormal objective findings, this diagnosis will be recorded as an adverse event and not the abnormal objective findings (e.g., viral hepatitis will be recorded as the adverse event and not the transaminase elevation). If a definitive diagnosis is not available, then the sign(s) (e.g., clinically significant elevation of transaminase levels) or symptom(s) (e.g., abdominal pain) will be recorded as the adverse event.

Adverse events fall into the categories "serious" and "non-serious."

11.1.2 Serious Adverse Event

A serious adverse event is one that at any time during the period of observation:

- Results in death
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is an important medical event, defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based on appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed above. Examples of such events include but are not limited to intensive treatment in an emergency department or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization. "Medically important" should be marked only if no other serious criteria are met.

An "unexpected SAE" is any SAE for which the nature, specificity or severity is not consistent with the currently known adverse event profile of cytarabine.

Clarification of the difference in meaning between "severe" and "serious"

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). Any grade ≥ 3 adverse event per CTCAE is generally considered severe AE. This is not the same as "serious," which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

11.1.3 Non-Serious Adverse Event

A non-serious adverse event is any adverse event not meeting any of the serious adverse event criteria.

11.2 Period of Observation

Following the subject's written consent to participate in the study, all SAEs must be collected, including those thought to be associated with protocol-specified procedures. Collection of all SAEs must continue for at least 28 days after the last dose of study treatment or until initiation of new chemotherapy regimen (whichever comes first).

The investigator will begin collecting ocular or neurologic toxicities of any grade related to cytarabine and other grade 3-5 non-hematologic and hematologic toxicities and associated toxicities, such as bleeding and infection once administration of cytarabine is initiated. Treated subjects, including those who were prematurely discontinued from the study, will be monitored for any adverse events that occur during the study until 28 days following the most recent dose of study treatment or initiation of a new chemotherapy treatment (whichever occurs first). If another course of anti-cancer therapy is initiated prior to the 28 day follow-up period visit, collection of adverse events will no longer be performed, with the exception of events that may be possibly, probably, or definitely related to cytarabine or are clinically significant. If AE is deemed related to the study intervention and ongoing at the end of the monitoring period, the event will be monitored until resolution, returns to baseline or in the investigator's opinion, deems the event is unlikely to resolve due to the patients underlying disease.

11.3 Documenting and Reporting of Adverse Events by Investigator

Ocular or neurologic adverse events of any grade related to cytarabine and other grade 3-5 non-hematologic and hematologic toxicities and associated toxicities, such as bleeding and infection must be fully recorded in the subject's case record form.

Revise documentation and recording requirements as required.

A laboratory test abnormality considered clinically relevant, e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event. Each event should be described in detail along with start and stop dates, severity, relationship to cytarabine, action taken and outcome.

Every attempt should be made to describe the adverse event in terms of a diagnosis that encompasses the component signs and symptoms. If only nonspecific signs or symptoms are present, then these should be recorded as separate diagnoses on the pages of the case report form.

All subjects who have adverse events, whether considered associated with the use of study drug or not, must be monitored to determine the outcome. The clinical course of the adverse event will be followed according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the Principal Investigator considers it medically justifiable to terminate follow-up. Should the adverse event result in death, a full pathologist's report should be supplied, if possible.

11.3.1 Assessment of Causal Relationship of Study Drug

The Investigator will provide an assessment of the potential causal relationship between adverse events and study medication by determining whether or not there is a reasonable possibility that the event was caused by the study medication. The relationship or association of the adverse event to the study medication will be characterized as not related, unlikely related, possibly related, probably related, or related:

Not Related: There is not a temporal relationship to the study drug administration or the adverse event is clearly due only to the progression of the underlying disease state, intercurrent illness, concomitant medication, concurrent therapy or other known cause.

Unlikely Related: There is little or no chance that the study drug administration caused the adverse event; the event is most likely due to another competing cause, including intercurrent illness, progression or expression of the disease state, or a reaction to a concomitant medication or concurrent therapy appearing to explain the reported adverse event.

Possibly Related: The association of the adverse event with the study drug administration is unknown; however, the adverse event is not reasonably attributed to any other condition.

Probably Related: When a reasonable temporal relationship exists between the adverse event and the study drug administration; significant symptoms abate upon discontinuation of the study drug and there is a reasonable explanation based on known characteristics of the study drug and there is no clear association with preexisting disease or therapy, intercurrent illness, concurrent therapy or other factor(s).

Related: When the adverse event is a known side effect of the study drug or there is a temporal relationship to the administration of the study drug; or the adverse event reappears upon re-administration of the study drug (rechallenge); or the significant symptoms of the adverse event abate upon discontinuation of the study drug (dechallenge).

