PrECOG Protocol Number: PrE0905

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

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

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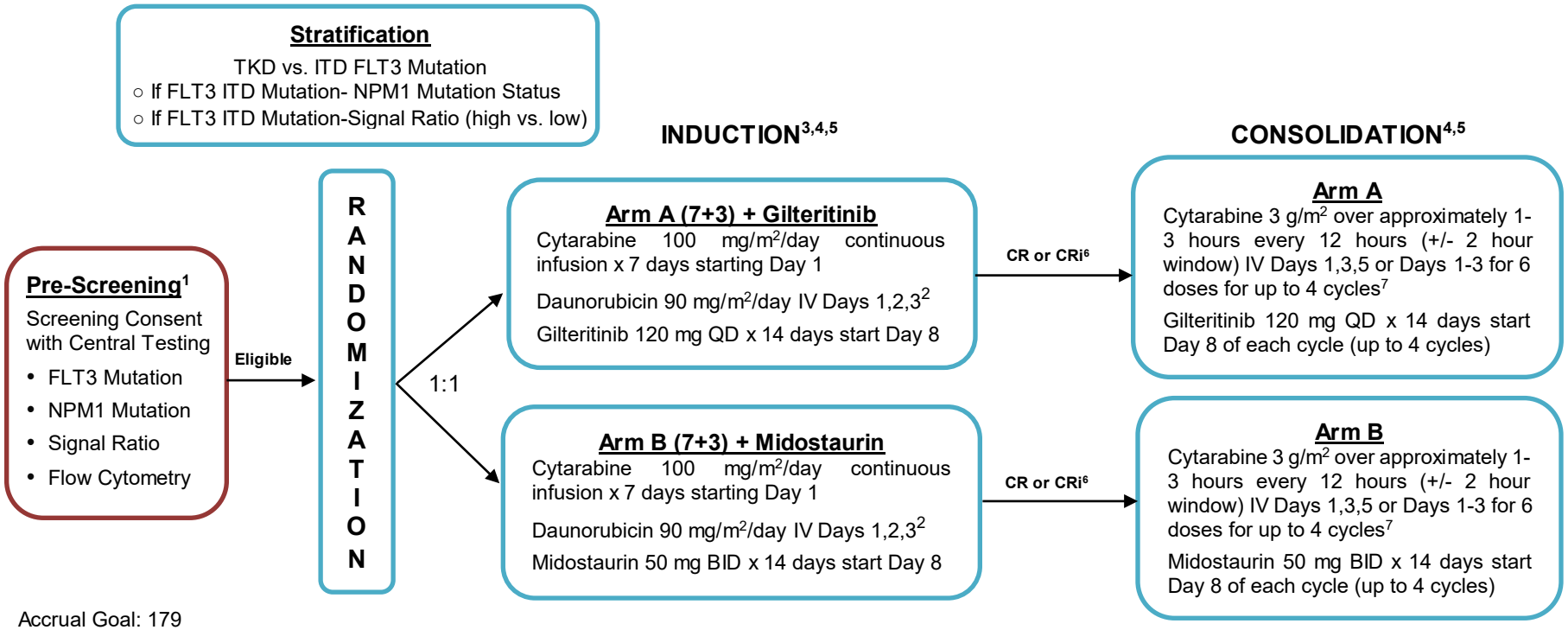
Brief Protocol Synopsis

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 per package insert or institutional guidelines or over 30-60 minutes Days 1,2,3 (45 mg/m^2/day if receives second cycle of induction).
3 Standard of care induction 7+3 chemotherapy may start prior to randomization using same regimen and doses as noted above while awaiting prescreening test results.
4 Patients may proceed to allogeneic TRANSPLANT after induction or after 0-4 cycles of consolidation.
5 Patients will go off treatment at the time of transplant or any non-protocol leukemia directed therapy.
6 If Complete Response (CR) or CR with incomplete hematologic recovery (CRi) is not achieved, a second induction cycle of therapy may be administered.
7 For patients age ≥ 55 or patients with decreased creatinine clearance recommend reducing consolidation cytarabine dose to 1.5 g/m^2.

List of Abbreviations

Abbreviation	Term
$5HT_{2B}R$	5-Hydroxytryptamine Receptor 2B.
AE	Adverse Event
AESI	Adverse Events Special Interest
ALK	Anaplastic Lymphoma Kinase
ALT	Alanine Aminotransferase
AML	Acute Myeloid Leukemia
AML-MRC	AML-Myelodysplasia-Related Changes
ANC	Absolute Neutrophil Count
APL	Acute Promyelocytic Leukemia
AST	Aspartate Aminotransferase
AUC	Area Under the Concentration-Time Curve
BCRP	Breast Cancer Resistance Protein
BID	Twice a Day
bp	Base Pair
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
C	Celsius
CBC	Complete Blood Count
CBPF	Central Biorepository Pathology Facility
CFR	Code of Federal Regulations
CHF	Congestive Heart Failure
CI	Confidence Interval
CK	Creatine Kinase
C_{max}	Maximum (Peak) Drug Concentration
CNS	Central Nervous System
CPK	Creatine Phosphokinase
CR	Complete Response
CRc	Composite Complete Remission
CRh	CR with Partial Hematologic Recovery

Abbreviation	Term
CRi	CR with Incomplete Hematologic Recovery
CRp	Complete Remission with Incomplete Platelet Recovery
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
C_{trough}	Trough Concentration
CYP	Cytochrome P450
DFS	Disease Free Survival
dL	Deciliter
DLT	Dose-Limiting Toxicity
DNA	Deoxyribonucleic Acid
DNMT3A	DNA Methyltransferase 3 Alpha
DNR	Daunorubicin
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
ECOG-ACRIN	Eastern Cooperative Oncology Group-American College of Radiology Imagine Network
eCRF	Electronic Case Report Form
eDC	Electronic Data Capture
EDTA	Ethylenediamine Tetraacetic Acid
EFS	Event Free Survival
ELN	European LeukemiaNet
F	Fahrenheit
FDA	Food and Drug Administration
FISH	Fluorescence In Situ Hybridization
FLT3	FMS-Like Tyrosine Kinase 3
FSH	Follicle-Stimulating Hormone
g	Gram
GCP	Good Clinical Practice

Abbreviation	Term
GFR	Glomerular Filtration Rate
GI	Gastrointestinal
HiDAC	High Dose Cytarabine
HIPAA	Health Information Portability and Accountability Act
hr	Hour
HRT	Hormone Replacement Therapy
HSCT	Hematopoietic Stem Cell Transplant
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDR	Idarubicin
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
ITD	Internal Tandem Duplication
IUD	Intrauterine Device
IUS	Intrauterine Hormone-Releasing System
IV	Intravenous; Intravenously
LDH	Lactate Dehydrogenase
LTK	Leukocyte Receptor Tyrosine Kinase
LVEF	Left Ventricular Ejection Fraction
m^2	Square Meter
mm^3	Cubic Millimeter
MDS	Myelodysplastic Syndrome
mg	Milligrams
min	Minute
mL	Milliliter
MLFS	Morphologic Leukemia-Free State
MPN	Myeloproliferative Neoplasms
MRD	Minimal Residual Disease

Abbreviation	Term
msec	Millisecond
MTD	Maximum Tolerated Dose
MUGA	Multigated Acquisition Scan
Mut+	Mutation Positive
n	Number
NCI	National Cancer Institute
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NE	Not Evaluable
ng	Nanogram
ng·h/mL	Hours Times Nanograms per Milliliter
NGS	Next Generation Sequencing
NPM1	Nucleophosmin 1-Mutated
NR	No Response
NSCLC	Non-Small Cell Lung Cancer
NYHA	New York Heart Association
OCT1	Organic Cation Transporter 1
OS	Overall Survival
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PDGF	Platelet-Derived Growth Factor
P-gp	P-glycoprotein
PIA	Plasma Inhibitory Activity
PO	*per os*; By Mouth (orally)
PK	Pharmacokinetics
PR	Partial Remission/Response
PRES	Posterior Reversible Encephalopathy
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QD	Daily

Abbreviation	**Term**
QTc	Corrected QT Interval
QTcF	Fridericia-Corrected QT Interval
ΔQTcF	Change from baseline in Fridericia-Corrected QT Interval
R_{ac}	Accumulation Index
SAE	Serious Adverse Event
T4	Thyroxine
t-AML	Therapy-Related Acute Myeloid Leukemia
TEAE	Treatment-Emergent Adverse Event
TK	Tyrosine Kinase
TKD	Tyrosine Kinase Domain
TKI	Tyrosine Kinase Inhibitor
t_{max}	Time to Reach Maximum (Peak) Plasma Concentration Following Drug Administration
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of the Normal Range
US	United States
vs	Versus
WBC	White Blood Cell
WOCBP	Women of Childbearing Potential
wt	Weight

1. Introduction- Background and Rationale

1.1 Acute Myeloid Leukemia (AML) – Disease Overview

The American Cancer Society estimates about 60,300 new cases of leukemia (all kinds) and 24,370 deaths from leukemia (all kinds) will occur in 2018.[1] The median age at diagnosis is 67 years of age, with 54% of patients diagnosed at 65 years or older.[2] It is estimated that 19,520 people will be diagnosed with Acute Myeloid Leukemia (AML), and 10,670 will die from the disease in 2018 in the United States.[1] While 60% to 80% of younger patients achieve a complete remission (CR) with standard therapy, only about 30% to 40% of the overall patient population has long-term disease-free survival.[3]

While patients under the age of 70 with AML are likely to achieve a remission with standard therapy, the majority will relapse and die of their disease. Cytogenetic and molecular factors have been found to be critical in prognosis. Currently, there is no effective cure for the disease.

1.1.1 Standard Chemotherapy for Induction and Consolidation

Induction therapy for previously untreated patients with AML has been fairly standardized over the past two decades with an anthracycline and cytarabine, followed by consolidation usually with high-dose cytarabine (HiDAC).

Based on the RATIFY trial (C10603) midostaurin was approved in 2017 for the treatment of patients with FLT3 mutated AML in combination with standard induction and consolidation.[4]

1.2 Background/Rationale

An overall CR rate of 50% to 80% can be expected with standard induction chemotherapy. Consolidation therapy is given with the goal of decreasing the risk of relapse and improving survival. However, presence of a FLT3 mutation in AML patients of any age is a poor prognostic factor.[5,6,7] In these patients, although the CR rate with standard chemotherapy regimens is generally equivalent, the relapse rate, disease free survival (DFS), event free survival (EFS), and overall survival (OS) at 5 years are significantly worse.[8] The addition of a targeted agent against FLT3 offers the possibility for significant therapeutic advantage in AML patients expressing FLT3,[9] through both an additive effect on leukemia cell kill, as well as through potentiation of chemotherapy-induced cell death.

1.2.1 FMS-Like Tyrosine Kinase (FLT3)

FMS-like tyrosine kinase (FLT3) is a member of the class III receptor tyrosine kinase (TK) family that is normally expressed on the surface of hematopoietic progenitor cells. FLT3 and its ligand play an important role in proliferation, survival and differentiation of multipotent stem cells. FLT3 is overexpressed in the majority of AML cases. In addition, activated FLT3 with internal tandem duplication (ITD) in and around the juxtamembrane domain and tyrosine kinase domain mutations at around D835 in the activation loop are present in 28% to 34% and 11% to 14% of AML cases, respectively.[10] These activated mutations in FLT3 are oncogenic and show transforming activity in cells.[11] Patients with FLT3-ITD mutation show poor prognosis in clinical studies, with a higher relapse rate, a shorter duration of remission from initial therapy (6 months versus 11.5 months for those without FLT3-ITD mutations) as well as reduced disease-free survival (16% to 27% versus 41% at 5 years) and OS (15% to 31% versus 42% at 5 years).[12,13,14,15,16] The incidence of relapse after hematopoietic stem cell transplant (HSCT) is also higher for patients with FLT3-ITD (30% versus 16% at 2 years for those without FLT3-ITD mutations).[17] Similar to their prognosis for first-line therapy, patients with relapsed/refractory FLT3 mutated AML have lower remission rates with salvage chemotherapy; shorter durations of remission to second relapse and

decreased OS relative to FLT3 mutation negative patients.[18,19,20] As such, efforts have been made to decrease the risk of relapse with allogeneic transplant in first remission and with the incorporation of novel agents in therapy.

