PrE0905 Statistical Analysis Plan (SAP)

Randomized Trial of Gilteritinib vs Midostaurin in FLT3 Mutated Acute Myeloid Leukemia (AML)

NCT#:	NCT03836209
Version Number:	3
Version Date:	09 November 2023

Statistical Analysis Plan

Study code PrE0905

Version Final V3

Date 09 November 2023

Randomized Trial of Gilteritinib vs Midostaurin in FLT3 Mutated Acute Myeloid Leukemia (AML)

DOCUMENT:	Statistical Analysis
Plan PROTOCOL Number:	PrE0905
Version:	V 3.0
Date:	October 5, 2022
Title:	Randomized Trial of Gilteritinib vs Midostaurin in FLT3 Mutated Acute Myeloid Leukemia (AML)
SAP STATUS	Final Version 3
SAP VERSION DATE	November 9, 2023

SPONSOR: PrECOG, LLC
1818 Market Street
Suite 3000
Philadelphia, PA 19103

PREPARED BY: [redacted]
Biostatistician

SIGNATURES/APPROVAL PAGE

AUTHORS

[redacted]

Biostatistician

Date

APPROVERS

[redacted]

Biostatistician

Date

[redacted]

Project Manager,

Date

TABLE OF CONTENTS

Contents

1. LIST OF ABBREVIATIONS

Abbreviation	Term
AE	Adverse Event
ALT	Alanine Aminotransferase
ALK	Anaplastic Lymphoma Kinase
AML	Acute Myeloid Leukemia
AML-MRC	AML-Myelodysplasia-Related Changes
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CI	Confidence Interval
CR	Complete Response
CRc	Composite Complete Remission
CRh	CR with partial hematologic recovery
CRi	CR with incomplete hematologic recovery
CRp	Complete Remission with Incomplete Platelet Recovery
CSR	Clinical Study Report
DFS	Disease Free Survival
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EFS	Event Free Survival
ELN	European LeukemiaNet
FDA	Food and Drug Administration
FLT3	FMS-Like Tyrosine Kinase 3
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous; Intravenously

Abbreviation	Term
MRD	Minimal Residual Disease
MTD	Maximum Tolerated Dose
MUGA	Multigated Acquisition Scan
Mut+	Mutation Positive
NCI	National Cancer Institute
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NE	Not Evaluable
NPM1	Nucleophosmin 1-Mutated
NR	No Response
OS	Overall Survival
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PR	Partial Remission/Response
QTc	Corrected QT Interval
QTcF	Fridericia-Corrected QT Interval
ΔQTcF	Change from baseline in Fridericia-Corrected QT Interval
SAE	Serious Adverse Event
SD	Stable Disease
TEAE	Treatment-Emergent Adverse Event
TKD	Tyrosine Kinase Domain
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of the Normal Range

2. INTRODUCTION

2.1. Objective of the Statistical Analysis Plan

This statistical analysis plan (SAP) describes the planned analysis of the safety and efficacy data from this study. A detailed description of the planned tables, figures and listings (TFLs) to be presented in the analysis is provided in the accompanying TFL template document.

The intent of this document is to provide guidance for the analysis of data related to safety and efficacy to describe any applicable statistical procedures. In general, the analyses come directly from the protocol, unless they have been modified by agreement between the Sponsor and Quality Data Services, Inc. (QDS). A limited amount of information concerning this study (e.g., objectives, study design) is summarized to help the reader interpret the accompanying TFL templates. That information is not a synopsis of the study and does not require review or approval because it is simply extracted from the protocol. Attached signatures indicate approval of the statistical analysis sections of the SAP, as well as accompanying TFL templates. These sections must be agreed upon prior to database lock. When the SAP and TFL templates are agreed upon and finalized, they will serve as the template for a portion of this study's report.

This SAP supersedes the statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified in the appropriate section of the study report. Any substantial deviations from this SAP will be agreed upon between the sponsor and QDS. Deviations from this SAP, both substantial and non-substantial, will be documented in the study report. Any updates to their respective analyses, study designs, and TFL presentations after this SAP is finalized and approved will be documented in a running Note to the SAP document.

3. STUDY OBJECTIVES

3.1. Primary Objective

To improve the FLT3 mutation negative (evaluated by polymerase chain reaction [PCR] at the end of induction) Composite Complete Response (CRc) [includes Complete Response (CR) or CR with incomplete hematologic recovery (CRi)] rate of patients with FLT3 mutated AML who receive gilteritinib compared to those who receive midostaurin in addition to standard therapy with cytarabine and daunorubicin during induction.

3.2. Secondary Objectives

The secondary objectives of this study are:

- To improve the FLT3 mutation negative Complete Response (CR) rate of patients with FLT3 mutated AML who receive gilteritinib compared to those who receive midostaurin in addition to standard therapy with cytarabine and daunorubicin during induction.

- To improve the MRD- CRc (evaluated by flow cytometry) rate of patients with FLT3 mutated AML who receive gilteritinib compared to those who receive midostaurin in addition to standard therapy with cytarabine and daunorubicin during induction.
- To improve CRc (CR or CRi) rate of patients with FLT3 mutated AML who receive gilteritinib compared to those who receive midostaurin in addition to standard therapy with cytarabine and daunorubicin during induction.
- To improve the Event Free Survival (EFS) of patients with FLT3 mutated AML who receive gilteritinib to those who receive midostaurin in addition to cytarabine and daunorubicin during induction and high dose cytarabine during consolidation.
- To improve the overall survival (OS) of patients with FLT3 mutated AML who receive gilteritinib to those who receive midostaurin in addition to cytarabine and daunorubicin during induction and high dose cytarabine during consolidation.
- To assess the toxicities of gilteritinib plus standard therapy during induction and consolidation in this patient population.
- To assess the toxicities of midostaurin plus standard therapy during induction and consolidation in this patient population.

3.3. Exploratory Objectives

- To evaluate the effect of gilteritinib on CRc rate, EFS and OS in patients with de novo AML, t- AML and AML-MRC separately.
- To evaluate the effect of gilteritinib on CRc rate, EFS and OS in patients with favorable, intermediate, and adverse risk separately, determined by 2017 ELN risk classification.
- To determine the predictive value of Minimal Residual Disease (MRD) positivity and negativity post induction therapy.
- To assess the MRD status of patients with AML by flow cytometry after induction therapy with a Tyrosine Kinase Inhibitor (TKI) and compare flow MRD to molecular studies in patients in CRc.
- To evaluate the FLT3 mutation negative CRc rate after first cycle of consolidation by treatment arm for those patients who are FLT3 mutation positive after induction and administered consolidation.
- To assess the feasibility of immediate transplant as post-remission therapy.
- Correlation of characteristics of FLT3 mutation and outcome.