11.3.2 Intensity of Adverse Events

The intensity of adverse changes in physical signs or symptoms will be graded according to the most up-to-date CTCAE version. For all other adverse events not described in the CTCAE, the intensity will be assessed by the Investigator using the following categories:

Mild (Grade 1) – transient or mild discomfort; no limitation in activity; no medical intervention/therapy required.

Moderate (Grade 2) – mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention/therapy required.

Severe (Grade 3) – marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible.

Life-threatening (Grade 4) – extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable.

Death (Grade 5) – the event resulted in death.

11.3.3 Action Taken with Study Drug

The action the Investigator took with study drug as a result of the event should be recorded as one of the following:

None – No action was taken with regard to chemotherapy as a result of the adverse event.

Interrupted – Chemotherapy was stopped due to the adverse event, but was later resumed at the same dose.

Dose decreased – The dose of cytarabine was decreased as a result of the adverse event.

Permanently discontinued – The subject was withdrawn from the study due to the adverse event.

Only one item should be chosen. If multiple actions apply, the following "worst case" scenario hierarchy should be used to determine the preferred entry:

permanently discontinued > dose decreased > interrupted.

11.3.4 Definition of Outcome

The outcome of the AE should be recorded as one of the following:

Resolved without sequelae – The subject fully recovered from the adverse event with no observable residual effects.

Resolved with sequelae – The subject recovered from the adverse event with observable residual effects.

Not resolved – The adverse event was present at the time of last observation.

Death – The subject died as a result of the adverse event.

11.4 Reportable Events

11.4.1 Serious Adverse Events

Serious (requiring hospitalization and/or increased level of care) adverse events (SAE's) that are unexpected (not in informed consent) and most likely related to cytarabine must be reported to the IRB within five working days. If there are more than three grade 3-5 neurologic adverse events, these will be reported to the IRB within 5 working days, and enrollment will be suspended until each case is evaluated for causality. If only limited details are known, these should be reported within that time frame and follow up reports can be submitted for elaboration, clarifications, or corrections. Any email correspondence must be kept in the trial file at the study site. The site investigator is responsible for informing the IRB and/or the Regulatory Authority of the SAE as per local requirements.

Follow-up information will be submitted to the IRB stating that this is a follow-up to a previously reported SAE and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the participant continued or withdrew from study participation.

12. STATISTICAL METHODS

The sections below provide an overview of the statistical considerations and analyses.

12.1 Data collection and management plan

Data will be collected using a CRF managed by the Oncology Clinical Pharmacy Specialists under the supervision of the PI. Information collected from the CRF will be entered into a secure, encrypted, password protected that only the PI and co-investigators have. Regulatory specialists and managers will be involved with the protocol once IRB application is submitted and while the protocol remains open with the IRB.

12.2 Sample Size Justification

This is a prospective single group study to determine the duration of neutropenia after consolidation chemotherapy with HiDAC 123. A historical control group will be selected by identifying patients who received cytarabine 1000 mg/m2 IV q12h D135 from 2/1/2017-

2/1/2019 by propensity matching factors: age (+/- 3 years), gender (male or female), Karnofsky PS (90-100/70-80/50-60), and number of HiDAC cycles. The hypothesis is that patients with HiDAC123 treatment schema have a shorter duration of neutropenia after consolidation chemotherapy, compared to matched historical controls who received HiDAC 135. Group sample sizes of 23 subjects from a prospective cohort and 23 subjects from historical baseline group will achieve 81% statistical power to reject the null hypothesis of equal means when the population mean difference in neutropenia duration is three days (16 vs. 19 days) with a standard deviation for both groups of four days and statistical significance level (alpha) of 0.05 using a one-sided, two sample, equal-variance t-test. Including 20% drop out rate, we will accrue 29 subjects per cohort.

12.3 Analysis of Primary Endpoint

When the required assumptions are met, duration of neutropenia will be analyzed by a one-sided, two sample, equal-variance t-test. If the normal distribution and equal variance are not validated, the corresponding non-parametric method will be used. Primary analysis will be for cycle 1 of consolidation only. Other cycles will be recorded and use descriptive statistics. Further, multivariable linear regression model of the neutropenia in days with group as the primary independent variable of interest will be fitted, while adjusting for some potential confounding variables, including AML type (WHO and FAB), documented infection in consolidation #1 (yes or no), ELN risk category (favorable, intermediate, high) and MRD positivity (yes or no), WBC at diagnosis (<50,000 or $\geq$ 50,000), delay between induction course and consolidation #1 (less than or more than 40 days), allogeneic HCT (yes or no).