Midostaurin is a small molecule that inhibits multiple receptor tyrosine kinases.[21] Midostaurin demonstrated the ability to inhibit FLT3 receptor signaling and cell proliferation, and it induced apoptosis in leukemic cells expressing ITD and TKD mutant FLT3 receptors or overexpressing wild type FLT3 and PDGF receptors.[21] Midostaurin also demonstrated the ability to inhibit KIT signaling, cell proliferation and histamine release and induce apoptosis in mast cells.[21] C10603 (Ratify) added midostaurin, a multikinase inhibitor, to induction and consolidation therapy and used midostaurin as maintenance therapy in patients with FLT3 mutated AML. Initial reports demonstrated a 5-year overall survival benefit (50.9% vs 43.9%, P=0.0078) with fewer patients experiencing events (lack of CR by day 28, relapse in 19.3% vs 27.5%).[4] Benefit was seen in patients with both FLT3-ITD and FLT3-TKD mutations. Based on the Ratify trial, midostaurin 50 mg orally twice a day (BID) has received Food and Drug Administration (FDA) approval for newly diagnosed AML that is FLT3 mutation-positive as detected by an FDA-approved test, in combination with standard cytarabine and daunorubicin induction and high-dose cytarabine consolidation.

1.3 Gilteritinib

Gilteritinib is a new chemical entity discovered by Astellas Pharma Inc. in collaboration with Kotobuki Pharmaceutical Co., Ltd. Gilteritinib has an inhibitory effect on TKs, mainly FLT3, tyrosine kinase receptor AXL (AXL), leukocyte receptor tyrosine kinase (LTK) and anaplastic lymphoma kinase (ALK).

AXL tyrosine kinase (AXL) is a member of TAM family (Tyro-3, AXL and Mer) receptor TKs and is normally expressed in cells of mesenchymal origin, such as osteoblasts, fibroblasts and blood cells. AXL has been reported to be overexpressed or activated in many cancers, including AML.[22] AXL overexpression in AML confers drug resistance[23] and is associated with adverse prognosis.[24,25] AXL inhibition suppresses the growth of human FLT3-positive AML in vivo.[26] In addition, AXL inhibition is also effective against FLT3-negative AML expressing AXL in vivo.[24]

Refer to the current Gilteritinib Investigator's Brochure (IB) for information on non-clinical data, in vitro, in vivo pharmacology, etc.

1.3.1 Clinical Pharmacokinetics (PK) and Pharmacodynamics[27]

Single and multiple dose gilteritinib PKs were characterized in subjects with relapse or refractory AML enrolled in Study 2215-CL-0101. Results indicated gilteritinib exhibits linear, dose-proportional PKs from 20 mg to 450 mg. Assessment of C_{trough} at various time points indicated steady state is achieved by day 15 after multiple dose administrations of gilteritinib from 20 to 450 mg once daily. An ex vivo Plasma Inhibitory Activity (PIA) assay was used to assess target inhibition in subjects treated with gilteritinib. Samples collected pre-dose and post-dose on days 1, 8, 15 and 29 demonstrated marked and sustained inhibition of phospho-FLT3 at doses 80 mg and higher.

The effect of strong and moderate cytochrome P450 (CYP)3A inhibitors on gilteritinib exposure was assessed in relapsed or refractory AML subjects (Study 2215-CL-0101) and healthy subjects (Study 2215-CL-0108). In relapsed or refractory AML subjects, there was a less than 2-fold increase in gilteritinib exposure when gilteritinib was co-administered with moderate or strong CYP3A4 inhibitors. In healthy subjects, gilteritinib exposure increased approximately 2.2-fold when gilteritinib was co-administered with itraconazole, a strong CYP3A4

and P-glycoprotein (P-gp) inhibitor. The effect of rifampin, a strong CYP3A and P-gp inducer, on gilteritinib PKs was evaluated in healthy adult subjects (Study 2215-CL-0108). Co-administration of gilteritinib with rifampin, resulted in an approximate 70% decrease in gilteritinib exposure. Collectively, these data support monitoring subjects who require concomitant medications that are strong CYP3A4 inhibitors and restricting use of concomitant medications that are strong CYP3A4 inducers.

A subset of relapsed or refractory AML subjects (2215-CL-0101) were co-administered gilteritinib and cephalexin a MATE 1 substrate to evaluate a potential drug-drug interaction. Cephalexin exposure and urinary excretion were comparable after single dose administration of cephalexin alone and in combination with gilteritinib (administered once daily). These results suggest co-administration of MATE1 substrates and gilteritinib is not expected to result in a clinically-relevant drug-drug interaction.

A preliminary analysis of the relationship between ASP2215 plasma concentration and Fridericia-corrected QT interval (QTcF) change from baseline (ΔQTcF) was performed on data from the 2215-CL-0101 study (data cutoff 06 Jun 2018). This assessment included 1885 observations from 251 patients. A model-averaging approach was used to develop a robust model to describe and predict the ASP2215 concentration-ΔQTcF relationship. A concentration-related increase in ΔQTcF was observed and the mean ΔQTcF at the mean steady-state C_{max} at 120 mg was predicted to be less than the 10 msec-threshold considered clinically significant. Additionally, 4.4% of relapse/refractory subjects had a maximum post-baseline QTcF interval >500 msec and 8.8% of patients had a >60 sec change in their maximum corrected QT interval (QTc) relative to baseline. These data indicate clinically-relevant corrected QT interval (QTc) prolongation is not anticipated.

With increasing dose of gilteritinib, increasing plasma concentrations of creatine kinase (CK) were observed. Comparison of day matched CK corrected relative to baseline with C_{trough} ASP2215 values showed a correlation with a positive slope. Similarly, comparison of the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) grade for CK elevations with gilteritinib C_{trough} values showed increasing incidence of higher CTCAE grades with increasing drug exposure. However, almost all of the elevations were grade 1 and grade 2, and the incidence of grade 3 AEs reported in the study population was low (4.8%). Overall, increasing CK plasma concentrations from baseline appeared to correlate with increasing ASP2215 plasma concentrations; the mechanism for this effect is unknown.

Gilteritinib clinical pharmacokinetics and pharmacology are summarized in the XOSPATA package insert. Additional details are described in the Gilteritinib IB.

1.3.2 Clinical Experience[27]

As of the data cutoff date of 06 Jun 2018, 1 study in AML patients, 3 studies in healthy subjects, 1 study in healthy subjects and hepatic impairment subjects and 1 study in patients with solid tumors were complete. A phase 1b/2 study in combination with erlotinib in NSCLC patients was terminated. Ongoing studies include, 8 studies in AML patients, a phase 1/2 rollover study, a taste profile study in healthy subjects and 2 expanded access studies. Overall, 170 healthy subjects, 16 subjects with hepatic impairment and 931 patients have received at least 1 dose of gilteritinib.

In Study 2215-CL-0101, a Phase 1/2 study in patients with relapsed and refractory AML, gilteritinib generally exhibited linear, approximately dose-proportional pharmacokinetics after once daily administration over the dose range evaluated (20 to 450 mg). Median t_{max} was observed between 2 and 6 hours following single and repeat dosing of gilteritinib. At a dose of 120 mg once daily, the median C_{max} at day 15 was approximately 282 ng/mL (n=3) and the median AUC_{24} at day 15 was approximately 6180 ng·h/mL (n=3). After multiple dose administration, gilteritinib exhibited a long half-life, ranging from 45 to 159 hours and up to 10-fold accumulation based on the accumulation index, R_{ac}.

Results from Study 2215-CL-0101 indicate that of the 252 patients who received at least 1 dose of gilteritinib, the majority of composite complete remission (CRc) and partial remission (PR) events were observed in FLT3 mutation-positive AML patients in dose groups of 80 mg and greater. The derived response rate (CRc + PR) at the end of treatment in the 191 FLT3 mutation-positive patients was 48.7% overall and 66.7%, 53.6%, 48.3%, 60.0% and 50.0% in the 80 mg, 120 mg, 200 mg, 300 mg and 450 mg dose groups, respectively.

Refer to the Gilteritinib IB for more details.

1.3.3 Clinical Safety[27]

Of the 252 patients in Study 2215-CL-0101 who received at least 1 dose of gilteritinib, the majority (249 [98.8%]) experienced at least 1 treatment-emergent adverse event (TEAE), and most (189 [75.0%]) patients experienced at least 1 TEAE considered by the investigator to be possibly or probably related to study drug. Common TEAEs (occurring in at least 10% of patients) included febrile neutropenia, anemia, thrombocytopenia, constipation, diarrhea, nausea, stomatitis, vomiting, abdominal pain, asthenia, fatigue, edema peripheral, pyrexia, pneumonia, sepsis, fall, alanine aminotransferase (ALT) increased, aspartate aminotransferase (AST) increased, blood alkaline phosphatase increased, blood creatinine increased, blood creatine phosphokinase increased, neutrophil count decreased, platelet count decreased, appetite decreased, hypoalbuminemia, hypocalcemia, hypokalemia, hypomagnesemia, hyponatremia, arthralgia, AML disease progression, dizziness, dysgeusia, headache, insomnia, acute kidney injury, cough, dyspnea, epistaxis, hypoxia, hypotension, pain in extremity and hypertension. No clear dose-dependent patterns were observed for overall TEAEs, TEAEs of grade 3 or higher, drug-related TEAEs, serious TEAEs or drug-related serious TEAEs. Overall, 105 patients experienced TEAEs leading to death. The incidence of deaths in the 20 mg, 40 mg, 80 mg, 120 mg, 200mg, 300 mg and 450 mg dose groups was 29.4%, 37.5%, 45.8%, 34.8%, 48.5%, 40.0% and 33.3%, respectively. The majority of the deaths were attributed to disease progression. Thirty-one patients experienced dose-limiting toxicities (DLTs). None of the doses below 450 mg met the criteria for pausing enrollment. Thus, the maximum tolerated dose (MTD) in Study 2215-CL-0101 is considered to be 300 mg.

Refer to the Gilteritinib IB for more details.

1.3.4 Dose Rationale

A Phase I/II trial in AML patients with relapsed and refractory disease treated with gilteritinib has recently been completed.[28] Gilteritinib was well tolerated in heavily pretreated patients. Grade 3 diarrhea and hepatic enzyme elevation were dose limiting at doses over 300 mg/day. At doses ≥ 80 mg/day, the derived response rate (CRc + PR) at the end of treatment in the 191 FLT3 mutation-positive patients was 48.7% overall and 66.7%, 53.6%, 48.3%, 60.0% and 50.0% in the 80 mg,

120 mg, 200 mg, 300 mg and 450 mg dose groups, respectively. The median duration of response in FLT3 mutation positive patients in ≥ 80 mg dose levels was 147.0 days (95% CI: 97.0, 307.0). In FLT3 mutation positive patients with a response of CR/CRh, the median duration of response was 383.0 days (95% CI: 136.0, Not Evaluable [NE]). The median overall survival (OS) from Kaplan-Meier estimates in FLT3 mutation positive patients in ≥ 80 mg dose levels was 218.0 days, with survival probabilities of 56.2% at 26 weeks and 24.9% at 1 year. This is in contrast to similar studies with midostaurin in which single agent therapy rarely resulted in a decrease in the bone marrow blast percentage and response was brief. The response rate to gilteritinib was furthermore not impacted by the presence of concurrent FLT3 D835 mutations. Based on this study a dose of 120 mg/day was chosen for future studies. A Phase 1 trial of gilteritinib + chemotherapy in newly diagnosed AML 2215-CL-0103 and a randomized trial of azacitidine +/- gilteritinib are ongoing.