4. STUDY DESIGN

4.1. General Study Design and Plan

This is a randomized, open-label Phase II study comparing gilteritinib to midostaurin investigational medications that combined with standard therapy in FLT3 mutated AML. Patients will be stratified according to FLT3-TKD vs. FLT3-ITD mutation. Patients with FLT3-ITD mutation will undergo further stratification with NPM1 mutation status (positive vs. negative) and signal ratio (high [≥ 0.5] vs. low [<0.5] of FLT3 Wild Type). Patients will be randomized in a ratio of 1:1 two arms treatments, arm A and B. Arm A is standard therapy plus gilteritinib, and arm B is standard therapy plus midostaurin. At each arm, if achieving CR or CRi, these patients will go on to consolidation treatment. Patients may proceed to allogeneic

transplant after induction or after 0-4 cycles of chemotherapy consolidation. The primary objective of this study is to assess the FLT3 mutation negative CRc (includes CR and CRi) rate of patients who receive arm A gilteritinib compared to those who receive arm B midostaurin in addition to standard therapy with cytarabine and daunorubicin after induction. FLT3 mutation negativity is evaluated by PCR at the end of induction.

We assume with the control arm of cytarabine, daunorubicin and midostaurin (arm B), the FLT3 mutation negative CRc rate after induction is 40%. With 170 eligible patients with allocation ratio of 1:1 to the two arms, the study will have 80% power to detect an improvement of 20% in the FLT3 mutation negative CRc rate in the arm A gilteritinib arm (i.e., from 40% to 60%) at the one-sided significance level of 0.05 based on Fisher's exact test.

Accounting for 5% ineligibility, we will need a total of 179 patients.
Assume 7 FLT3 mutated patients will be accrued each month, the accrual of the study will be completed in 2.1 years.

4.2. Study Population

FLT3 mutated acute myeloid leukemia (AML) adult patients with age between 18 to 70 years. There will total of 179 patients enroll into the study.

4.3. Randomization and Blinding

Patients who then meet eligibility requirements may be randomized for study participation. Patients will be stratified prior to randomization by criteria noted below based on central laboratory assessment of prescreening samples.

Patients will be stratified according to FLT3-TKD vs. FLT3-ITD FLT3 mutation. Patients with FLT3-ITD mutation will undergo further stratification with NPM1 mutation status (positive vs. negative) and signal ratio (high [≥ 0.5] vs. low [<0.5] of FLT3 Wild Type). Patients will be randomized to receive standard induction treatment (cytarabine and daunorubicin) with either gilteritinib or midostaurin.

Patients will be assigned in 1:1 randomization to Arm A or Arm B (treatment assignments are not blinded):

- TLT3-TKD vs. FLT3-ITD FLT3 Mutation
  - If FLT3-ITD Mutation: NPM1 Mutation Status (positive vs. negative)
  - If FLT3-ITD Mutation: Signal Ratio (high [≥ 0.5] vs. low [<0.5] of FLT3 Wild Type)

Patients that are negative for FLT3 mutated, will be considered screen failures.

Standard of care induction 7+3 chemotherapy may start prior to randomization while awaiting prescreening test results. Protocol treatment will begin no later than 3 days after randomization.

This is an open-label study. Both patients and investigator know the treatment after the

randomization.

4.4. Treatment Administration

All patients will receive standard of care 7+3 induction chemotherapy with cytarabine and daunorubicin and standard consolidation with high dose cytarabine. Patients will be randomized to receive either open label gilteritinib or midostaurin and will receive the assigned study drug during induction and consolidation therapy.

4.4.1. Study Treatment Schedule

There are 2 treatment arms. The treatment with midostaurin is the control arm, denoted as Arm B. The treatment with gilteritinib is the investigation medication arm, denoted as Arm A.

Arm A treatment: cytarabine + daunorubicin +

Gilteritinib. Arm B treatment: cytarabine +

daunorubicin + Midostaurin. Induction: There are 1- 2

cycle of therapy in induction.

Consolidation: There are 0 - 4 cycles of therapy in Consolidation.

Consolidation cycle 1, patients who achieve a CR or CRi and have no residual significant toxicities from the induction course are eligible for consolidation therapy. In general, consolidation therapy should start within 1 month of CR/CRi.

Consolidation Cycle 2-4, Patients must have maintained remission during the consolidation cycles. Patients who do not begin consolidation Cycle 2-4 within 30 days of recovery from previous cycle are ineligible for further protocol therapy.

4.4.2. Discontinue Treatment

Patients who do not achieve a CR or CR with incomplete hematologic recovery (CRi) by Day 60 if one cycle and Day 84-105 if 2 cycles of induction therapy will be removed from study.

Patients who achieve CR or CRi can proceed to post remission consolidation therapy on study or may proceed to allogeneic transplant if desired.

Patients who go to transplant or any other non-protocol leukemia directed therapy will complete an end of treatment visit and will be followed for relapse and survival only.

Reasons that a patient may discontinue treatment in a clinical study are considered to constitute one of the following:

1. Failure to achieve CR or CRi after 1- 2 cycles of induction.
2. Patient requires treatment with therapy not allowed per protocol, or patient goes on to receive HSCT.
3. Recurrence of disease or documented progression of disease.

4. Intercurrent illness that prevents further administration of treatment per investigator discretion.
5. Unacceptable adverse events.
6. Investigator and/or patient discontinue chemotherapy.
7. Pregnancy.
8. Develops a second malignancy (except for non-melanoma skin cancer or cervical carcinoma in-situ) that requires treatment, which would interfere with this study.
9. The patient may choose to withdraw from the study at any time for any reason.
10. General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator.
11. Severe non-compliance to protocol as judged by the investigator.
12. Lost to follow-up.
13. Death.
14. Closure of study by PrECOG.

4.4.3. Duration of Follow-Up

Patients will be followed for adverse events for 30 days after their last dose of study medication. If AE is possibly related to study therapy, then even >30 days, this AE should be reported.

Patients should be followed every 3 months from end of treatment for 2 years, then every 4 months for a year, then every 6 months for 1 year, then annually for progression and survival until death or study closure whichever comes first.