12.4 Analysis of Secondary Endpoints

All secondary endpoints will be summarized by descriptive statistics. Group comparisons will be carried out using appropriate statistical tests in an exploratory fashion.

12.5 Analysis of Exploratory Endpoints

Exploratory endpoints will be descriptive.

12.6 Analysis of Safety Data

Safety data will be descriptive as we expect a low number of non-hematologic adverse effects. Hematologic adverse effects and associated infections are common with this regimen in standard practice, including hospitalizations. We do not expect any additional adverse effects with the change in this schedule.

12.7 Interim Analysis and Stopping Rules

No interim analysis for efficacy will be planned. However, the study will be monitored and stopped following statistical rules. Sequential boundaries will be used to monitor neurological dose-limiting toxicities (DLT) (Grade 3-5) rate. The accrual will be halted if

excessive numbers of DLTs are seen, that is, if the number of DLT is equal to or exceeds the Boundary (b_n) out of the accrued subjects (n) with full follow-up (see table 12.7.1). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 5% when the rate of DLT is equal to the acceptable rate 10%. This boundary is equivalent to testing the null hypothesis, after each subject, that the event rate is equal to 10%, using a one-sided level 0.01 test.

Table 12.7.1

The trial will be stopped if the number of neurological DLT (grade 3-5) is equal to or exceeds the **Boundary (b_n)** out of the total **Number of Subjects (n)** participating in the trial with completed follow-up.

Number of subjects (n)								
1	2	3-6	7-10	11-16	17-21	22-28	29	30
Boundary (b_n)								
-	2	3	4	5	6	7	8	-

12.8 Data Integrity and Safety Committee

This protocol will be reviewed and monitored by the University of Florida Health Cancer Center Data Integrity and Safety Committee (UFHCC DISC) in accordance with their policies and procedures. They will review and monitor study progress, toxicity, safety and other data from this trial. Questions about subject safety or protocol performance will be addressed with the sponsor-investigator, statistician and study team members. Should any major concerns arise, the DISC will offer recommendations regarding whether or not to suspend the trial.

UFHCC DISC data and safety monitoring activities include:

- Review of clinical trial conducted for progress and safety
- Review of all adverse events requiring expedited reporting as defined in the protocol
- Review of reports generated by data quality control review process
- Notification of the sponsor-investigator of recommended action
- Notification of sites coordinated by the UFHCC of adverse events requiring expedited reporting and subsequent committee recommendations for study modifications

In compliance with the UFHCC data and safety monitoring plan, the PI will provide a Data Integrity and Safety Committee Report to DISC at the predetermined timelines for the level of risk category assigned during the initial SRMC (Scientific Review and Monitoring Committee) review, which occurs prior to initial IRB approval.

UFHCC investigator-initiated protocols will be classified into one of the following categories

of risk by the SRMC (see SRMC manual for further details):

Level 1 – **Low risk** Investigator Initiated interventional trials.

Level 2 – **Moderate risk** Investigator Initiated or externally sponsored interventional (such as drug, biologic or device) trials using FDA approved or commercially available compounds or interventions.

Level 3 – **High risk** Investigator Initiated or externally sponsored interventional trials (such as investigator-sponsored INDs, Phase I trials, studies requiring biosafety approval, or other areas that may be designated by NIH as high risk).

Level 4 – Complex trials involving **very high risk** to subjects and a high level of complexity such as first in human or gene transfer studies.

The risk level assigned by SRMC will determine the appropriate level of DISC monitoring required, with increased monitoring required for higher-risk trials.

Findings will be communicated to all study sites by UFHCC CRO.

12.9 Data Monitoring

UFHCC (University of Florida Health Cancer Center) Quality Assurance team and/or project management officers will perform remote monitoring and may make monitoring visits to the trial sites periodically during the trial to confirm that all sites are complying with the protocol. Source documents will be reviewed for completion and validated against data submitted electronically via Electronic Data Capture. The site investigator/institution guarantee access to source documents by UFHCC or its designee and appropriate regulatory agencies. As part of the responsibilities assumed by conducting this study, the Principal Investigator (PI) agrees to maintain and have available for monitoring adequate case records (accurate source documents and CRFs) for the subjects treated under this protocol.

The trial site may also be subject to quality assurance audit by any collaborating sponsors or their designee as well as inspection by appropriate regulatory agencies.

It is important for the site investigator and their relevant personnel to be available during the monitoring visits and possible audits and for sufficient time to be devoted to the process.

12.10 Principal Investigator Responsibilities

Per UF IRB requirements, the PI is personally responsible for conducting and supervising the conduct of human research subjects by "protecting the rights, safety, and welfare of subjects under the investigator's care." The PI also must ensure that all the research conducted is done so in an ethical manner and in accordance with all federal, state, and local laws and regulations, institutional policies, and the requirements of the IRB.