Data provided from the manufacturer, Astellas (1 Apr 2019): A Phase 1 trial of gilteritinib + chemotherapy in newly diagnosed AML 2215-CL-0103 (NCT02236013) is ongoing with most recent results presented at American Society of Hematology in 2018.[29] At the time of the presentation, 62 subjects were enrolled at gilteritinib dose levels ranging from 40 mg to 200 mg/day. Two subjects in the 200 mg/day cohort experienced DLTs (neutropenia, neutropenic enterocolitis). The maximum tolerated dose and the recommended expansion dose were established at 120 mg/day.

The end-of-treatment investigator-reported rate of composite complete remission (CRc) for response evaluable FLT3^{mut+} subjects receiving gilteritinib 120 mg on Schedule 1 (n=17) was 100%. The CRc rate in FLT3^{mut+} subjects receiving Schedule 2 induction with daunorubicin was also 100%. Among subjects who received ≥ 80 mg/day gilteritinib (n=51), CRc rates for FLT3^{mut+} subjects were 90.3% (n=28/31).

Response by dose level is outlined below:

Table 1-1: Antileukemic Response: FLT3+ Patients

Response*	S1 40 mg (N=3)	S1 80 mg (N=1)	S1 120 mg (N=17)	S1 200 mg (N=2)	S1 Total (N=23)	S2 IDR (N=5)	S2 DNR (N=6)
CR n (%)	2 (67%)	1 (100%)	12 (71%)	1 (50%)	16 (70%)	4 (80%)	2 (33%)
CRp n (%)			1 (6%)		1 (4%)		
CRi n (%)	1 (33%)		4 (23%)		5 (22%)	1 (20%)	2 (33%)
CRc‡ n (%)	3 (100%)	1 (100%)	17 (100%)	1 (50%)	22 (96%)	5 (100%)	4 (67%)
NR/NE n (%)				1 (50%)	1 (4%)		2 (33%)

*Response parameters were defined according to the International Working Group Criteria for AML (Cheson B, et al. J Clin Oncol. 2003; 12 (24):4642–4649).

‡CRc included patients who achieved CR, CRp, and CRi.

Abbreviations: CR, complete remission; CRc, composite complete remission; CRi, complete remission with incomplete hematologic recovery; CRp, complete remission with incomplete platelet recovery; FLT3, fms-like tyrosine kinase 3; mut+, mutation positive; NR, no response; NE, not evaluable; IDR, Idarubicin; DNR, Daunorubicin.

Grade ≥ 3 AEs in ≥ 10% of patients were febrile neutropenia (63.3%), thrombocytopenia (18.3%), decreased platelet count (16.7%), neutropenia (15.0%), bacteremia (10.0%), sepsis (10.0%), and decreased white blood cell count (10.0%). Serious drug-related AEs in >1 subject were febrile neutropenia (n=9), small intestinal obstruction, lung infection, sepsis, and decreased ejection fraction (all n=2).

1.3.4.1 Gilteritinib Pharmacokinetics and Pharmacodynamics

In an ongoing Phase 1/2 study (Study 2215-CL-0103), gilteritinib is administered in combination with combination 7+3 chemotherapy regimens to newly diagnosed patients with AML.[29] The maximum tolerated dose was determined to be 120 mg. Preliminary analysis of gilteritinib pharmacokinetics was performed and found to be comparable to that observed in the monotherapy setting.[30] Gilteritinib concentrations after once-daily 120 mg gilteritinib in combination with 7+3 chemotherapy generally exceed 100 ng/mL, a threshold level associated with improved clinical response in subjects with relapsed or refractory AML administered 120 mg gilteritinib as monotherapy. These data coupled with the safety, efficacy and tolerability result described above, support the proposed dose of 120 mg gilteritinib in combination with 7+3 chemotherapy.

Refer to Gilteritinib IB for more details.

1.4 Summary of Rationale for Proposed Study

Activating mutations of the receptor tyrosine kinase FLT3 are one of the most common molecular abnormalities in AML and are present in about 30% of newly diagnosed patients.[31] The presence of a FLT3-ITD mutation in an AML patient implies a poor prognosis with only 22% of patients maintaining a remission for two years in a Phase III national study.[32] The prognostic impact of the FLT3-ITD mutation does appear to be lessened in those with a lower allelic burden (signal ratio) who are also Nucleophosmin 1- Mutated (NPM1) mutated.

FLT3-ITD AML is rarely cured with chemotherapy alone. Current approaches often incorporate allogeneic bone marrow transplant in first remission (CR1) for those patients who have a matched donor and are medically qualified for transplantation. The first hurdle to achieve on the road to potential curative therapy is achievement of remission with induction therapy. Intensification of anthracycline (daunorubicin) dose has been shown to improve survival in newly diagnosed AML when compared to standard dose.[32] In subgroup analysis of this Phase III study, FLT3-ITD patients demonstrated a prolonged median survival (15.2 months vs 10.2 months, P=0.09) with high dose daunorubicin which was demonstrated to be statistically significant on longer follow-up.[33] Hematopoietic stem cell transplant (HSCT) holds the most potential of cure for patients with high risk leukemias. In a single institution retrospective study of FLT3 -ITD patients who underwent early HSCT in CR1, 75% remained disease free at 3 years post transplantation.[34] However, patients who do not achieve remission or relapse early on do not have the opportunity to proceed to allogeneic transplant.

Midostaurin is known to be a nonselective inhibitor with low FLT3 inhibitory potency compared to newer agents. Gilteritinib has been designed as a FLT3 inhibitor that is both selective and potent. In a recently reported Phase I/II trial, 252 patients with relapsed and refractory AML were treated with this agent. It was well tolerated and at dose levels ≥ 80 mg/day, FLT3 inhibition was consistently seen with overall response rates >50% in this heavily pretreated population. We are hoping gilteritinib, a more potent FLT3 inhibitor than midostaurin, will have further improvement in both FLT3-ITD and FLT3-TKD mutations.

Despite advances in AML therapy, the majority of patients die of their disease. The first hurdle to achieve on the road to potential curative therapy in AML is achievement of remission with induction therapy. Pharmacologic targeting of FLT3 (both ITD and TKD) represents an important potential approach for improving these outcomes. While C10603 demonstrated an improvement in CR and in survival in FLT3 mutated AML patients, gilteritinib as a more potent FLT3 inhibitor has in Phase I/II studies in induction had promising CR rate and will hopefully allow for more Minimal Residual Disease (MRD) negative CRs and decreased risk of relapse after CR.

2. Study Objectives

2.1 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.

2.2 Secondary Objectives

- 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.

2.3 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.

3. Selection of Patients

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. For each patient, this checklist may be photocopied, completed and maintained in the patient's chart.

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday four weeks later would be considered Day 28.

PrECOG Patient No. _____

Patient's Initials (F, M, L) _____

Physician Signature and Date _____

NOTE: PrECOG does not allow waivers to any protocol specified criteria. All eligibility criteria listed in Section 3 must be met, without exception. All questions regarding clarification of eligibility criteria must be directed to the Medical Monitor or PrECOG Study Contact.

NOTE: Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to registration by the treating physician; however source must be the first documentation. In some cases the checklist will therefore only serve as confirmation of the information in the patient records.

REGISTRATION ELIGIBILITY: Patients age ≥ 18 years to ≤ 70 years.

Any patient undergoing bone marrow biopsy with suspicion of or known diagnosis of acute myeloid leukemia (AML) will be asked to sign a Prescreening Consent to allow for centralized testing of bone marrow/peripheral blood samples and for ongoing storage of research samples for future evaluations. Patients will be asked to consent to collect minimal demographic information for screening and for future optional evaluation of samples.

The following will be performed at a Central Laboratory:

- Flow cytometry to confirm blasts are myeloid
- FLT3 mutation status (includes signal ratio)
- NPM1 mutation status

Patients negative for FLT3 mutated AML will not be eligible for further evaluation for study entry (will be counted as a screen failure).

A diagnosis of acute myeloid leukemia will be documented by the study investigator from site evaluation.

RANDOMIZATION ELIGIBILITY CRITERIA:

_____ 3.1 Patient must have previously untreated FLT3 mutated Non M3 AML (FLT3-TKD or FLT3-ITD allowed).

Date of Diagnosis (i.e., date of diagnostic bone marrow biopsy): _____

FLT3 Mutation Status (Invivoscribe Report): _____

ITD vs TKD Status (Invivoscribe Report): ☐ ITD ☐ TKD

If ITD, Signal Ratio (Invivoscribe Report): _____

☐ High (≥ 0.5) ☐ Low (<0.5)

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

_____ 3.2 Patient must have had no prior systemic therapy for AML, except as noted below:

- Hydroxyurea and emergent leukapheresis or preemptive treatment with retinoic acid prior to exclusion of Acute Promyelocytic Leukemia (APL) allowed.
- Prior therapy for myelodysplastic syndrome (MDS) or myeloproliferative neoplasms (MPN) (e.g., thalidomide or lenalidomide, interferon, jakafi, cytokines, 5-azacytidine or decitabine, histone deacetylase inhibitors).
- Initiation of standard of care 7+3 induction chemotherapy using same regimen and doses as defined in protocol (Section 5.2.1) while awaiting prescreening test results.

_____ 3.3 Patients may not have received hypomethylating agent within 21 days.

Date of Last Hypomethylating Agent Treatment (if applicable): _____

_____ 3.4 The patient may not have M3 AML.

_____ 3.5 The patient may not have AML with known Core Binding Factor –t(8;21), inv(16), t(16;16) (if tested prior to registration [testing not required]).

If available: FISH: ☐ Date of Test: _____ **AND/OR**

Cytogenetics: ☐ Date of Test: _____

NOTE: If results return with known Core Binding Factor –t(8;21), inv(16), t(16;16) after patient is registered, patient may be allowed to remain on-study per investigator's discretion after discussion with PrECOG.

_____ 3.6 The patient may not have known active Central Nervous System (CNS) leukemia.

NOTE:
- Prophylaxis with intrathecal chemotherapy is allowed prior to or during induction/consolidation.
- If prophylactic lumbar puncture is performed, recommend scheduling so results are available prior to Day 8 or wait until Day 21 to perform.

_____ 3.7 Patient must have an ECOG performance status of 0-3 (Appendix I).

_____ 3.8 Patient must be age ≥ 18 years to ≤ 70 years.

_____ 3.9 Patient must be able to understand and willing to sign IRB-approved informed consent.

_____ 3.10 Patient must be willing to provide mandatory bone marrow and blood samples for research (Section 13.0).

_____ 3.11 Patient must have adequate organ function as measured by the following criteria, obtained ≤ 48 hours prior to randomization except ECG and left ventricular ejection fraction (LVEF) which can be done ≤ 2 weeks prior to randomization:

- Serum creatinine ≤ 1.5x institutional upper limit of normal (ULN), or if serum creatinine outside normal range, then glomerular filtration rate (GFR) >40 mL/min as measured by Cockcroft-Gault formula (Appendix II).

  Creatinine: _____ Institution ULN: _____ Date of Test: _____

  GFR (if applicable): _____ Date of Test: _____
- ALT and AST ≤ 3x ULN, unless secondary to leukemia.

  ALT: _____ Institution ULN: _____ Date of Test: _____

  AST: _____ Institution ULN: _____ Date of Test: _____
- Serum total or direct bilirubin <2 mg/dL, unless due to Gilbert's, hemolysis or leukemic infiltration.