If a patient is removed from treatment for reason(s) other than progression or full consent withdrawal, follow for survival/relapse until death or study closure.

5. EFFICACY MEASUREMENTS

Measurement Scheduled Visits Delay due to holidays, weekends, bad weather or other unforeseen circumstances will be permitted.

5.1. FLT3 mutation status

FLT3 mutation status, positive or negative is evaluated by PCR.

5.2. Bone Marrow Aspirate/Biopsy

A bone marrow aspirate/biopsy is required on all patients on Day 21 after initiating the first cycle of induction. Patients who have residual disease will be eligible to receive a second cycle of induction.

A repeat bone marrow aspirate/biopsy should be done to assess for response and MRD upon count recovery (Absolute Neutrophil Count (ANC) ≥ 1000/mm3, platelets ≥ 100,000/mm3) but no later than Day 60 after initiation of the cycle of induction therapy (i.e., Day 60 or patients who receive one cycle of induction and Day 84-105 for patients that receive 2 cycles of induction).

Patients who do not achieve a CR or CRi by Day 60 after initiation of the last cycle of induction therapy will be removed from study.

Patients are not eligible to proceed to Consolidation unless bone marrow samples are obtained for MRD at the time of CR/CRi.

A bone marrow aspirate/biopsy is also required at end of Consolidation to assess disease status.

5.3. Treatment Response

The treatment response can be following categories: Complete Remission (CR), Complete Remission with incomplete hematologic recovery (CRi), Morphologic Leukemia-Free State (MLFS), Partial Remission (PR), Stable Disease, Treatment Failure, Progressive Disease (PD), Relapse.

5.4. Minimal Residual Disease (MRD)

MRD will be detected by using PCR or flow cytometry. The results will be reported in negative (MRD-) or positive (MRD). To achieve MRD negative is one of goals for the treatment.

5.5. Hematologic recovery

Hematologic recovery or peripheral blood count recovery is Absolute Neutrophil Count (ANC) ≥ 1000/mm3 and platelets ≥ 100,000/mm3

5.6. Event-Free Survival (EFS)

EFS is defined as time from study randomization to the date of induction treatment failure, relapse after CRc or to death from any cause after CRc, whichever comes first. For patients alive without induction treatment failure or relapse after CRc, EFS will be censored at the date of the last disease evaluation.
The main analysis of EFS is to set the event date for induction treatment failure on day 1 of randomization.

5.7. Overall Survival (OS)

OS is defined as the duration of time from start of treatment to time of death.

6. SAFETY MEASUREMENTS

Safety will be evaluated by

- Adverse events
- Concomitant medications and procedures
- Clinical laboratory tests,
- Physical examination findings,
- Vital signs measurements, and
- Electrocardiogram

6.1. Adverse Events

Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient administered a medicinal product in a clinical investigation and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (e.g., including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product (investigational or marketed), whether considered related to the product (investigational or marketed).

After either informed consent, but prior to initiation of study treatment (cytarabine or daunorubicin), only AEs/SAEs caused by a protocol-mandated intervention not considered standard of care will be collected (e.g., SAEs related to invasive procedures such as biopsies). After the initiation of study treatment, any changes from baseline which meet (CTCAE V 5.0) Grade ≥ 3 AEs and SAEs must be recorded on the appropriate page of the electronic Case Report Form (eCRF). In addition, AEs less than Grade 3 that meet dose interruption, reduction or discontinuation must also be recorded, including: ≥ Grade 2 pancreatitis, QTc interval, PRES, cerebellar toxicity of any grade, creatinine >2 mg/dL and direct or total bilirubin ≥ 2 mg/dL.

The following information should be included for all AEs: date of onset and resolution, severity of the event; the investigator's opinion of the relationship to study drug; intervention or treatment required for the AE; action taken with study drug; cause of the event; and information regarding resolution/outcome.

The categories and definitions of severity used for clinical trials AEs are defined in the NCI's Common
Terminology Criteria (CTCAE) V5.0 (http://www.ctep.cancer.gov).

Categories 'definite', 'probable' and 'possible' are considered study drug related. Categories 'unlikely' and 'unrelated' are considered not study drug related.

AEs related to cytarabine, daunorubicin, gilteritinib or midostaurin should be followed for 30 days after last dose of study therapy until ≤ grade 1 or stabilization. Any AE's (serious or not) that occur more than 30 days after the last dose of study therapy but that are deemed to be at least possibly related to study therapy shall be reported.

All laboratory results required by protocol will be recorded directly on the laboratory eCRF pages.

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the eCRF (e.g., abnormalities that require study drug dose delay, discontinuation of study treatment, more-frequent follow-up assessments, further diagnostic investigation, etc.).

6.2. Concomitant Medications and Procedures

Concomitant medications and procedures will be collected during the trial and recorded in the CRF page.

6.3. Clinical Laboratory Assessments

Clinical laboratory tests will be collected at screening, prior cycles of treatments, twice weekly during induction, weekly in consolidation, and at end of treatment.

6.4. Physical Examination and Medical History

Medical history will be collected at screening. Physical examination findings will be collected at screening, prior treatment cycles, daily after induction treatment or weekly after consolidation, and at end of treatment.

6.5. Electrocardiogram

Perform 12-lead ECG in triplicate (3 separate ECGs recorded at least 5 minutes apart) following a 10- minute resting period prior to treatment. The mean QTcF of the triplicate ECG tracings will be used for final treatment decisions and AE reporting.

7. GENERAL STATISTICAL CONSIDERATIONS

This section will go into detail about the statistical approaches and methodology for this study analysis. Statistical analysis and programming of tables and listings will be conducted by QDS, using SAS® Release 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

7.1. Methodology

In general, listings will be presented by patient. Tables will be summarized and presented by treatment arm in specific analysis populations.

The results of this study will be reported using summary tables, figures, and data listings. Continuous variables will be summarized using mean, SD, median, minimum, and maximum. Categorical variables will be summarized by presenting the number (frequency) and percentage in each treatment arm.

7.2. Handling of Repeat, Dropouts or Missing Data

All attempts will be made to prevent any missing values. Missing or invalid data will be treated as missing, not imputed.

If collected AE onset date is missing, then the Treatment-emergent AE (TEAE) is assumed. If the AE onset date is partial, a conservative approach will be applied. The date will be compared as far as possible with the date of first dose date. If missed date and the same month, then the TEAE is assigned. If only onset year available and the same year of first dose date, then TEAE is assigned.
If the relationship with study medication is missing, then related is assigned.