Oversight is defined as "management by overseeing the performance or operation of a person or group; watchful care, superintendence, general supervision". Any person serving as a PI has voluntarily accepted these responsibilities and is expected to fully comply with these requirements, as outlined in the UFHCC Guidance: *Principal Investigator Responsibilities and Oversight.*

The PI will be primarily responsible for continuous monitoring of adverse events, protocol violations, and other immediate protocol issues. The study coordinator will collect information on subjects enrolled through the use of electronic or paper adverse event (AE) forms, CRFs, and Informed Consent forms.

13. EMERGENCY PROCEDURES

13.1 Emergency Contact

In emergency situations, the treating physician should contact the Principal Investigator by telephone at the number listed on the title page of the protocol.

13.2 Emergency Treatment

During and following a subject's participation in the study, the treating physician and/or institution should ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the study.

14. ADMINISTRATIVE, ETHICAL, AND REGULATORY CONSIDERATIONS

14.1 Good Clinical Practice

The procedures set out in this study protocol pertaining to the conduct, evaluation, and documentation of this study are designed to ensure that the Principal Investigator and Co-Investigators abide by Good Clinical Practice (GCP), as described in International Conference on Harmonization (ICH) Guideline E6 and in accordance with the general ethical principles outlined in the Declaration of Helsinki.

The study will be conducted in compliance with the protocol. The protocol, any amendments, and the subject informed consent will receive Institutional Review Board (IRB) approval before initiation of the study.

The Principal Investigator will conduct all aspects of this study in accordance with applicable national, state, and local laws of the pertinent regulatory authorities.

All potential serious breaches must be reported immediately to the UFHCC Project Management Office (PMO, pmo@cancer.ufl.edu, who will then report the breach to UFHCC DISC) and the IRB of record, if applicable. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

14.2 Institutional Review Board

Before study initiation, the investigator must have written and dated approval from the IRB for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects. The investigator should also provide the IRB with a copy of the Investigator Brochure or product labeling, information to be provided to subjects, and any updates. The investigator should provide the IRB with reports, updates, and other information (e.g., amendments, and administrative letters) according to regulatory requirements or institution procedures.

14.3 Compliance with Laws and Regulations

It is intended that the proposed study be conducted according to the International Conference on Harmonization E6 Guideline for Good Clinical Practice (GCP) and the Declaration of Helsinki.

> Please refer to the International Conference on Harmonization and GCP: http://www.fda.gov/oc/gcp/guidance.html; Declaration of Helsinki: http://www.fda.gov/oc/health/helsinki89.html; Code of Federal Regulations, Title 21: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch. cfm]

All UF Health Cancer Center investigator-initiated trials, meeting the criteria of the FDAAA's applicable clinical trials, will be registered with ClinicalTrials.gov by the Project Management Officer (PMO) or assigned designee. All studies must be registered prior to enrollment of the first participant. The Project Management Officer or assigned designee will maintain the responsibility of updating trials registered with ClinicalTrials.gov. Per FDA requirement, information must be updated at least every twelve months and the registry must be updated within thirty days of any changes in recruitment status or completion of the study. The PMO will determine if registration and updates to the NCI CTRP are required.

14.4 Delegation of Investigator Responsibilities

The Principal Investigator will ensure that all persons assisting with the study are adequately informed about the protocol, any amendments to the protocol, the study treatments, and their study-related duties and functions. The Principal Investigator will maintain a list of Co-Investigators and other appropriately qualified persons to whom he has delegated significant study-related duties.

Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks. This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure; debarment). Systems with procedures that ensure the quality of every aspect of the study will be implemented.

14.5 Subject Information and Informed Consent

Before being enrolled in this clinical trial, the subject must consent to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to him or her. An informed consent document that includes both information about the study and the consent form will be prepared and given to the subject. This document will contain all ICH, GCP, and locally required regulatory elements. The document must be in a language understandable to the subject and must specify the person who obtained informed consent.

After reading the informed consent document, the subject must give consent in writing. The written informed consent will be obtained prior to conducting any study-related procedures or tests. The subject's consent must be confirmed at the time of consent by the personally dated signature of the person conducting the informed consent discussions and a copy of the consent form (preferably signed) must be given to the subject for their records.

The PI will retain the original signed consent document. The PI will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

14.6 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

Subjects will be told that the IRB, UF Health DISC, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection law.

14.7 Protocol Amendments

Once the study has started, amendments should be made only in exceptional cases. Protocol amendments will not be implemented without prior written IRB approval. All amendments

will be submitted to the IRB and SRMC (as applicable), and written verification that the amendment was submitted and subsequently approved is to be obtained, and notification will sent out to the applicable study teams, prior to implementing the amendment.