  Total Bilirubin: _____ Date of Test: _____ **OR**

  Direct Bilirubin: _____ Date of Test: _____

- QTcF interval ≤ 500 msec (using Fridericia's correction).
  QTcF: _____ Date of Test: _____
- Left Ventricular Ejection Fraction >45%.
  Multigated Acquisition Scan (MUGA): ☐ Date of Test: _____ **OR**
  Echocardiogram (ECHO): ☐ Date of Test: _____

_____ 3.12 The patient may not be known to have hypokalemia and/or hypomagnesemia that does not respond to supplementation.

_____ 3.13 A female patient is eligible to participate if she is not pregnant and at least one of the following conditions apply:

- Not a woman of childbearing potential (WOCBP) as defined in Appendix III **OR**
- WOCBP who agrees to follow the contraceptive guidance as defined in Appendix III throughout the treatment period and for at least 180 days after the final study drug administration.

A blood test to rule out pregnancy ≤ 2 weeks prior to randomization.

Is the patient a woman of childbearing potential? _____ (yes/no)

If yes, Date of Test: _____ Results: _____

_____ 3.14 Female patient must agree not to breastfeed or donate ova starting at treatment and throughout the study period, and for at least 180 days after the final study drug administration.

_____ 3.15 A male patient must agree not to donate sperm starting at treatment and throughout the study period, and for at least 120 days after the final study drug administration.

_____ 3.16 A male patient with female partner(s) of child-bearing potential must agree to use contraception as detailed in Appendix III during the treatment period, and for at least 120 days after the final study drug administration.

_____ 3.17 Male patient with a pregnant or breastfeeding partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy or time partner is breastfeeding throughout the treatment period, and for at least 120 days after the final study drug administration.

_____ 3.18 Patients may not have another malignancy that could interfere with the evaluation of safety or efficacy of this combination. Patients with a prior malignancy will be allowed in the following circumstances:

1) Not currently active and diagnosed at least 3 years prior to the date of enrollment.
2) Non-invasive diseases such as low risk cervical cancer or any cancer in situ.

_____ 3.19 The patient may not have a history of Long QT Syndrome.

_____ 3.20 The patient may not have evidence of uncontrolled angina, severe uncontrolled ventricular arrhythmias, electrocardiographic evidence of acute ischemia, or congestive heart failure (CHF) New York Heart Association (NYHA) Class 3 or 4 (Appendix IV). Patient may also not have a history of CHF NYHA Class 3 or 4 in the past, unless a prescreening echocardiogram (ECHO) or multigated acquisition scan (MUGA) performed within 2 weeks prior to study entry with results of left ventricular ejection fraction >45%.

_____ 3.21 The patient may not have had major surgery or radiation therapy within 4 weeks of registration.

_____ 3.22 The patient may not require treatment with concomitant drugs that are strong inducers of CYP3A and P-gp (Appendix V).

_____ 3.23 Patients with a known allergy to any of the study medications, their analogues, or excipients in the various formulations of any agent are not eligible.

_____ 3.24 Patients with known gastrointestinal (GI) disease or prior GI procedure that could interfere with the oral absorption or tolerance of gilteritinib or midostaurin including difficulty swallowing are not eligible.

_____ 3.25 Patients with any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the completion of the treatment according to the protocol are not eligible.

_____ 3.26 Patients may not participate in any other therapeutic clinical trials, including those with other investigational agents not included in this trial during treatment on this study without prior approval from PrECOG.

4. Registration Procedures

4.1 Ethics

This study will be conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki and will be consistent with applicable US regulatory requirements and International Conference on Harmonization/Good Clinical Practice (ICH/GCP).

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

Freely given written informed consent must be obtained from every patient or their legally acceptable representative prior to clinical trial participation, including informed consent for any screening procedures conducted to establish patient eligibility for the trial.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s). This trial will not use the services of investigators or study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure, debarment). Investigators are responsible for the conduct of the study at their study site.

4.2 Regulatory Requirements

Before a site may enroll patients, protocol-specific regulatory and other documents must be submitted to PrECOG as noted in study materials. Detailed information regarding document submission and control is provided to each site in separate study materials.

Once required documents are received, reviewed, and approved by PrECOG or their representative, study materials will be forwarded to the site. Any changes to site regulatory documents must be submitted by the investigator to the responsible party in a timely manner. Initial study drug shipment will not occur until PrECOG approval. Once PrECOG activates a site, enrollment make occur. No patients will begin protocol therapy without formal registration as per the process below.

4.3 Patient Registration

Patients may not undergo any study-required procedures that are not part of standard of care, until signed informed consent is obtained (prescreening and main consent).

Prescreening

Any patient undergoing bone marrow biopsy with suspicion of or known diagnosis of AML will be asked to sign a Prescreening Consent form. Patients must provide written consent to submission of bone marrow and/or peripheral blood samples for centralized testing for immunophenotyping at baseline for later MRD assessment, FLT3, NPM1 and DNMT3 mutation status. Patients will also confirm if they agree to the future use of optional research samples.

After prescreening consent, the patient will be registered in the study by site personnel via an electronic data capture (eDC) system. Demographic and diagnostic information will be collected on registered patients and recorded on electronic Case Report Forms (eCRFs) regardless of their FLT3 mutation status or final diagnosis.

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

Patients that are negative for FLT3 mutated AML by Central Laboratory testing, will be considered screen failures.

Screening Eligibility

Once patient results are positive for FLT3 mutation by Central Laboratory testing and are Non M3 AML, the study site will have the patient sign the main consent and proceed to confirmation of eligibility and randomization.

Eligibility requirements per Section 3 will be confirmed by the investigator site during the registration process. This includes receipt and review of the central laboratory assessments as well as institutional diagnosis of acute myeloid leukemia.

4.4 Patient Randomization

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

- TKD vs. 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)

Treatment will begin no later than 3 days after randomization.

Full information regarding registration/randomization procedures can be found in the materials provided to study sites. Documentation from the web randomization system including the treatment assignment will be placed in the patient record. Correspondence regarding patient registration/randomization must be kept in the study records.

4.5 Research Bone Marrow and Blood Samples

Mandatory bone marrow and blood samples are required during the study.

Time points for bone marrow and blood samples are outlined in the study parameters (Section 10) and specific preparation and shipment requirements are outlined in the correlative section of this protocol (Section 13) and the lab manuals.

5. Treatment Plan

5.1 Overview

Eligible AML patients with FLT3 mutation by central laboratory testing prior to randomization 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).

All patients will receive standard induction chemotherapy with cytarabine and daunorubicin and standard consolidation with high-dose cytarabine.

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

Patients will be randomized to receive either open label gilteritinib or midostaurin and will receive the assigned study drug during induction and consolidation therapy.

5.2 Treatment and Administration Schedule

End of Cycle = Count Recovery

5.2.1 Induction

Day 1 of treatment will begin no later than 3 days after randomization with the first dose of induction chemotherapy, and each day is 24 hours in length. Therefore, if chemotherapy were to have begun in the evening, then it is possible that the scheduled gilteritinib or midostaurin administration on day 8 may occur on calendar day 9.

Prophylaxis with intrathecal chemotherapy is allowed prior to or during induction/consolidation.

If prophylactic lumbar puncture is performed, recommend scheduling so results are available prior to Day 8 or wait until Day 21 to perform.

Arm A (7+3) + Gilteritinib

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

ARM B (7+3) + Midostaurin

- **Cytarabine** 100 mg/m^2/day will be administered by continuous IV infusion for a total of 7 days beginning Day 1
- **Daunorubicin** 90 mg/m^2/day will be administered IV per package insert or per institutional guidelines or over 30-60 minutes Days 1,2,3.
- **Midostaurin** 50 mg orally BID x 14 days beginning on day 8

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.

NOTE: If second cycle of induction is needed, daunorubicin dose will be decreased to 45 mg/m^2/day IV Days 1,2,3.

A bone marrow aspirate/biopsy will be performed on all patients on Day 21 (+/-1 day window; delays due to holidays, weekends, or other unforeseen circumstances will be permitted) to determine the need for a second induction cycle. If the marrow aspirate/biopsy is inadequate to make a determination, consider repeating the bone marrow assessment in 5-7 days.

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

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

A repeat bone marrow aspirate/biopsy should be done to assess for response and MRD status upon peripheral blood count recovery (Absolute Neutrophil Count (ANC) ≥ 1000/mm^3, platelets ≥ 100,000/mm^3) but no later than Day 60 after initiation of the first cycle of induction therapy for patients who receive 1 cycle of induction or no later than Day 60 after initiation of the second cycle of induction therapy (i.e., Day 84-105) for patients who receive 2 cycles of induction (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).

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 (if otherwise eligible) or may proceed to allogeneic transplant if desired. Patients who do not achieve CR/CRi are removed from study.

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.

5.2.2 Consolidation

Patients deemed suitable by the treating investigator may proceed to allogeneic TRANSPLANT at any time after induction or after 0-4 cycles of chemotherapy consolidation.

Patients will continue to receive the same study drug (gilteritinib or midostaurin) assigned during induction for consolidation.

NOTE: Patients are not eligible to proceed to consolidation on study unless bone marrow samples are obtained for Minimal Residual Disease (MRD) at the time of CR/CRi.

Consolidation Cycle 1

Patients who achieve a CR or CRi and have no residual significant toxicities from the induction course are eligible for further protocol therapy. In general, consolidation therapy should start within 1 month of documentation of CR/CRi, after the resolution of any non-hematologic toxicity of previous chemotherapy (≤ Grade 1 or baseline). Patients must have maintained peripheral blood evidence of a remission as defined in Section 9 and must have an ANC ≥ 1000/mm^3. For patients who have not recovered from the toxicities of chemotherapy within 30 days of documentation of remission, and thus require additional time, a repeat marrow should be obtained to confirm remission and PrECOG must be contacted for approval to continue on study.

NOTE: Patients who achieve a CRi post remission therapy are eligible to proceed to consolidation once their ANC recovers to 1000/mm^3 even if that happens more than 60 days after the initiation of their last induction cycle.

ARM A

- **Cytarabine** 3 g/m^2* over approximately 1-3 hours every 12 hours (+/- 2 hour window) 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/m^2* over approximately 1-3 hours every12 hours (+/- 2 hour window) 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/m^2.

A repeat bone marrow aspirate/biopsy is required at the end of the first consolidation cycle for repeat response assessment and MRD testing.

Consolidation Cycle 2-4

Patients must have maintained peripheral blood evidence of a remission as defined in Section 9. Consolidation Cycle 2-4 should commence within 2 weeks following recovery of peripheral blood counts after previous consolidation. Patients who do not begin consolidation Cycle 2-4 within 30 days of recovery from previous cycle are ineligible for further protocol therapy.

ARM A

- **Cytarabine** 3 g/m^2* over approximately 1-3 hours every 12 hours (+/- 2 hour window) 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/m^2* over approximately 1-3 hours every12 hours (+/- 2 hour window) 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/m^2.

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.

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, and will be followed for relapse and survival only.

5.2.3 Arm A: Gilteritinib Administration

Gilteritinib 120 mg tablets (three 40 mg tablets) are taken once daily by mouth on days 8-21 (if chemotherapy were to have begun in the evening, then it is possible that the scheduled gilteritinib administration on day 8 may occur on calendar day 9). Patients will be instructed to take the daily gilteritinib with water as close to the same time each morning as possible. Tablets should not be chewed, crushed or broken.

Gilteritinib will be self-administered at home when patients are not hospitalized. If a patient forgets to take a dose in the morning and are within 6 hours of the planned dosing time, they should be instructed to take their dose. If the patient forgets to take their daily dose and are more than 6 hours has passed the planned dosing time, they should be instructed to wait for the next morning to dose. If vomiting occurs after dosing, the patient should not receive another dose, but wait until the next morning to dose.