If any post-dose safety data is repeated, the measurement taken first at the visit in question will be used in the analysis.

7.3. Endpoints

7.3.1. Primary Endpoint

The primary endpoint of this study is the FLT3 mutation negative (evaluated by polymerase chain reaction [PCR]) Composite Complete Response (CRc) [includes CR and CRi] rate after induction treatment and complete MRD assessment. The cut points used for FLT3 mutation negative are 1% (equivalent to 10^{-2}) for FLT3-TKD and 10^{-4} for FLT3-ITD.

The final analysis of FLT3 mutation negative CRc rate will be performed after the last patient has completed the induction treatment and all patients have MRD response data available. Patients who drop out prior to MRD response assessment or without MRD assessment will be counted as non-responders in the analysis. The FLT3 mutation negative CRc rate will be compared between gilteritinib and midostaurin arms using Fisher's exact test. All eligible and treated patients will be included into the analysis. Two-sided 90% confidence interval on the difference in the FLT3 mutation negative CRc rate will be provided based on the normal approximation to the difference of two independent binomial proportions with continuity correction.

7.3.2. Secondary Endpoints

The secondary objective endpoints are:

1. The FLT3 mutation negative CR rate of patients with FLT3 mutated AML.
2. The MRD- CRc rate of patients with FLT3 mutated AML
3. The CRc rate of patients with FLT3 mutated AML
4. Event Free Survival (EFS)

The FLT3 mutation negative Complete Response (CR) rate of patients with FLT3 mutated AML who receive gilteritinib will be compared to those who receive midostaurin in addition to standard therapy with cytarabine and daunorubicin during induction. The final analysis of FLT3 mutation negative CR rate will be performed after the last patient has completed the induction treatment and all patients have MRD response data available. Patients who drop out prior to MRD response assessment will be counted as non-responders in the analysis. The FLT3 mutation negative CR rate will be compared between gilteritinib and midostaurin arms using Fisher's exact test. Two-sided 90% confidence interval on the difference in the FLT3 mutation negative CR rate will be provided based on the normal approximation to the difference of two independent binomial proportions with continuity correction.

7.4. Analysis Populations

The FLT3 mutated AML adult patients are this study population. There will be a total of 179 patients enrolled into this study.

Patients negative for FLT3 mutated AML will not be eligible to enroll the study and counted as screen failure.

7.4.1. Safety Population

Safety population is used for safety analysis. The safety population is defined as all patients treated with at least one dose of study medication during the study. All safety data collected up to the end of the study are included in the safety analysis.

7.4.2. Efficacy Population

Efficacy population will be all eligible and treated subjects. Ineligible and never started treatment will be excluded from this population. The eligible patients are subjects already pre-screened, enrolled with signed consent and randomized into treatment arms.

7.5. Safety Analysis

The safety evaluations include AEs, concomitant medication, clinical laboratory assessments. Safety analysis will be assessed by induction and consolidation for the safety population.

7.5.1. Disposition

Disposition data e.g., informed consent, screen failure, randomized, induction patients, consolidation, etc. will be summaries descriptive by treatment arm and overall, for the patients who enrolled into the study. Patients who signed informed consent and randomized are considered as enrolled into the study.

7.5.2. Demographics and Baseline Disease Characteristics

Demographic and baseline disease data, e.g., age, gender, race, WHO classification, disease category, Extra Medullary Disease, baseline blood parameters (including WBC, hemoglobin, platelet, PB blast counts, marrow blast counts, etc), pathology, cytogenetics, ELN risk classification, height, weight etc., will be summarized descriptively (number of patients (frequency), mean, SD, median, minimum, and maximum) by treatment arm and overall, in the analysis population.

Medical history will be summarized by treatment arm and overall, in the safety population.

7.5.3. Medical History

Prior cancer therapy for previous cancer and current cancer, medical history, and signs and symptoms that collected during screening will be summaries by treatment arm and overall.

7.5.4. Concomitant Medications and Procedure

The use of concomitant medications and procedure will be recorded on the CRFs. The concomitant medications will be coded to a World Health Organization Drug Dictionary (WHO-DD) term (Version WHODRUG Version - March 2016).

The use of concomitant medications and procedures will be summarized by arm and overall, for the safety population for induction and consolidation separately. In each of these summary tables, the number and percentage of patients taking each medication will be presented by ATC Classification.

7.5.5. Study Treatment Exposure

A summary of number of induction and consolidation cycles received by treatment arms will be displayed.

7.5.6. Adverse Event

An overall summary of adverse events by 2 treatment arms will be performed for induction and consolidation separately. It will include number and percentage of treatment-related AE (TrAE), Grade 3 or higher TrAE, serious TrAE, treatment-related grade 3 or higher AE leading to stop treatment and study discontinuation, death, etc. The worst grade of treatment-related non-hematological AE for each patient will also be summarized by treatment arms for induction and consolidation separately.
The following AEs are considered as hematological AEs:
Anemia, CD4 lymphocytes decreased, Lymphocyte count decreased, Neutrophil count decreased, Platelet count decreased, and White blood cell decreased.
All others that are not in the above list will be classified as Non-hematological AEs.

All collected adverse events are coded in MedDRA version 23.0 The treatment-emergent adverse events (TEAE) are defined as database collected AE that onset on or after the first protocol treatment (Gilteritinib or Midostaurin) date or worsening of a pre-existing AE after treatment. TrAE is defined as the investigators' assessed drug-related adverse events and their causality after drug is administered. All TrAE will be summarized by system organ class and preferred term and 2 treatment arms and displayed in frequency order.
All TrAEs that onset before the first dose of consolidation treatment will be summarized in induction tables. The TrAEs that onset on or after the first dose of consolidation treatment will be summarized in consolidation tables.

7.5.7. Clinical Laboratory Data

The Lab shift from baseline table will be displayed by treatment arm at End of Induction, End of Consolidation and End of Study for each laboratory test.

Laboratory results will be graded by CTCAE (v5.0) and summary the maximum grade in each lab category.

7.5.8. Physical Exam Data

All abnormal findings from physical exam are recorded in medical history, adverse event or other appropriated CRF pages. There are no data display for physical exam data.

7.5.9. ECG Data

ECG data collected value and change from baseline will be summarized by treatment cycle and displayed by treatment arm and overall treatment group. For the triplicate measurements in a timepoint, the average of the 3 records will be represented for that timepoint value in analysis.

7.6. Efficacy Analysis

All Efficacy analyses are performed for efficacy population.