On an emergency-basis, to eliminate an immediate safety hazard to a subject, a protocol deviation may be implemented immediately, provided the IRB and UFHCC CRO PMO (pmo@cancer.ufl.edu) are notified within **5** business days with a full justification and description of the event.

14.8 Case Report Forms

The Principal Investigator and/or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document protocol-required outcomes for safety monitoring and data analysis. All study data will be entered electronically in an Electronic Data Capture system in accordance with the protocol schedule of events and guidelines developed in the Data Management Plan for the study, using a secure access account.

All protocol data is the sole property of UFHCC and should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from UFHCC.

14.9 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

UF Health Cancer Center requires that all study documentation be maintained for at least 6 years from the date of final study publication. No study records may be destroyed without

prior authorization from UF.

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16. APPENDICES

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Appendix A: Infection Definitions

Type of Infection	Clinical/Radiographic Criteria	Laboratory/Microbiological
Major Infections		
Primary Blood BSI [20]	Organism(s) identified in blood is not related to an infection at another site	• Pathogen cultured from ≥ 1 blood cultures • Cultured organism(s) are other than common skin contaminants
Primary BSI with Skin Colonizers[20]	• Patient has at least one of the following signs or symptoms: fever (> 38.0°C), chills, or hypotension AND • Organism(s) identified from blood is not related to an infection at another site **AND** • The same common commensal (i.e., diphtheroids [Corynebacterium spp. not C. diphtheriae], Bacillus spp. [not B. anthracis], Propionibacterium spp., coagulase-negative staphylococci [including S. epidermidis], viridans group streptococci, Aerococcus spp., and Micrococcus spp.) is identified	• ≥ 2 blood specimens drawn on separate occasions (collected on the same or consecutive calendar days) • Collected in a manner which suggests that 2 separate blood draw site preparations were performed (e.g., different venipunctures, a combination of venipuncture and lumen withdrawal, or different lumens of the same central line)
Pneumonia[21]	**All** of the following criteria are met: • Chest imaging (either chest X-ray or computed tomography scan) consistent with pneumonia; **AND**	• The presence of the clinical pneumonia syndrome in conjunction with the isolation of a probable pulmonary pathogen from the blood, sputum, BAL fluid, or lung tissue.

Type of Infection	Clinical/Radiographic Criteria	Laboratory/Microbiological
	• No evidence of pulmonary edema based on echocardiographic and pulmonary artery catheter data, fluid balance, and radiographic response to diuresis; **AND** • Temperature greater than 38°C*, **OR** dyspnea, **OR** cough with purulent sputum *Fever may be absent in a minority of patients and some may even be hypothermic.	• Coagulase-negative Staphylococcus and Candida species will be excluded unless there is biopsy-proven invasion or concomitant isolation of the organism in respiratory secretions and blood in the absence of a more likely pulmonary pathogen. • A virus will be considered a pathogen if it is detected by antibody or culture or polymerase chain reaction from respiratory secretions in the absence of a more likely etiologic agent
CMV Pneumonia (Proven)[20]	• Clinical symptoms and/or signs of pneumonia such as new infiltrates on imaging, hypoxia, tachypnea, and/or dyspnea **AND** • CMV documentation in lung tissue	CMV documented in lung tissue by virus isolation, rapid culture, histopathology, immunohistochemistry, or DNA hybridization techniques
CMV Pneumonia (Probable)[22]	• Clinical symptoms and/or signs of pneumonia **AND** • Detection of CMV	CMV by viral isolation, rapid culture of BAL fluid, or the quantitation of CMV DNA in BAL fluid. A definite cut-off for CMV DNA load is not established
Proven Lower RVI[23]	New pulmonary infiltrates per CT chest or chest X-ray with or without lower respiratory tract symptoms	Respiratory virus detected with BAL or biopsy specimen by multiplex-PCR
Probable Lower RVI[23]	No new pulmonary infiltrates per CT chest or chest X-ray with lower respiratory tract symptoms, with or without pulmonary function decline	Respiratory virus detected in BAL or lung biopsy sample by multiplex-PCR

Type of Infection	Clinical/Radiographic Criteria	Laboratory/Microbiological
Proven IFI[24] Only laboratory/ microbiological criteria	**Microscopic analysis** ***Mold:*** Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage ***Yeast:*** Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy from a normally sterile site (other than mucous membranes) showing yeast cells—for example, Cryptococcus spp. indicated by encapsulated budding yeasts or Candida spp. showing pseudohyphae or true hyphae ***Culture (sterile material)*** ***Mold:*** Recovery of a mold or "black yeast" by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL fluid, a cranial sinus cavity specimen, and urine	• Blood culture that yields a mold (e.g., Fusarium species) in the context of a compatible infectious disease process • Blood culture that yields yeast (e.g., Cryptococcus or Candida spp.) or yeast like fungi (e.g., Trichosporon species) • Cryptococcal antigen in CSF indicates disseminated cryptococcosis