When patients are hospitalized for treatment (i.e. induction, aplasia or events), gilteritinib will be administered per site procedures for inpatient study management. Instructions to patients and study staff regarding the above recommendations for administration should be included in orders or related patient record documentation, including drug handling and disposal.

Patient compliance for gilteritinib administration will be assessed by pill counts and IRB reviewed medication diary provided to sites. Bottles containing gilteritinib tablets will be given to patients for self-administration, when not hospitalized, and at scheduled visits. Previously distributed bottles will be returned to the study site and tablets counted by the study staff. Investigational product accountability will be maintained on the drug accountability record as per regulatory requirements.

Any discrepancy will be reviewed with the patient during the visit and documented as needed. The medication diary will be maintained in the patient records and available for review at site monitoring visits.

Dose reductions are permitted (Section 6). Missed doses are not made up. Assigned treatment should continue through induction and consolidation cycles until unacceptable toxicity occurs, or the patient meets a treatment discontinuation criterion as provided in Section 6 and/or Section 7.3.

5.2.4 Arm B: Midostaurin Administration

Midostaurin 50 mg (two 25 mg capsules) twice a day by mouth on days 8-21 (if chemotherapy were to have begun in the evening, then it is possible that the scheduled midostaurin administration on day 8 may occur on calendar day 9). Patients should take their doses at approximately the same time each day, and approximately 12 hours should elapse between the morning and evening doses. Each daily dose should be given with food and a glass of water (~240 mL). Patients should be instructed to swallow capsules whole and not open, break or chew capsules. If vomiting occurs, no re-dosing is allowed prior to the next scheduled dose.

When patients are hospitalized for treatment (i.e. induction, aplasia or events), midostaurin will be administered per site procedures for inpatient study management. Instructions to patients and study staff regarding the above recommendations for administration should be included in orders or related patient record documentation, including drug handling and disposal.

Compliance in taking assigned dose of midostaurin (commercial stock) when the patient is not hospitalized, will be assessed by a medication diary. Patients should be instructed to bring medication diaries to the site at each visit, for staff review. Patients may be counseled by the study staff regarding adherence concerns.

Dose interruptions are permitted (Section 6). Missed doses are not made up. Assigned treatment should continue through induction and consolidation cycles until unacceptable toxicity occurs, or the patient meets a treatment discontinuation criterion as provided in Section 6 and/or Section 7.3.

6. Dose Modifications

6.1 Dose Interruptions & Reductions

All toxicities should be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE V5.0). A copy of the CTCAE V5.0 can be downloaded from the CTEP website (http://www.ctep.cancer.gov).

Delays due to holidays, weekends, or other unforeseen circumstances will be permitted.

6.1.1 Arm A and Arm B: Daunorubicin

Initial and subsequent daunorubicin doses should be reduced as follows for hepatotoxicity (Table 6-1):

Table 6-1: Daunorubicin Hepatotoxicity Dose Reduction

Direct Bilirubin	Dose Reduction
<2	None
2-3	25%
>3	50%

For patients with evidence of hepatic dysfunction, reassess regularly per institutional standards during induction treatment.

6.1.2 Arm A: Gilteritinib

The dose levels include the following (Table 6-2):

Table 6-2 Gilteritinib Dose Levels

Dose Level (DL)	Gilteritinib Dose
DL 1 (starting dose)	120 mg
DL -1	80 mg
DL -2	40 mg

The gilteritinib dose may be initially reduced by 1 dose level for a described instance meeting criteria for dose reduction. The study drug dose can be further reduced by a second dose level. Note that dose reductions should occur in a step-wise manner. Only 2 dose level reductions are permitted. Dose re-escalation is not allowed following dose reduction unless there is a specific reason not related to gilteritinib for temporarily holding or reducing gilteritinib.

Gilteritinib will be interrupted per Table 6-3. When the grade of the adverse event (AE) decreases to ≤ 1, study drug may be resumed at the next lower dose level, unless the investigator deems the AE was, in retrospect, unlikely to be or not related to the study drug, at which point the original dose may be resumed.

Additionally, if the investigator deems it necessary to ensure subject safety, dosing may be interrupted or reduced for reasons other than those provided in Table 6-3. In the unusual circumstance that dosing is interrupted or reduced for reasons not specified in the table, the investigator should promptly inform PrECOG.

Missed doses of gilteritinib will not be made up.

Table 6-3 Guidelines for Gilteritinib Dose Interruption or Reduction Event	
Event	**Action**
First occurrence: Grade ≥ 3 nonhematological AE possibly, probably or definitely related to the study drug (excluding pancreatitis, see below)	Dosing will be interrupted. If the AE resolves to ≤ grade 1 within 14 days, the patient may resume dosing at the reduced dose during this or subsequent cycles. If the AE does not resolve to ≤ grade 1 within 14 days, the subject will be discontinued from treatment and an end of treatment visit will be performed.
Second occurrence: Recurrence of the same nonhematological AE or appearance of other new nonhematological AE Grade ≥ 3 probably, possibly or definitely due to the study drug (excluding pancreatitis, see below)	Dosing will be interrupted. If the AE resolves to ≤ grade 1 within 14 days, the patient may resume dosing at the reduced dose during this or subsequent cycles. If the AE does not resolve to ≤ grade 1 within 14 days, the subject will be discontinued from treatment and an end of treatment visit will be performed.
Third occurrence: Recurrence of a prior nonhematological AE or appearance of other new nonhematological AE Grade ≥ 3 probably, possibly or definitely due to the study drug (excluding pancreatitis, see below)	The study drug will be discontinued.
At any point, in retrospect, if the investigator determines prior non-hematological Grade ≥ 3 AE which was initially attributed as possibly, probably or definitely related to study drug is now deemed unrelated	The study drug will be re-escalated to dose that the participant was on at the time of the AE.
Pancreatitis	Dosing will be interrupted until pancreatitis is resolved, then resume gilteritinib at 80 mg during this or subsequent cycles.
QTcF >500 ms	If the QTcF is >500 ms at any time point, the ECG will be repeated (within 2 hours or as soon as possible). Confirm that potassium and magnesium abnormalities are corrected. A cardiology consult will be obtained as medically indicated. If the repeat ECG confirms a QTcF >500 ms, dosing of study treatment will be interrupted. If QTcF resolves to ≤ 480 ms within 14 days from study drug interruption, subsequent dosing will be reduced to 80 mg (or if currently at 80 mg, reduction to 40 mg).
QTc interval increased by >30 msec over screening on ECG on Day 15 of Cycle 1	Confirm with ECG on Day 16. If confirmed, consider dose reduction to 80 mg. If currently at 80 mg, dose reduction to 40 mg.
Posterior Reversible Encephalopathy Syndrome (PRES)	Contact PrECOG, discontinue gilteritinib.

6.1.3 Arm B: Midostaurin

Interrupt midostaurin for Grade 3/4 non-hematologic toxicity thought to be possibly related to midostaurin and restart at same dose when toxicity resolves to ≤ Grade 1. If the toxicity resolves prior to day 21, then restart at same dose to complete current cycle. Missed doses will not be made up.

For QTcF >500, confirmed on repeat study, check potassium and magnesium and correct any abnormalities. If at that time QTcF is >480, restart at 50 mg daily, until QTcF is ≤ 480. Once QTcF ≤ 480 restart at 50 mg BID.

6.1.4 Arm A and Arm B: Cytarabine Consolidation

For patients age ≥ 55 or patients with decreased creatinine clearance recommend reducing consolidation cytarabine dose to 1.5 g/m^2.

Table 6-4 Guidelines for Cytarabine Dose Interruption or Discontinuation

Dose Interruption or Discontinuation	Induction	Consolidation
Cerebellar Toxicity (any grade)	Discontinue	Discontinue
Direct Bilirubin ≥ 2 mg/dL	NA	Interrupt*
Creatinine >2 mg/dL	NA	Interrupt*

* Cytarabine may be interrupted for 72 hours. Gilteritinib/Midostaurin cannot be started until at least 48 hours after last dose of Cytarabine. If Cytarabine is not completed by Day 6, contact PrECOG to discuss schedule.

6.2 Concurrent Therapies

6.2.1 Permitted

Patients should receive full supportive care, including transfusions of blood and blood products, antibiotics, antiemetics, allopurinol, growth factors, etc., when appropriate.

NOTE: Prophylaxis with intrathecal chemotherapy is allowed prior to or during induction/consolidation.

6.2.2 Not Permitted

- Treatment with concomitant drugs that are strong inducers of CYP3A and P-gp are prohibited unless essential for the care of the patient.

6.2.3 Cautionary Use

Caution is advised when considering the following:

- Treatment with concomitant drugs that are strong inhibitors of P-gp and concomitant drugs that target serotonin $5HT_{2B}R$ or sigma nonspecific receptor are to be avoided with the exception of drugs that are considered absolutely essential for the care of the subject.
- Treatment with concomitant drugs that are strong inhibitors of CYP3A should be avoided with the exception of antibiotics, antifungals and antivirals that are used as standard of care to prevent or treat infections. Grapefruit juice should not be ingested during study treatment. If strong CYP3A inhibitors are used concomitantly, patients should be closely monitored for AEs.
- Precaution should be used in use of gilteritinib with concomitant drugs that are known to prolong QT intervals or QTc. A cardiology consult should be obtained as medically indicated.
- Precaution should be used in use of gilteritinib with concomitant drugs that are substrates of P-gp, BCRP and OCT1, since these transporters have been shown to be inhibited by gilteritinib in in vitro studies.
- The investigator should consult individual labels for all drugs that the subject is taking to evaluate if they fall into any of the above named categories. (Appendix V: Additional Excluded and Cautionary Medications).

- Any other treatments of AML (including but not limited to chemotherapy, radiotherapy, surgery, HSCT, immunotherapy or cellular therapy) are prohibited during therapy.
  - Initiation of standard of care 7+3 induction chemotherapy using same regimen and dose as defined in protocol (Section 5.2.1) while awaiting prescreening test results is allowed.
  - Prophylaxis with intrathecal chemotherapy is allowed prior to or during induction/consolidation.
- Participation in another interventional study with an investigational agent while on treatment is prohibited.

7. Study Duration and Discontinuation of Therapy

7.1 Treatment Duration

Patients will receive protocol therapy as outlined unless:

1. Disease progression per Section 9 guidelines or clinical progression.
2. Toxicities considered unacceptable by either the patient or the investigator, despite optimal supportive care and dose modifications as per Section 6.
3. Patient proceeds to bone marrow transplant or any non-protocol leukemia directed therapy.
4. Development of an inter-current illness that prevents further administration of study treatment.
5. Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, protocol treatment should be discontinued.
6. Patient withdraws consent or is unable to comply with study procedures.

7.2 Duration of Follow-Up

Patients will be followed for adverse events for 30 days after their last dose of study medication. However, if a patient experiences an adverse event >30 days after their last dose of study medication that is felt to be, in the opinion of the investigator, possibly, probably or definitely related to study therapy, the adverse event should be reported.

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.

Patients should be followed 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 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.

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. 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 per Section 10 will also be collected.

7.3 Criteria for Removal from Study Treatment

A genuine effort will be made to determine the reason(s) why a patient fails to return for the necessary visits or is discontinued from the trial, should this occur. It will be documented whether or not each patient completed the clinical study. If for any patient study treatment or observations were discontinued, the reason will be recorded on the appropriate electronic case report form. Reasons that a patient may discontinue treatment in a clinical study are considered to constitute one of the following:

1. If the patient has already initiated midostaurin or gilteritinib therapy and CNS involvement is detected, the patient may either come off protocol therapy immediately or complete therapy through Day 21 then will be removed from protocol treatment.
2. Failure to achieve CR or CRi after 2 cycles of induction.