7.6.1. FLT3 mutation negative Composite Complete Response (CRc) rate in induction

This is primary endpoint. The composite complete response includes CR and CRi. Subjects who are FLT3 mutation negative by PCR at the end of induction and response of CR or CRi are counted as responders in this analysis. The rates are derived from responders over each treatment arms.

The final analysis of FLT3 mutation negative CRc rate will be performed after the last patient has completed the induction treatment and all patients have MRD response data available. Patients who drop out prior to MRD response assessment will be counted as non-responders in the analysis. The FLT3 mutation negative CRc rate will be compared between gilteritinib and midostaurin arms using Fisher's exact test. All eligible and treated patients will be included into the analysis. Two-sided 90% confidence interval on the difference in the FLT3 mutation negative CRc rate will be provided based on the normal approximation to the difference of two independent binomial proportions with continuity correction.
Multivariable logistic regression modeling will be used to adjust for the stratification factors and other possible baseline clinical and biological risk factors (eg, age, WBC, hemoglobin, platelet, disease categories, and cytogenetics).

Comparison of CRc rate between treatment arms will also be conducted in patients with de novo AML, t- AML and AML-MRC separately; and in patients with favorable, intermediate, and adverse risk separately, determined by 2017 ELN risk classification.

composite responder = FLT3 mutation negative and response CR or CRi after induction treatment
responder rate = responder / all efficacy population
composite responder rate in arm A = responder in arm A / arm A efficacy
population composite responder rate in arm B = responder in arm B / arm
B efficacy population

A sensitivity analysis will also be done among those with MRD assessment after induction
responder rate = responder / (all efficacy population subject – subject dropouts prior to MRD assessment) composite responder rate in arm A = responder in arm A / (arm A – subjects' dropout prior to MRD assessment) composite responder rate in arm B = responder in arm B / (arm B – subjects' dropout prior to MRD assessment)

7.6.2. FLT3 mutation negative Complete Response (CR) rate in induction

This is one of secondary endpoints. The FLT3 mutation negative Complete Response (CR) rate of patients with FLT3 mutated AML who receive gilteritinib will be compared to those who receive midostaurin in addition to standard therapy with cytarabine and daunorubicin during induction.

responder = FLT3 mutation negative and response CR after induction
treatment responder rate = responder / all efficacy population
responder rate = responder in arm A / arm A efficacy
population responder rate = responder in arm B / arm
B efficacy population

The final analysis of FLT3 mutation negative CR rate will be performed after the last patient has completed the induction treatment and all patients have MRD response data

available. Patients who drop out prior to MRD response assessment will be counted as non-responders in this analysis. The FLT3 mutation negative CR rate will be compared between gilteritinib and midostaurin arms using Fisher's exact test. Two-sided 90% confidence interval on the difference in the FLT3 mutation negative CR rate will be provided based on the normal approximation to the difference of two independent binomial proportions with continuity correction.

Multivariate logistic regression modeling will be used to adjust for the stratification factors and other possible baseline clinical and biological risk factors (eg, age, WBC, hemoglobin, platelet, disease categories, and cytogenetics).

7.6.3. MRD- (evaluated by flow) CRc rate

The MRD- CRc responders are subjects who respond CR or CRi and MRD response is negative as evaluated by flow.

The MRD- CRc rate will be compared by 2 treatment arms using Fisher's exact test. Two-sided 90% confidence interval on the difference in the MRD- CRc rate will be provided based on the normal approximation to the difference of two independent binomial proportions with continuity correction.

Multivariate logistic regression modeling will be used to adjust for the stratification factors and other possible baseline clinical and biological risk factors (eg, age, WBC, hemoglobin, platelet, disease categories, and cytogenetics).

7.6.4. CRc (CR or CRi) Rate

The CRc (CR or CRi) rates will also be compared between 2 treatment arms by using Fisher's exact test. Two-sided 90% confidence interval on the difference in the CRc rate will be provided based on the normal approximation to the difference of two independent binomial proportions with continuity correction.

Multivariate logistic regression modeling will be used to adjust for the stratification factors and other possible baseline clinical and biological risk factors (eg, age, WBC, hemoglobin, platelet, disease categories, and cytogenetics).

7.6.5. FLT3 mutation negative CRc (CR or CRi) ate after first consolidation cycle

This analysis is for the patients who are FLT3 mutation positive after induction and administered consolidation. The CRc (CR or CRi) rates will also be compared between 2 treatment arms by using Fisher's exact test. Two-sided 90% confidence interval on the difference in the CRc rate will be provided based on the normal approximation to the difference of two independent binomial proportions with continuity correction.

7.6.6. Event-Free Survival Analysis (EFS)

EFS is defined as time from study randomization to the date of induction treatment failure,

relapse after CRc or to death from any cause after CRc, whichever comes first. For patients alive without induction treatment failure or relapse after CRc, EFS will be censored at the date of the last disease evaluation.

The main analysis of EFS is to set the event date for induction treatment failure on day 1 of randomization.

A sensitivity analysis of EFS will be performed where follow-up will be censored at the start of transplantation (if transplant was done before the date of the last disease evaluation).

7.6.7. Overall Survival (OS)

OS is defined as the time between randomization and death from any cause. The censored follow-up time for patients without death information is the date of last contact.

The final analysis of EFS and OS will be performed when the full information of EFS is reached (i.e., 110 events have occurred). Estimates of EFS and OS, including medians and confidence intervals, will be calculated using the Kaplan-Meier method. Comparison of EFS and OS between treatment arms will be conducted using the one-sided stratified log-rank test with FLT3 mutation (TKD vs ITD), FLT3-ITD mutation signal ratio (high [≥ 0.5] vs. low [<0.5] of FLT3 Wild Type), and NPM1 mutation status, the same stratification factors used in randomization. A multivariate Cox proportional hazards model, stratified on the same factors above, will also be used to assess the treatment effect.

Comparison of EFS and OS between treatment arms will also be conducted in patients with de novo AML, t-AML and AML-MRC separately; and in patients with favorable, intermediate, and adverse risk separately, determined by 2017 ELN risk classification.

7.6.8. Predictive value of Minimal Residual Disease (MRD) positivity and negativity post induction therapy

To determine the predictive value of Minimal Residual Disease (MRD) positivity and negativity post induction therapy. EFS and OS of the patients who achieved MRD negative after induction will be compared with those who did not. All CRc patients who have MRD response evaluated post induction will be included into this analysis. The interaction of treatment and MRD status will be tested in the Cox regression model. If a strong interaction effect is detected, the treatment effect will be looked at within MRD+ and MRD- patients separately.