Type of Infection	Clinical/Radiographic Criteria	Laboratory/Microbiological
	Yeast: Recovery of a yeast by culture of a sample obtained by a sterile procedure (including a freshly placed [<24 h ago] drain) from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process	
Probable IFI[24] Subjects **MUST** have one each (at a minimum) of host, clinical, and microbiologic criteria	**Host criteria:** • Recent history of neutropenia (< 500 cells/mm3 for > 10 days) temporally related to onset of fungal disease; **OR** • Received an allogeneic HSCT; **OR** • Corticosteroid use for > 3 weeks (average dose 0.3 mg/kg/d prednisone equivalent); **OR** • Treatment with T-cell immune suppressants (e.g., cyclosporine, TNFα blocker, specific monoclonal antibodies or nucleoside analogues) within the last 90 days; **OR** • Inherited severe immunodeficiency; **Clinical criteria**: • Lower respiratory tract fungal disease (1 of 3 on CT scan): o Dense, well circumscribed lesion(s) with or without a halo sign; **OR**	**Microbiologic criteria** • Mold in sputum, BAL fluid, bronchial brush, or sinus aspirate samples, indicated by 1 of either presence of fungal elements indicating a mold or recovery of a mold by culture (e.g., Aspergillus, Fusarium, Zygomycetes, or Scedosporium spp.); **OR** • Positive evidence of GM in serum, BAL or CSF; **OR** • Positive evidence of β-D-glucan in serum

Type of Infection	Clinical/Radiographic Criteria	Laboratory/Microbiological
	○ Air-crescent sign; **OR** ○ Cavity; **OR** • Tracheobronchitis (mark the finding(s) per bronchoscopic evaluation) ○ Tracheobronchial ulceration; **OR** ○ Nodule; **OR** ○ Pseudo-membrane; **OR** ○ Plaque or eschar; **OR** • Sinonasal Infection (imaging showing sinusitis plus 1 of the following 3 signs): ○ Acute localized pain (including pain radiating to the eye); **OR** ○ Nasal ulcer with black eschar; **OR** ○ Extension of the paranasal sinus process across bony barriers; **OR** • CNS Infection (1 of the following): ○ Focal lesions on imaging; **OR** ○ Meningeal enhancement on MRI or CT;	

Type of Infection	Clinical/Radiographic Criteria	Laboratory/Microbiological
	OR • Disseminated candidiasis (at least 1of the followings after an episode of candidemia within the previous 2 weeks): o Small target-like abscesses (bull's eye lesions) in liver or spleen; **OR** o Progressive retinal exudates on ophthalmologic examination	
Clostridium difficile infection (CDI)[25] Diagnosis will be if the clinical criterion is met AND there is microbiological confirmation	Presence of at least 3 unformed bowel movements in a 24-hour period or fewer consecutive hours	• A stool test result positive for the presence of toxigenic C. difficile or its toxins or colonoscopic **OR** histopathologic findings demonstrating pseudomembranous colitis • Patients presenting with ileus or megacolon will need a perianal specimen test positive for C. difficile or its toxins
Typhilitis[26] Diagnosis will be if the clinical criteria are met AND there is microbiological confirmation	• Patients with fever > 38.3°C) * or > 38°C sustained for 1 hour, neutropenia and abdominal signs such as abdominal cramping, abdominal pain (> 3 on VAS), abdominal distention, diarrhea, GI bleeding **AND** • Exclusion of other diagnoses such as C. difficile associated colitis or other biopsy proven diagnosis	• ANC ≤ 500/mm3 **AND** • Bowel wall thickening on CT exam or US exam > 4 mm (transverse scan) thickening in any segment of the bowel for at least 30 mm length (longitudinal scan)