3. Patient requires treatment with therapy not allowed per protocol, or patient goes on to receive HSCT.
4. Recurrence of disease or documented progression of disease.
5. Intercurrent illness that prevents further administration of treatment per investigator discretion.
6. Unacceptable adverse events.
7. Investigator and/or patient discontinue chemotherapy.
8. Pregnancy.
9. Develops a second malignancy (except for non-melanoma skin cancer or cervical carcinoma in-situ) that requires treatment, which would interfere with this study.
10. The patient may choose to withdraw from the study at any time for any reason.
11. General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator.
12. Severe non-compliance to protocol as judged by the investigator.
13. Lost to follow-up.
14. Death.
15. Closure of study by PrECOG.

Patients who discontinue study treatment early should be followed for response assessments and survival, if possible. Follow-up will continue per Section 10, as applicable.

8 Adverse Event Reporting

8.1 Collection of Safety Information

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 or not considered related to the product (investigational or marketed).

After either informed consent, but prior to initiation of induction, 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). Please note, if induction is started prior to randomization, AE/SAE collection will start at time of randomization. After the initiation of treatment on-study, 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 criteria per Section 6.1 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. All laboratory results required by protocol (Section 10) will be recorded directly on the laboratory eCRF pages. Per Sections 8.2 to 8.7 additional events will be recorded as AEs and/or SAEs (meeting definitions) on the appropriate eCRF.

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than individual symptoms. The following information should be included for all documented AEs: date of onset and resolution, severity of the event; the investigator's opinion of the relationship to study drug (see definitions below); intervention or treatment required for the AE; action taken with study drug; cause of the event (if known); and information regarding resolution/outcome.

If the Grade ≥ 3 laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5x the ULN associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event eCRF.

If the Grade ≥ 3 laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the eCRF. If the laboratory abnormality can be characterized by a precise clinical term in CTCAE Version 5.0, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7.0 mEq/L should be recorded as "hyperkalemia".

Observations of the same Grade ≥ 3 laboratory abnormality from visit to visit should not be repeatedly recorded unless their severity, seriousness, or etiology changes.

Severity

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).

Attribution

The following categories and definitions of causal relationship or attribution to study drug should be used to assess Adverse Events:

- Definite: There is a reasonable causal relationship between the study drug and the event. The event response to withdrawal of study drug (dechallenge) and recurs with rechallenge, if clinically feasible.

- Probable: There is a reasonable causal relationship between the study drug and the event. The event responds to dechallenge. Rechallenge is not required.
- Possible: There is a reasonable causal relationship between the study drug and the event. Dechallenge information is lacking or unclear.
- Unlikely: There is doubtful causal relationship between the study drug and the event.
- Unrelated: There is clearly not a causal relationship between the study drug and the event or there is a causal relationship between another drug, concurrent disease, or circumstances and the event.

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

The development of a new cancer should be regarded as an AE. New cancers are those that are not the primary reason for administration of study treatment and have been identified after inclusion of the patient into the clinical study.

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, and reported as SAEs if they become serious. 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.

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.

8.2 Serious Adverse Events (SAEs)

A ***serious AE*** is any untoward medical occurrence occurring after initiation of study treatment or that at any dose:

- results in death (i.e., the adverse event actually causes or leads to death)
- is life-threatening (defined as an event in which the study patient 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 a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above).

Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

8.3 Drug-Induced Liver Injury Adverse Events

The following adverse events must be reported to PrECOG as Serious Adverse Events, irrespective of regulatory seriousness criteria.

- Liver specific adverse events leading to study (study drug) discontinuation
- Hepatic laboratory abnormalities meeting potential Drug-induced Liver Injury criteria including:

- Any potential Hy's Law case*, including those where review and adjudication concluded they did not meet the criteria
- All patients who died of hepatic illness
- All patients who discontinued trial drugs for hepatotoxicity, including patients with abnormalities consistent with protocol-specified stopping rules
- ALT and/or AST>3x ULN AND Total Bilirubin >2x ULN

***Criteria for Hy's Law[35]**

Hy's law is defined as drug-induced jaundice caused by hepatocellular injury, without a significant obstructive component, has a high rate of bad outcomes, from 10% to 50% mortality (or transplant). The 2 "requirements" for Hy's Law are:

- Evidence that a drug can cause hepatocellular-type injury, generally shown by an increase in transaminase elevations higher than 3x ULN (2x ULN elevations are too common in treated and untreated patients to be discriminating).
- Cases of increased bilirubin (at least 2x ULN) with concurrent transaminase elevations at least 3x ULN and no evidence of intrahepatic or extrahepatic bilirubin obstruction (elevated alkaline phosphatase) or Gilbert's syndrome.

8.4 SAE Reporting Requirements

Serious adverse events (SAE) are defined above. The investigator should inform PrECOG of any SAE within 24 hours of being aware of the event. The date of awareness should be noted on the report. This must be documented on the SAE form provided for this trial. This form must be completed and supplied to PrECOG within 24 hours or 1 business day at the latest on the following working day. The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up SAE report form. A final report to document resolution of the SAE is required. The investigator is responsible for following all SAEs until resolution, until the subject returns to baseline status, or until the condition has stabilized with the expectation that it will remain chronic, even if this extends beyond study participation. A copy of the transmission confirmation of the SAE report should be attached to the SAE and retained in the patient records.

Collection of complete information concerning SAEs is extremely important. Full descriptions of each event will be followed. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report.

SAEs should be scanned and emailed to PrE0905SAE@qdservices.com as per the instructions found in study materials provided to the investigator site.

[redacted]
Medical Monitor
During normal business hours:
(8:30 am-5:00 pm EST):
Phone: 610-354-0404
After normal business hours:
Phone: [redacted]

Manager, Clinical Safety
During normal business hours:
(8:30 am-5:00 pm EST):
Phone: 610-354-0404
After normal business hours:
Cell: [redacted]

PrECOG will have accountability for both initial and follow-up reporting of SAEs to the investigational product manufacturer and regulatory authorities within appropriate timelines.

Investigators should also report event(s) to their IRB as required.

All SAEs, regardless of causality, must be collected which occur within 30 days of last dose of study treatment. This includes all deaths within 30 days of last dose of cytarabine, daunorubicin, gilteritinib or midostaurin regardless of attribution. In addition, the Investigator should notify PrECOG or designee of any SAE that may occur after this time period which they believe to be definitely, probably or possibly related to investigational product.

NOTE: After study closure, study-drug related SAEs should be reported voluntarily by the treating physician directly to the manufacturer.

Serious adverse event reporting to regulatory authorities and all participating investigators will be conducted by PrECOG (or designee) in accordance with 21CFR312.32, local requirements and international regulations, as appropriate. FDA reporting requirement timelines will be followed. PrECOG will also concurrently forward any such reports to Astellas.

8.5 Reporting of Other Second Primary Cancers

New cancers are those that are not the primary reason for administration of study treatment and have been identified after inclusion of the patient into the clinical study.

All cases of new primary cancers that occur during or after protocol treatment must be reported to PrECOG on a Second Primary Cancer form within 30 days of diagnosis, regardless of relationship to protocol treatment. Secondary primary malignancies should also be reported as a SAE. The SAE form is not for use for reporting recurrence or development of metastatic disease. A copy of the pathology report, if applicable, should be sent, if available.

NOTE: Once data regarding survival and remission status are no longer required by the protocol, no follow-up data should be submitted.

8.6 Procedures in Case of Pregnancy

Prior to study enrollment, women of childbearing potential (WOCBP-not surgically sterilized and between menarche and 1 year post menopause) and male patients with a female partner of childbearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy, documented in the informed consent. In addition, all WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

Pregnancy of a female patient or the female partner of a male patient occurring while the patient is receiving study drug or within 180 days for female patient or 120 days for female partner after the patient's last dose of study drug will be reported to PrECOG on a Pregnancy Form within 24 hours of the investigator's knowledge of the pregnancy.

All reports of congenital abnormalities/birth defects and spontaneous miscarriages should also be reported and handled as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth including health of the newborn or congenital abnormality) must be followed and documented on the Pregnancy Form even if the subject was discontinued from the study treatment. Should pregnancy occur during a subject's participation, the subject will immediately be discontinued from the treatment and followed per protocol.

The study-specific Pregnancy Form can be found in the Study Reference Manual.

8.7 Reporting Guidelines in the Case of Overdose

In the event of an overdose with any of the drugs on-study (cytarabine, daunorubicin, gilteritinib [defined as more than 300 mg for a single dose] or midostaurin), defined as any dose higher than the patient allocated dose in the study, unless otherwise noted, should be captured as an adverse event. Both symptomatic and non-symptomatic overdose must be reported. For any accidental or intentional overdose with the study treatment that is symptomatic, **even if not fulfilling a seriousness criterion**, a SAE form should also be completed and reported to PrECOG within 24 hours of the investigator's knowledge of the overdose.

9 Measurement of Effect[36]

Response assessments will require a bone marrow aspirate/biopsy to be performed. The 2017 European LeukemiaNet (ELN) recommendations will be used to assess response.[36]

End of Cycle = Count Recovery

A bone marrow aspirate/biopsy is required on all patients on Day 21 (+/- 1 day window; delays due to holidays, weekends, or other unforeseen circumstances will be permitted) after initiating the first cycle of induction. Patients who have residual disease will be eligible to receive a second cycle of induction identical to the first (Section 10).

A repeat bone marrow aspirate/biopsy should be done to assess for response and MRD upon count recovery (Absolute Neutrophil Count (ANC) ≥ 1000/mm^3, platelets ≥ 100,000/mm^3) 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. (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).

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 the end of the first Consolidation cycle to assess disease and MRD status.

9.1 Complete Remission (CR)

Complete remission is defined as the following:

- Bone marrow blasts <5%
- Absence of circulating blasts and blasts with Auer rods
- Absence of extramedullary disease
- ANC ≥ 1000/mm^3
- Platelet count ≥ 100,000/mm^3

9.2 Complete Remission with incomplete hematologic recovery (CRi)

Requires that all the same response criteria in peripheral blood and bone marrow as CR with the exception of the following:

- Residual neutropenia (<1000/mm^3) or
- Residual thrombocytopenia (<100,000/mm^3 independent of platelet transfusions)

9.3 Morphologic Leukemia-Free State (MLFS)

- Bone marrow blasts <5%
- Absence of blast 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%.

9.4 Partial Remission (PR)

- All hematologic criteria of CR
- Decrease of bone marrow blast percentage to 5% to 25%

- Decrease of pretreatment bone marrow blast percentage by at least 50%

9.5 Stable Disease

Absence of CR, CRi, PR, MLFS; and criteria for PD not met.

Period of stable disease should last at least 3 months.

9.6 Treatment Failure

Primary Refractory Disease: No CR or CRi after 2 courses of intensive induction treatment or recovery with blasts/loss of CR/CRi within 28 days of documentation of initial CR/CRi. Exclude patients with death in aplasia or death due to indeterminate cause.

Death in Aplasia: Deaths occurring ≥ 7days 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.

9.7 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 500/mm^3 and/or platelet count to >50,000/mm^3 non-transfused); or
- >50% increase in peripheral blasts (White Blood Count (WBC) × % blasts) to >25 000/mm^3 (in the absence of differentiation syndrome); or
- New extramedullary disease

"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.

9.8 Relapse

Relapse following CR/CRi is defined as (only if this happens >28 days after CR/CRi):

Bone marrow blasts ≥ 5%; or reappearance of blasts in the blood; or development of extramedullary disease.

10. Study Parameters

1. Pre-study assessments should be done ≤ 48 hours prior to randomization (except for bone marrow biopsy, thyroid function testing, pregnancy testing, ECG and left ventricular ejection fraction which can be done ≤ 2 weeks).