The correlation between MRD by molecular studies and morphology will be assessed. McNemar's test will be used to assess the concordance of the measurements.

To assess the correlation of FLT3 mutation with outcome, the EFS and OS of those patients with FLT3- TKD mutation will be compared with patients without FLT3-ITD mutation. Among those patients with FLT3-ITD mutation, EFS and OS will be compared among those with ITD signal ratio >0.5 with those ≤ 0.5 of FLT3 Wild Type.

7.7. Interim Analysis

There is an Interim Analysis of FLT3 Mutation Negative CRc Rate and EFS in this study.

An interim futility analysis of FLT3 mutation negative CRc rate will be performed when 90 (i.e., 50% of
179) patients have MRD response data available. The PrECOG Data Safety Monitoring Board (DSMB) may consider stopping the study for inefficacy if the observed FLT3 mutation negative CRc rate in the gilteritinib arm is lower than the control arm. The FLT3 mutation negative CRc rate (and CR rate) with two treatment arms combined will also be calculated. Upon the approval of the DSMB, it may be released to the study team to guide the design in other AML Phase III trials.

When the last patient has completed the induction treatment and all patients have MRD response data available, the final analysis of FLT3 mutation negative CRc rate will be performed. At that time, if the information fraction of EFS >50% (i.e., more than 55 EFS events have occurred), an interim futility analysis of EFS will be performed as well. Otherwise, the interim analysis of EFS will be performed later until 50% of information is reached. In the interim analysis of EFS time, the Kaplan-Meier curve for each arm and the point estimate and two-sided 90% confidence interval of hazard ratio (gilteritinib arm vs. control arm) will be provided. If the lower bound of the 90% confidence interval of HR is above 1, the study may be permanently terminated for inefficacy in EFS.

8. SUMMARY OF CHANGES FROM PROTOCOL-SPECIFIED ANALYSES

No changes are planned.

9. REFERENCES

References are provided in the protocol.

10. TABLES, FIGURES, AND LISTINGS

See separate template document.

11. APPENDIX

11.1. Appendix I. Study Procedure and Parameters

Procedures	Pre-screen	Screening/ Prior to Randomization	Induction*					Consolidation*			End of Treatment[2]	Follow - Up[2]
			Daily	Weekly	Day 21 from Start of Cycle 1 Induction	Prior to 2nd Induction, if Applicable	End of Induction	Prior to Each Consolidation Cycle	Weekly	End of Consolidation		
Written Informed Consent	X[1]	X										
Disease Characteristics[2]		X										
Medical/Surgical History		X										
Assessment of Baseline Signs and Symptoms		X										
Height		X										
Physical Exam		X	X[3]			X		X	X		X	
Vital Signs (Temperature, Pulse, Blood Pressure)		X	X[3]			X		X			X	
Weight[4]		X				X		X			X	
BSA		X				X		X			X	
Performance Status		X		X		X		X			X	
CBC/Differential/ Platelet[4,5]		X	X[6]			X		X	X[6]		X	
Chemistry[4,7]		X		X[8]		X		X	X[8]		X	
Liver Functions[4,9]		X		X[8]		X		X	X[8]		X	
Thyroid Function Test (T4 & TSH)[10]		X									X	
Serum Pregnancy Test[11]		X										
Left Ventricular Ejection Fractions (MUGA or ECHO)[12]		X				X[12]						
12-Lead ECG (QTcF Interval)		X[13]		X[14]	X[14]	X[14]		X[14]				
Local Bone Marrow Aspirate Biopsy (Mandatory)	X[2]				X[15]		X[17]			X		
Research Bone Marrow Samples Submission (Mandatory)[19]	X[1]						X			X		

Protocol Therapy Administration[20]			X					X				
Concomitant Medications		X	X[3]			X		X				
Adverse Events Assessment			X[3]			X		X			X	
Survival Status											X	

* Scheduled Visits: Delay due to holidays, weekends, bad weather or other unforeseen circumstances will be permitted.

1 Any patient undergoing bone marrow biopsy with suspicion of or known diagnosis of AML will be asked to sign a Prescreening Consent in order to obtain baseline immunophenotyping and determination/confirmation of FLT3 status and obtain research samples for the study prior to randomization. FLT3 (includes signal ratio), NPM1 and DNMT3 mutation status will be performed on all patients. These tests will be completed at Central Labs. See Section 13.1, Invivoscribe Lab Manual and PrE0905 Lab Manual for details. FLT3 and NPM1 mutation status results must be available on all patients. Patients must have known FLT3 mutation and will be stratified according to TKD vs. ITD FLT3 mutation. Patients with FLT3-ITD mutation will undergo further stratification with NPM1 mutation status (positive vs. negative) and signal ratio (high [≥ 0.5] vs. low [<0.5] of FLT3 Wild Type).
For patients who are registered with tissue submitted for the study and are FLT3 negative or other condition, no further information will be collected after baseline demographics, sample submission, sample results and final diagnosis.

2. Record date of diagnosis (date of diagnostic bone marrow biopsy), primary tumor type, histology, cytogenetics and molecular markers.

3 **Induction:** Daily if admitted to hospital, otherwise weekly until count recovery.
Consolidation: May be done locally after Day 8 visit per institutional guidelines.

4. Per Study Parameters and as clinically indicated.

5 CBC with differential and platelet count which includes WBC, ANC, Platelets, Hemoglobin, and Hematocrit. Additionally, required within 24 hours prior to each cycle of chemotherapy.

6 **Induction:** Differential daily until disappearance of peripheral blasts and then again when WBC over 500. Differential twice weekly at nadir, after peripheral blasts gone and while WBC <500.
Consolidation: Twice weekly.

7 BUN/creatinine, uric acid, sodium, potassium, chloride, glucose, calcium, magnesium, and phosphorus. Additionally, required within 24 hours prior to each cycle of chemotherapy.

8 **Induction:** Twice weekly.
Consolidation: Weekly.

9 AST, ALT, and total bilirubin. If total bilirubin ≥ 2, obtain direct bilirubin (if direct bilirubin ≥ 2, refer to Section 6.1 for Dose Modifications/Interruptions). Additionally, required within 24 hours prior to each cycle of chemotherapy.