Type of Infection	Clinical/Radiographic Criteria	Laboratory/Microbiological
	*Fever may be absent in a minority of patients and some may even be hypothermic.	
Other Infections		
Febrile Neutropenia[27] Both clinical and laboratory criteria should be met	• A single oral temperature of > 38.3°C (101°F) **OR** • A temperature of > 38.0°C (100.4°F) sustained for > 1 hour	• ANC < 500 cells/mm^3 **OR** • ANC that is expected to decrease to < 500 cells/mm^3 during the next 48 hours
Upper RVI[23]	• No new pulmonary infiltrates per CT chest or chest X-ray • Symptoms of upper respiratory tract infection	Respiratory virus detected in a nasopharyngeal swab or sputum by multiplex-PCR
Possible Lower RVI[23]	New pulmonary infiltrates per CT chest or chest X-ray (but without confirmation of respiratory virus in the lower respiratory tract) with or without LRTD signs or symptoms (e.g., cough, wheezing, rales, tachypnea, shortness of breath, dyspnea, or hypoxia).	Respiratory virus detected in a nasopharyngeal swab or sputum by multiplex-PCR
Catheter-associated BSI for Non-skin common skin contaminants[20] BOTH clinical and microbiological criteria should be met	• A CVC must be present at the time of the BSI or removed within the 48 hours prior to onset, unless clinical evidence links the BSI to a catheter removed > 48 hours before onset (e.g., purulent thrombophlebitis) **AND** • Absence of other focus of infection as a source of the BSI	Recognized pathogen cultured (other than skin contaminants) from one or more blood cultures, unrelated to infection at another site
Septic Shock[28]	• Any documented infection **AND**	Lactate >2 mmol/L (>18 mg/dL)

Type of Infection	Clinical/Radiographic Criteria	Laboratory/Microbiological
	• Requirement of vasopressors and have a lactate >2 mmol/L (>18 mg/dL)	
Catheter associated BSI for common skin contaminants[20] (e.g. diphtheroids, Bacillus spp, Propionibacterium spp, coagulase negative staphylococci, and micrococci) BOTH clinical and microbiological criteria should be met	**At least one of the following:** • Fever (> 38°C) • Chills • Hypotension • Signs of infection of catheter insertion site or tunnel	• Organisms (diphtheroids, Bacillus spp., Propionibacterium spp., coagulase negative staphylococci, and micrococci) cultured from: o At least two separate blood draws collected on the same or consecutive calendar days, **OR** • Collected in a manner which suggests that 2 separate blood draw site preparations were performed (e.g., different venipunctures, a combination of venipuncture and lumen withdrawal, or different lumens of the same central line)

Appendix B: UF Health Shands/BMT Program Blood Product Support Guidelines – Adult

Product:	**Situation:**	**Current BMT Program Recommendations:**
Platelet	Leukemia – Routine	≤ 10,000/μL No change for fevers
Platelet	• Bleeding – Not CNS • Prior to and post-invasive procedure (not CNS)	>50,000/μL for LP
Platelet	• CNS bleeding • Prior to and post-invasive CNS procedure	≤100,000/μL
Platelet	Subjects receiving therapeutic anticoagulation	≤50,000/μL
Red Cells	Routine	Hct ≤ 20%
Red Cells	Asymptomatic with history of cardiovascular disease	Hct ≤ 25%
Red Cells	Symptomatic cardiovascular or pulmonary disease	Hct 26-30%

Appendix C: European Leukemia Net (ELN) Guidelines

Response criteria in acute myeloid leukemia

Category	Definition	Comment
Response		
CR without minimal residual disease (CR_{MRD-})	If studied pretreatment, CR with negativity for a genetic marker by RT–qPCR, or CR with negativity by MFC	Sensitivities vary by marker tested, and by method used; therefore, test used and sensitivity of the assay should be reported; analyses should be done in experienced laboratories (centralized diagnostics)
Complete remission (CR)	Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC ≥1.0 × 10^9/L (1000/microL); platelet count ≥100 × 10^9/L (100,000/microL)	MRD^+ or unknown
CR with incomplete hematologic recovery (CR_i)	All CR criteria except for residual neutropenia (<1.0 × 10^9/L [1000/microL]) or thrombocytopenia (<100 × 10^9/L [100,000/microL])	
Morphologic leukemia-free state (MLFS)	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required	Marrow should not merely be "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%
Partial remission (PR)	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5 to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%	Especially important in the context of phase 1/2 clinical trials
Treatment failure		
Primary refractory disease	No CR or CR_i after 2 courses of intensive induction treatment; excluding patients with death in aplasia or death due to indeterminate cause	Regimens containing higher doses of cytarabine are generally considered as the best option for patients not responding to a first cycle of 7+3; the likelihood of responding to such regimens is lower after failure of a first
Death in aplasia	Deaths occurring ≥7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia	
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring ≥7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available	
Response criteria for clinical trials only		
Stable disease	Absence of CR_{MRD-}, CR, CR_i, PR, MLFS; and criteria for PD not met	Period of stable disease should last at least 3 months
Progressive disease (PD)*¶	Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood: • >50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with <30% blasts at baseline; or persistent marrow blast percentage of >70% over at least 3 months; without at least a 100% improvement in ANC to an absolute level (>0.5 × 10^9/L [500/microL], and/or platelet count to >50 × 10^9/L [50,000/microL] nontransfused); or • >50% increase in peripheral blasts (WBC × % blasts) to >25 × 10^9/L (>25,000/microL) (in the absence of differentiation syndrome)¶; or • New extramedullary disease	Category mainly applies for older patient given low-intensity or single-agent "targeted therapies" in clinical trials In general, at least 2 cycles of a novel agent should be administered Some protocols may require blast increase in 2 consecutive marrow assessments at least 4 weeks apart; the date of progression should then be defined as of the first observation date Some protocols may allow transient addition of hydroxyurea to lower blast counts "Progressive disease" is usually accompanied by a decline in ANC and platelets and increased transfusion requirement and decline in performance status or increase in symptoms
Relapse		
Hematologic relapse (after CR_{MRD-}, CR, CR_i)	Bone marrow blasts ≥5%; or reappearance of blasts in the blood; or development of extramedullary disease	
Molecular relapse (after CR_{MRD-})	If studied pretreatment, reoccurrence of MRD as assessed by RT–qPCR or by MFC	Test applied, sensitivity of the assay, and cutoff values used must be reported; analyses should be done in experienced laboratories (centralized diagnostics)