Procedures	Pre-screen	Screening/ Prior to Randomization	Induction*					Consolidation*			End of Treatment[24]	Follow-Up[25]
			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 Cycle 1		
Written Informed Consent	X[1]	X										
Disease Characteristics[2]		X										
Medical/Surgical History		X										
Assessment of Baseline Signs & Symptoms		X										
Height		X										
Physical Exam		X	X[3]			X		X	X[3]		X	
Vital Signs (Temperature, Pulse, Blood Pressure)		X	X[3]			X		X			X	
Weight[4]		X				X		X			X	
BSA		X				X		X				
Performance Status		X		X		X		X			X	
CBC/Differential/ Platelets[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 Tests (T4 & TSH)[10]		X									X	
Serum Pregnancy Test[11]		X										
Left Ventricular Ejection Fraction (MUGA or ECHO)[12]		X				X[12]						

Procedures	Pre-screen	Screening/ Prior to Randomization	Induction*					Consolidation*			End of Treatment[24]	Follow-Up[25]
			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 Cycle 1		
12-Lead ECG (QTcF Interval)[4]		X[13]		X[14]	X[14]	X[14]			X[14]		X[15]	
Local Bone Marrow Aspirate/Biopsy (Mandatory)	X[2]				X[16]		X[17]			X	X[18] Optional	X[19]
Research Bone Marrow Samples Submission (Mandatory)[20]	X[1]						X			X		X[19]
Protocol Therapy Administration[21]			X					X				
Concomitant Medications[22]		X	X[3]			X		X			X	
Adverse Events Assessment			X[3]			X		X			X[23]	
Survival Status												X

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

** If prophylactic lumbar puncture is performed, recommend scheduling so results are available prior to Day 8 or wait until Day 21 to perform.

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, then twice weekly at nadir, after peripheral blasts gone and while WBC <500. If the patient is discharged prior to recovery of WBC (≥ 500), obtain differential twice weekly until end of induction, then per institutional guidelines until consolidation starts.

Consolidation: Twice weekly or per institutional guidelines.

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 or per institutional guidelines.

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-qt[37,38,39]

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 8 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, after last cycle of consolidation per institutional guidelines.

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

20 **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, then two 10 mL EDTA tubes of peripheral blood will be allowed if patient has any circulating blasts.

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 First Cycle 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.

21 **INDUCTION:**

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/m^2/day will be administered by continuous IV infusion for a total of 7 days beginning Day 1
- **Daunorubicin** 90 mg/m^2/day will be administered IV per package insert or institutional guidelines or over 30-60 minutes Days 1,2,3 (45 mg/m^2/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/m^2/day will be administered by continuous IV infusion for a total of 7 days beginning Day 1
- **Daunorubicin** 90 mg/m^2/day will be administered IV per package insert or institutional guidelines or over 30-60 minutes Days 1,2,3 (45 mg/m^2/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.

Prophylaxis with intrathecal chemotherapy is allowed prior to or during induction.

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 of CR/CRi.

CONSOLIDATION:

ARM A

- **Cytarabine** 3 g/m^2* over approximately 1-3 hours every 12 hours (+/- 2 hour window) 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/m^2* over approximately 1-3 hours every12 hours (+/- 2 hour window) 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/m^2.

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.

Prophylaxis with intrathecal chemotherapy is allowed prior to or during consolidation.

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.

22 Please review all concomitant medications. Section 6.2.2 for medications not permitted and Section 6.2.3 for medications to use with caution. Only record concomitant medications for SAE and/or AESI in eCRF.

23 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.

24 Upon count recovery after last cycle of chemotherapy and completion of gilteritinib or midostaurin.

25 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.

11 Drug Formulation and Procurement

11.1 Gilteritinib[27]

Please refer to the current Investigator's Brochure for additional information.

11.1.1 Other Names

[redacted]

11.1.2 Classification

Gilteritinib is a tyrosine kinase inhibitor under development for the treatment of AML. Gilteritinib has an inhibitory effect on FMS-like tyrosine kinase 3 (FLT3), AXL, leukocyte receptor tyrosine kinase (LTK) and anaplastic lymphoma kinase (ALK).

11.1.3 Storage and Stability

Gilteritinib is an oral drug that is available in a 40 mg tablet. In addition to the active ingredient, gilteritinib 40 mg tablets contain well-characterized excipients. Gilteritinib tablets are round light-yellow, debossed, film-coated tablets. The tablets are contained within a high-density polyethylene bottle. Each bottle of gilteritinib contains 30 tablets.

The storage conditions of gilteritinib tablets 40 mg packed into bottles is 20-25°C (68-77°F) with excursions permitted to 15°C and 30°C (59°F and 86°F). Do not freeze. Protect from light.

11.1.4 Dose Specifics

Gilteritinib 120 mg tablets (three 40 mg tablets) are taken once daily by mouth on days 8-21. Patients will be instructed to take the daily gilteritinib with water as close to the same time each morning as possible. Tablets should not be chewed, crushed or broken.

Gilteritinib will be self-administered at home when patients are not hospitalized, and when scheduled for clinic visits. If a subject forgets to take a dose in the morning and within 6 hours of the planned dosing time, they should be instructed to take their dose. If the subject forgets to take their daily dose and more than 6 hours has passed the planned dosing time, they should be instructed to wait for the next morning to dose. If vomiting occurs after dosing, the subject should not receive another dose, but just wait until the next morning to dose.

Sites should review the medication diary instructions at each visit.

Dose reductions are permitted (Section 6).

11.1.5 Drug Interactions

Treatment with concomitant drugs that are strong inducers of CYP3A and P-gp (e.g., rifampin, phenytoin, St. John's Wort) are prohibited as they can decrease the plasma exposure of gilteritinib. Treatment with concomitant drugs that are strong inhibitors of CYP3A and P-gp (e.g., voriconazole, itraconazole, posaconazole, clarithromycin, erythromycin, captopril, carvedilol, ritonavir, azithromycin) should be avoided with the exception of antibiotics, antifungals and antivirals that are used as standard of care to prevent or treat infections. If CYP3A inhibitors are used concomitantly, patients should be monitored closely for AEs.

Treatment with concomitant drugs that target serotonin $5HT_{2B}R$ or sigma nonspecific receptor should be avoided with the exception of drugs that are considered absolutely essential for the care of the patient.

Precaution should be used in treatment of gilteritinib with concomitant drugs that are substrates of P-gp (e.g., digoxin, dabigatran etexilate), BCRP (e.g. mitoxantrone, rosuvastatin) and OCT1 (e.g., metformin), since these transporters have been shown to be inhibited by gilteritinib in vitro.

Caution should be used when treating patients concomitantly with gilteritinib and drugs that are known to prolong QT or QTc intervals.

Additionally, patients with moderate or severe hepatic dysfunction as measured by laboratory tests should be closely monitored.

11.1.6 Agent Availability

Gilteritinib will be supplied by Astellas and provided to PrECOG's designated depot. PrECOG designated depot will distribute to sites.

The initial supply of gilteritinib will be sent directly to the site upon site activation. As needed, gilteritinib may be requested by the Principal Investigator (or their authorized designees) at each participating institution. The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return/destruction (site's drug destruction policy must be reviewed and approved by PrECOG before any study drug can be destroyed at a site) of gilteritinib.

11.1.7 Agent Ordering

PrECOG will be responsible for ordering drug for re-supply to the site.

Requests for shipments of gilteritinib to the PrECOG designated depot will be coordinated between PrECOG and Astellas.

11.1.8 Agent Accountability

Gilteritinib will be stored in a secure location. Only authorized pharmacy and study staff will have access to this agent. Drug accountability will be reviewed by PrECOG.

11.1.9 Important Identified Risks

11.1.9.1 Posterior Reversible Encephalopathy Syndrome

Posterior Reversible Encephalopathy Syndrome (PRES) is an important identified risk for gilteritinib. During the 2215-CL-0101 study, 2 patients, 1 in the 120 mg dose group and 1 in the 200 mg dose group, developed PRES. Both events were assessed by the investigator to be possibly related to gilteritinib. One patient (120 mg) also experienced seizure. This patient continued taking gilteritinib. At the time of reporting, the PRES was resolving and the seizure had resolved. The other patient (200 mg) experienced seizure and confusional state (both events started 1 day prior to the onset of PRES). This patient discontinued gilteritinib and the events of PRES, seizure and confusional state resolved.

Study drug discontinuation should be considered if PRES is diagnosed in patients receiving gilteritinib.

11.1.9.2 QT Prolongation

Arrhythmias due to QT prolongation is a potential risk for gilteritinib. 4.4% of relapse/refractory patients had a maximum post baseline QTcF interval >500 msec and approximately 8.8% of patients had a >60 msec change in their maximum QTc relative to baseline. Although clinically relevant QTc prolongation is not anticipated, additional eligibility criteria

for enrollment (exclusion of patients with QTcF >500 ms, long QT syndrome, hypokalemia or hypomagnesemia) and ECG assessments during induction and consolidation have been implemented.

11.1.9.3 Other Identified Risks

Other identified risks of gilteritinib include peripheral edema, increased blood creatine phosphokinase, myopathy, increased liver transaminase and diarrhea.

11.1.10 Important Potential Risks

11.1.10.1 Cardiac Failure

Cardiac Failure has been reported in oncology clinical studies. Although these patients had other potentially contributing factors including a medical history of cardiac failure, prior chemotherapy treatment (anthracyclines) and concurrent bacteremia/sepsis, the role of gilteritinib could not be completely excluded. Patients should be monitored for signs and symptoms of cardiac failure while being treated with gilteritinib.

11.1.10.2 Pericarditis and Pericardial Effusions

Pericarditis and pericardial effusions has been reported in oncology clinical studies. Some of these patients had concurrent lung infection or sepsis, the role of gilteritinib could not be completely excluded. Patients should be monitored for signs and symptoms of pericarditis and pericardial effusion while being treated with gilteritinib.

11.1.10.3 Pancreatitis Acute

Pancreatitis and pancreatitis acute have been reported in oncology clinical studies. Patients should be monitored for signs and symptoms of pancreatitis and pancreatic acute while being treated with gilteritinib.

11.1.11 Other Potential Risks

Other potential risks of gilteritinib include gastrointestinal obstruction, gastrointestinal perforation, gastrointestinal hemorrhage, differentiation syndrome and squamous cell skin carcinoma.

Please refer to the current Investigator's Brochure for additional information.

11.1.12 Overdose

Neither the effects of overdose of gilteritinib (defined as more than 300 mg for a single dose) nor an antidote to overdose are known. In the case of an overdose, the patient should be closely monitored for adverse reactions and supportive treatment should be administered.

11.2 Midostaurin[21]

Midostaurin will be obtained by the individual study sites as standard of care treatments from commercial stock. Please refer to the commercial package insert for full prescribing information.

11.2.1 Other Names

RYDAPT

11.2.2 Classification

Midostaurin is a kinase inhibitor.

11.2.3 Storage and Stability

Midostaurin is an oral drug that is available in 25 mg capsules.

Store in the original package to protect from moisture at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F).

11.2.4 Dose Specifics

Midostaurin 50 mg (two 25 mg capsules) twice a day by mouth on days 8-21. Patients should take their doses at approximately the same time each day, and approximately 12 hours should elapse between the morning and evening doses. Each daily dose should be given with food and a glass of water (~240 mL). Patients should be instructed to swallow capsules whole and not open, break or chew capsules. If vomiting occurs, no re-dosing is allowed prior to the next scheduled dose.

Dose interruptions are permitted (Section 6).

11.2.5 Drug Interactions

Midostaurin is metabolized by CYP3A4 to active compounds. Co-administration of drugs which are potent inducers (e.g., phenytoin) or, especially, potent inhibitors (e.g., itraconazole) of this isoenzyme could decrease or increase midostaurin concentrations, and, potentially, decrease the effectiveness or increase toxicity, respectively.

Potent CYP3A4 inhibitors are thought to represent the most significant potential for drug interactions with midostaurin (Appendix V).

11.2.6 Side Effects

Please refer to the Package Insert.