10 Thyroid Function test results not required for randomization.

11 Required for sexually active females of child-bearing potential.

12 LVEF must be obtained before initiating second induction cycle (if applicable) and must use same method as initial assessment.

13 Perform 12-lead ECG in triplicate (3 separate ECGs recorded at least 5 minutes apart) following a 10-minute resting period. Patient may not have QTcF interval >500 msec or Long QT Syndrome.

Calculation of QTc interval using the Fridericia formula:

https://qxmd.com/calculate/calculator_48/ecg-corrected-qt37,38,39

14 Perform 12-lead ECG in triplicate (3 separate ECGs recorded at least 5 minutes apart) following a 10-minute resting period prior to gilteritinib or midostaurin administration (ECG within 1 hour of dosing preferred). The mean QTcF of the triplicate ECG tracings will be used for final treatment decisions and AE reporting. If the mean of the triplicate QTcF is >500 msec, then triplicate ECGs will be repeated (within 2 hours). If the repeat ECGs confirm a mean QTcF >500 msec, refer to Section 6.1.2 and Section 6.1.3 for further guidance on treatment decision. In addition, on Cycle 1, Day 15 if QTcF is increased >30 msec over screening, repeat ECG on Day 16 and refer to Section 6.1.2 for further guidance on treatment decision.

Induction (First Induction)

Day 8, Day 15 and Day 21

Second Induction and Consolidation

Day 1 of each cycle

See footnote 13 for Calculation of QTc interval using the Fridericia formula.

15 Perform 12-lead ECG in triplicate (3 separate ECGs recorded at least 5 minutes apart) following a 10-minute resting period.

See footnote 13 for Calculation of QTc interval using the Fridericia formula.

16 +/- 1 day window (Delays due to holidays, weekends, or other unforeseen circumstances will be permitted).

17 For patients that receive 1 cycle of induction, no later than Day 60 after initiation of first cycle of induction therapy. For patients that receive 2 cycles of induction, no later than Day 60 after initiation of the second cycle of induction therapy (i.e., Day 84-105). [MRD sample will be obtained by Day 60 on all patients even if counts have not recovered. The only exception is a patient who has been declared a relapse based on blood counts.]

18 Optional, at time of relapse, bone marrow samples are requested to be sent for correlative studies (Section 13.1).

19 Mandatory Research Bone Marrow Samples: See Section 13.1, Invivoscribe Lab Manual and PrE0905 Lab Manual for details.

Prescreening

Invivoscribe: One 4 mL sodium heparin green top tube

- If bone marrow sample (preferred) is not available, then one 4 mL Sodium Heparin green top tube of peripheral blood will be allowed if patient has any circulating blasts.

CBPF: Two 10 mL EDTA tubes

- If diagnostic bone marrow was done prior to study enrollment or patient is inaspirable with a minimum of 10% blasts AND a minimum of 1000 WBC/mm3 peripheral blood, then two 10 mL EDTA tubes of peripheral blood may be sent instead of bone marrow.

End of Induction (First and Second Induction, as applicable)

Invivoscribe: If FLT3 ITD+, one 4 mL EDTA tube (bone marrow)

CBPF: All patients (ITD+ and/or TKD+), one 10 mL EDTA tube (bone marrow) If FLT3 TKD+, one additional 10 mL EDTA tube (bone marrow)

NOTE: Patients are not eligible to proceed to consolidation unless bone marrow samples are obtained for MRD at time of CR/CRi.

End of Consolidation

Invivoscribe: If FLT3 ITD+, one 4 mL EDTA tube (bone marrow)

CBPF: All patients (ITD+ and/or TKD+), one 10 mL EDTA tube (bone marrow) If FLT3 TKD+, one additional 10 mL EDTA tube (bone marrow)

Relapse (OPTIONAL)

CBPF: Two 10 mL EDTA tubes (bone marrow)

Optional: Any leftover tissue banked for future research.

INDUCTIONS:

Standard of care induction 7+3 chemotherapy may start prior to randomization using same regimen and dose as defined in Section 5.2.1 while awaiting prescreening test results.

Arm A (7+3 + Gilteritinib)

- Cytarabine 100 mg/m2/day will be administered by continuous IV infusion for a total of 7 days beginning Day 1
- Daunorubicin 90 mg/m2/day will be administered IV per package insert or institutional guidelines or over 30-60 minutes Days 1,2,3 (45 mg/m2/day if receives second cycle of induction)
- Gilteritinib 120 mg orally QD x 14 days starting on day 8

If second cycle of induction is needed, treatment will begin after Day 28 (i.e., ≥ 7 days after completing gilteritinib) but no later than Day 45.

ARM B (7+3) + Midostaurin

- Cytarabine 100 mg/m2/day will be administered by continuous IV infusion for a total of 7 days beginning Day 1
- Daunorubicin 90 mg/m2/day will be administered IV per package insert or institutional guidelines or over 30-60 minutes Days 1,2,3 (45 mg/m2/day if receives second cycle of induction)
- Midostaurin 50 mg orally BID x 14 days beginning on day 8

If second cycle of induction is needed, treatment will begin after Day 24 (i.e., ≥ 3 days after completing midostaurin) but no later than Day 45.

If there is a delay in starting FLT3 inhibitors in Arm A or Arm B (due to obtaining drug, i.e. insurance, formulary, etc.), the FLT3 inhibitor will need to stop after Day 21 dose (i.e., Day 21 or Day 22 [if FLT3 inhibitor started on Day 9]). No missed doses will be made up.

See Section 5 for dosing instructions and additional information for gilteritinib and midostaurin administration and Section 6 for dose delays/modifications. If CR or CRi is not achieved, a second induction cycle of therapy may be administered.

NOTE: Patients are not eligible to proceed to consolidation unless bone marrow samples are obtained for MRD at time oCR/CRi.

CONSOLIDATION:

ARM A

- Cytarabine 3 g/m2* over approximately 1-3 hours every 12 hours on days 1,3,5 or days 1-3 for a total of 6 doses for up to 4 cycles
- Gilteritinib 120 mg orally QD x 14 days beginning on day 8 of each cycle (up to 4 cycles)

ARM B

- Cytarabine 3 g/m2* over approximately 1-3 hours every12 hours on days 1,3,5 or days 1-3 for a total of 6 doses for up to 4 cycles
- Midostaurin 50 mg orally BID x 14 days beginning on day 8 of each cycle (up to 4 cycles)

* For patients age ≥ 55 or patients with decreased creatinine clearance recommend reducing consolidation cytarabine dose to 1.5 g/m2.
If there is a delay in starting FLT3 inhibitors in Arm A or Arm B (due to obtaining drug, i.e., insurance, formulary, etc.), the FLT3 inhibitor will need to stop after Day 21 dose. No missed doses will be made up.
See Section 5 for dosing instructions and additional information for gilteritinib and midostaurin administration and Section 6 for dose delays/modifications.