CR: complete response; MRD: measurable residual disease (also known as minimal residual disease); ANC: absolute neutrophil count; MLFS: morphologic leukemia-free state; WBC: white blood cell; IDH: isocitrate dehydrogenase; RT-qPCR: real-time quantitative polymerase chain reaction; MFC: multiparameter flow cytometry.

* The authors acknowledge that this new provisional category is arbitrarily defined; the category aims at harmonizing the various definitions used in different clinical trials.

¶ Certain targeted therapies, for example, those inhibiting mutant IDH proteins, may cause a differentiation syndrome, that is, a transient increase in the percentage of bone marrow blasts and an absolute increase in blood blasts; in the setting of therapy with such compounds, an increase in blasts may not necessarily indicate PD.

Appendix D: Protocol Version History / Summary of Changes

Protocol Version	Change History	Date of Changes
v1.0	**N/A – Original Protocol**	
v1.1	**Protocol Signature Page** • PI name and site information *added*. **Exploratory Objectives** • Monitoring time period after starting induction has been *updated* to match other areas of the protocol. **4.3 Exclusion Criteria** • Criteria (G) has been *added* to match the exclusion criteria listed under the **Protocol Synopsis** section.	14-Sep-2021
V2.0	**Protocol Synopsis** • Inclusion criteria language added to (B), and a sub-paragraph regarding usage of non-site collected laboratory sample results added under criteria (C) **4.2 Inclusion Criteria** • Sub-paragraph below criteria (C) has been added to match Inclusion Criteria listed in **Protocol Synopsis** section • Minor language updates to criteria (B) **6.1 Schedule of Events** • Foot note 8 had language added • New footnote (10) created and superscript placed on 'Interval History/Physical Exam'	09-May-2022
V3.0	**Protocol Schema** • Updated subject pool to adults aged 55 years and older **Protocol Synopsis/Inclusion Criteria/Exclusion Criteria** • Updated study recruitment criteria to adults aged 55 years and older **6.1 Schedule of events**	08-February-2023

	• Footnote #7: Amended language to avoid a scenario where subject is indefinitely followed for an Adverse Event **Section 6.2 End of Treatment Evaluations** • Clarified language regarding what defines End of Treatment date. • Defined hematologic recovery	
V4.0	**Protocol Synopsis / Rationale / Inclusion Criteria / Exclusion Criteria / Efficacy Assessment** • Updated definition of 'recovery' to incomplete hematologic recovery • Updated transfusion language to match REDCap • Returned subject age inclusion criteria to >= 61 years of age **Section 6.1 Schedule of Events** • Created (+/- 14 days) window for End of Treatment visit • Added +/- to 28 day window in Follow-Up visits • Amended footnote 7 to adjust AE collection period • Amended footnote 8 to include relapsed disease **Sections 6.2 End of Treatment Evaluations** • Clarified EOT criteria to be met. • Added 'Relapsed disease' • Clarified hematologic recovery defined as complete or incomplete • Added reference to Appendix C. ELN guideline **Section 8.2 Criteria for Study Treatment Discontinuation** • Clarified treatment discontinuation procedures for subjects in complete hematologic recovery **Section 11.2 Period of Observation** • Redefined Period of Observation SAE collection to go until 28 days after last	06-April-2023

	infusion or 'subject starts new chemo therapy treatment' (whichever comes first) **Appendix C** • Addition of new appendix for ELN Guideline	