NOTE: Patients deemed suitable by the treating investigator may proceed to allogeneic TRANSPLANT after induction or after 0-4 cycles of consolidation. Patients will go off treatment at the time of transplant or any non-protocol leukemia directed therapy after completion of the end of treatment visit.

21 Patients will be followed for adverse events for 30 days after their last dose of study medication. However, an adverse event occurring at any time after discontinuation of study therapy that is felt to be at least possibly related to study therapy should be recorded.

NOTE: Pregnancy within 180 days for WOCBP and for 120 days for a female partner of child-bearing potential after the final study drug administration will be reported and followed per Section 8.6.

22 Upon count recovery after last cycle of chemotherapy.

23 Every 3 months from end of treatment for 2 years, then every 4 months for 1 year, then every 6 months for 1 year, then annually for progression and survival until death or study closure (+/- 1 month) whichever comes first. Patients should be seen up to the 2-year point at the treating institution. If the patient is unable to be seen at the treating institution, copies of office visit notes including labs with local oncologist should be obtained until year 5 per schedule above. Initiation of all anticancer therapy for current remission and first anticancer therapy for relapse will be documented. If patient is removed from treatment for reason(s) other than progression or full consent withdrawal, follow for relapse/survival until death or study closure.

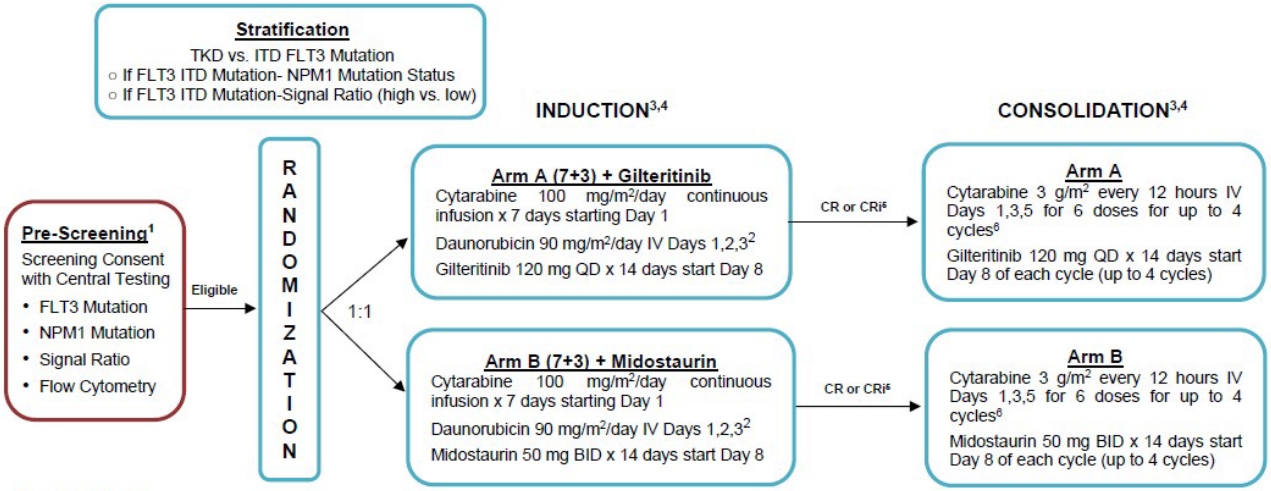
NOTE: For patients who are registered with tissue submitted for the study and are FLT3 negative, no further information or other condition will be collected after baseline demographics, sample submission, sample results and final diagnosis. For eligible patients randomized to the trial, but do not receive any protocol therapy, baseline, safety [as applicable] and end of treatment follow-up information will also be collected.

Appendix II. Brief Protocol Synopsis

Brief Protocol Synopsis

See Protocol Document Sections for complete details

Study Schema



Accrual Goal: 179

1 Any patient undergoing bone marrow biopsy with suspicion of or known diagnosis of AML will be asked to sign a Prescreening Consent in order to confirm diagnosis and determination/confirmation of FLT3 status at central laboratory and obtain research samples for the study prior to randomization.
2 Daunorubicin 90 mg/m^2/day will be administered IV over 30-60 minutes Days 1,2,3 (45 mg/m^2/day if receives second cycle of induction).
3 Patients may proceed to allogeneic TRANSPLANT after induction or after 0-4 cycles of consolidation.
4 Patients will go off treatment at the time of transplant or any non-protocol leukemia directed therapy.
5 If Complete Response (CR) or CR with incomplete hematologic recovery (CRi) is not achieved, a second induction cycle of therapy may be administered.
6 For patients age ≥ 55 reduce consolidation cytarabine dose to 1.5 g/m^2.

13. DOCUMENT HISTORY

Version Date	Modified By	Summary of Changes
16Mar2020	Wei Chen	First draft, version 1.0
17Sep2020	Wei Chen	updated per the version 2.0 protocol and PrECOG review comments. The major changes: 1. added a new exploratory objective 2. Update TEAE definition per standard care 7+3 therapy can start prior randomization 3. AE/ConMed analysis separated by induction/consolidation 4. Non-hematology AE
03Nov2020	Wei Chen	1. Do the similar as AE analysis for Lab, Vital, ECG, separate Induction and Consolidation. 2. Specified Non- hematology AE list 3. Implement induction/Consolidation separate analysis to the mock tables. Added more tables and Figures 4. Unified SAP, Table Mock and Listing Mock 3 files in same version and date.
15Nov2021	Dapo Olaitan	1. Eligible patients' definition modified and added to the mock table. 2. Added 2017 ELN Risk Classification to the mock table 3. Data inclusion on vital sign and physical examination modified 4. Drug related adverse events (TrAE) modified 5. Vital sign summary modified and dropped from the mock table 6. MRD CRc secondary endpoint definition modified by flow cytometry and updated in the mock table. 7. Models are updated accordingly and by request in the mock table. 8. Unified updates within SAP, Table Mock and Listings.
09Nov2023	Li Chen	1. Update on Author of the Document. 2. Updates to sections 7.6.2, 7.6.3, 7.6.4 3. Change in Reporting Conventions.