11.3 Daunorubicin[40,41,42]

Daunorubicin will be obtained by the individual study sites as standard of care treatments from commercial stock. Daunorubicin is generic and may be obtained from multiple manufacturers. Please refer to the commercial package insert for full prescribing information.

11.3.1 Other Names

Daunorubicin hydrochloride, Daunomycin, Rubidomycin, Cerubidine

11.3.2 Classification

Anthracycline topoisomerase inhibitor.

11.3.3 Storage and Stability

Store unopened vials in refrigerator, 2° to 8°C (36° to 46°F). Store prepared solution for infusion at room temperature, 15° to 30°C (59° to 86°F) for up to 24 hours. Contains no preservative. Discard unused portion. Protect from light.

11.3.4 Dose Specifics

Induction: Daunorubicin 90 mg/m^2/day will be administered IV per package insert or institutional guidelines or over 30-60 minutes Days 1, 2, and 3 (45 mg/m^2/day if receives second cycle of induction).

If patient does not achieve CR or CRi after first cycle of induction, then a second cycle of induction will be given. If second induction is needed, treatment will begin after day 24 for midostaurin (i.e., ≥ 3 days after completing midostaurin) and day 28 for gilteritinib (i.e., ≥ 7 days after completing gilteritinib).

Dose modifications are permitted (Section 6).

11.3.5 Preparation

Daunorubicin is commercially available. Preparation will be per package insert and institutional guidelines.

11.3.6 Drug Interactions

Use of daunorubicin in a patient who has previously received doxorubicin increases the risk of cardiotoxicity. Daunorubicin should not be used in patients who have previously received the recommended maximum cumulative doses of doxorubicin or daunorubicin. Cyclophosphamide used concurrently with daunorubicin may also result in increased cardiotoxicity.

Hepatotoxic medications may impair liver function and increase the risk of toxicity.

Daunorubicin may decrease the effect of ciprofloxacin absorption by altering the intestinal mucosa.

11.3.7 Side Effects

Please refer to Package Insert.

11.4 Cytarabine[43,44]

Cytarabine will be obtained by the individual study sites as standard of care treatments from commercial stock. Cytarabine is generic and may be obtained from multiple manufacturers. Please refer to the commercial package insert for full prescribing information.

11.4.1 Other Names

Cytosar-U, Ara-C, Arabinosyl, cytosine arabinoside

11.4.2 Classification

Antimetabolite.

11.4.3 Storage and Stability

Store at 20°C to 25°C (68°F to 77°F). Protect from light.

The dry powder is stored at room temperature. After reconstitution, cytarabine is stable for 7 days at room temperature and 15 days refrigerated. Solutions with a slight haze should be discarded.

11.4.4 Dose Specifics

Induction: Cytarabine 100 mg/m^2/day will be administered by continuous IV infusion for a total of 7 days beginning Day 1.

If patient does not achieve CR or CRi after first cycle of induction, then a second cycle of induction will be given. If second induction is needed, treatment will begin after day 24 for midostaurin (i.e., ≥ 3 days after completing midostaurin) or day 28 for gilteritinib (≥ 7 days after completing gilteritinib).

Consolidation: Cytarabine 3 g/m^2* over approximately 1-3 hours every 12 hours (+/- 2 hour window) on days 1, 3, and 5 or days 1-3 for a total of 6 doses for up to 4 cycles.

***** For patients age ≥ 55 or patients with decreased creatinine clearance recommend reducing consolidation cytarabine dose to 1.5 g/m^2.

Dose interruptions are permitted (Section 6).

11.4.5 Preparation

Cytarabine is commercially available. Preparation will be per package insert and institutional guidelines.

11.4.6 Drug Interactions

Reversible decreases in steady-state plasma digoxin concentrations and renal glycoside excretion were observed in patients receiving beta-acetyldigoxin and chemotherapy regimens containing cyclophosphamide, vincristine and prednisone with or without cytarabine injection or procarbazine.

Steady-state plasma digitoxin concentrations did not appear to change. Therefore, monitoring of plasma digoxin levels may be indicated in patients receiving similar combination chemotherapy regimens. The utilization of digitoxin for such patients may be considered as an alternative.

An in vitro interaction study between gentamicin and cytarabine showed a cytarabine related antagonism for the susceptibility of K. pneumoniae strains. This study suggests that in patients on cytarabine being treated with gentamicin for a K. pneumoniae infection, the lack of a prompt therapeutic response may indicate the need for re-evaluation of antibacterial therapy.

Clinical evidence in one patient showed possible inhibition of fluorocytosine efficacy during therapy with cytarabine injection. This may be due to potential competitive inhibition of its uptake.

11.4.7 Side Effects

Please refer to Package Insert.

12 Statistical Considerations

12.1 Study Design & Sample Size Considerations

This is a randomized, open-label Phase II study comparing gilteritinib to midostaurin in patients receiving standard induction therapy with cytarabine and daunorubicin 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 to receive standard induction treatment (cytarabine and daunorubicin) with either gilteritinib or midostaurin. If achieving CR or CRi, these patients will go on to receive standard consolidation treatment of high-dose cytarabine with the study drug received during induction (gilteritinib or midostaurin). 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 gilteritinib compared to those who receive midostaurin in addition to standard therapy with cytarabine and daunorubicin after induction. FLT3 mutation negativity is evaluated by PCR at the end of induction.

Based on a retrospective study performed at John-Hopkins,[45] we assume with the control arm of cytarabine, daunorubicin and midostaurin, 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 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.

12.2 Statistical Analysis

12.2.1 Primary Objectives

The primary endpoint of this study is the FLT3 mutation negative Composite Complete Response (CRc) [includes CR and CRi] rate. The cut points used for FLT3 mutation negativity are 1% (equivalent to 10^{-2}) for FLT3-TKD and 10^{-4} for FLT3-ITD (please see Sections 13.1.2.1 and 13.1.2.2 for details). 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.

12.2.2 Secondary and Exploratory Objectives

As a secondary objective, 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 after 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.

The MRD- CRc rate of patients with FLT3 mutated AML who receive gilteritinib will be compared to those who receive midostaurin. MRD will be measured by flow cytometry. The MRD- CRc rate will be compared 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.

The CRc (CR or CRi, as defined in Section 9) rate of patients with FLT3 mutated AML who receive gilteritinib will also be compared to those who receive midostaurin. The CRc rate will be compared 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. One of the secondary objectives of this study is to compare event free survival (EFS) of patients who receive gilteritinib to those who receive midostaurin in addition to cytarabine and daunorubicin after induction and high-dose cytarabine after consolidation. 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.

We assume with the control arm of cytarabine/daunorubicin plus midostaurin, for FLT3 mutated patients, the cure rate of EFS is 25% and the median EFS for the non-cure patients is 6 months. With approximately 170 eligible patients, the study will have 80% power to detect 38% reduction in hazard rate in EFS in the gilteritinib arm, using one-sided log rank test at the significance level of 0.05 and assuming 2 years of follow-up. The number of EFS events needed is about 110.

We will also assess OS of patients who receive gilteritinib to those who receive midostaurin in addition to cytarabine and daunorubicin after induction and high-dose cytarabine after consolidation. 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. Cox proportional hazards models, stratified on the same factors above, will also be used to assess the treatment effect.

A sensitivity analysis of EFS will be performed where follow-up will be censored at the start of transplantation.

Comparison of CRc rate, 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.

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.

For patients who are FLT3 mutation positive after induction and administered consolidation, the FLT3 mutation negative CRc rate after first cycle of consolidation and their corresponding 90% confidence intervals will be given by treatment arm.

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 with 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.

As per NCI CTCAE Version 5.0, the term toxicity is defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment. The maximum grade for each type of toxicity will be recorded for each patient, and frequency tables will be reviewed to determine toxicity patterns during induction and consolidation separately by treatment arm.

12.3 Planned Interim Futility Analyses

12.3.1 Interim Analysis of FLT3 Mutation Negative CRc Rate and EFS

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.

The interim futility analysis of FLT3 mutation negative CRc rate is complete and stopping criteria was not met. The study will continue as planned.

12.3.2 Study and Safety Monitoring Plan

This study will be monitored by the PrECOG DSMB. The DSMB meets twice each year. For each meeting, all monitored studies are reviewed for toxicity and progress toward completion. When appropriate, the DSMB will also review interim analyses of outcome data. Only the study statistician and the DSMB members will have access to interim analyses of outcome data. Prior to completion of this study, any use of outcome data will require approval of the DSMB.

13. Laboratory and Pathology Correlative Studies

13.1 Correlative Studies: Mandatory Bone Marrow Samples

13.1.1 Overview

13.1.1.1 Central Biorepository Pathology Facility (CBPF)

Prescreening: Central Biorepository Pathology Facility (CBPF) will perform the following:

- Baseline immunophenotype for later MRD assessment.

Additional testing will be done for DNMT3A mutation status testing using next generation sequencing on all FLT3 mutated patients.

Bone marrow samples will be sent to the CBPF after induction to perform:

- AML MRD by flow cytometry on all patients.
- MRD for FLT3-TKD mutation analysis on FLT3-TKD mutated patients.

Bone marrow samples will also be sent to the CBPF after first consolidation cycle to perform:

- MRD for FLT3-TKD mutation analysis on FLT3-TKD mutated patients.

Additional analyses performed will depend on resources, sample quality and availability:

- Assess correlation between MRD by molecular studies and morphology (blast count).
- Assess outcomes of patients who are MRD positive/negative by morphology vs. flow cytometry.
- Assess outcomes of patients who have mutations in NPM1.
- Assess outcomes of patients who have FLT3 mutations and mutations in NPM1 and DNMT3A.

Any left-over samples will be stored at the CBPF for future research.

13.1.1.2 **Invivoscribe**

Prescreening: Invivoscribe will perform the following:

- FLT3 ITD and TKD testing, includes signal ratio.
- NPM1 mutation status
- Prescreening results will be returned to the site (no other research sample results will be returned to sites)

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

- FLT3-ITD MRD testing

End of First Consolidation Cycle

- FLT3-ITD MRD testing

Any left-over samples will be batched and shipped to CBPF for future research.

13.1.2 Methodology

13.1.2.1 CBPF: Baseline Immunophenotype, DNMT3A and MRD

Prescreening Flow Cytometry

Flow cytometry will be performed at baseline.

Multicolor, multiparameter flow cytometric immunophenotyping will be performed using antibodies to CD2, surface CD3, cytoplasmic CD3, CD4, CD5, CD7, CD13, CD14, CD15, CD19, CD25, CD33, CD34, CD36, CD38, CD45, CD54, CD56,CD64, CD117, CD123, CD133, HLA-DR, MPO, TdT to characterize the immunophenotype of the blasts. Approximately 50,000 events will be collected for this assay.

Baseline DNMT3A

DNMT3A will be interrogated using next generation sequencing technology. Sequencing libraries will be prepared from genomic DNA obtained from bone marrow aspirate or peripheral blood samples using hybridization capture-based target enrichment of the genomic regions of interest [DNMT3A (NM_022552) exons 8-22 (codons 286-862), exon 23 (codons 866-913)]. Bidirectional paired-end sequencing will be performed using a next generation sequencing (NGS) platform to screen for single nucleotide variants and insertions/deletions (up to 52 base-pairs). The genomic reference sequence used is genome GRCh37/hg19.

Analytical Sensitivity: 5% (one mutant allele in the background of nineteen wild type alleles).

Variants are reported per the standardized mutation nomenclature guidelines by the human genome variation society (HGVS, www.hgvs.org <http://www.hgvs.org>).

Post-Induction

Flow Cytometry (all patients)

Minimal residual acute myeloid leukemia will be detected using multicolor, multiparameter flow cytometry using antibodies to CD4, CD5, CD7, CD13, CD14, CD15, CD19, CD25, CD33, CD34, CD36, CD38, CD45, CD54, CD56, CD64, CD117, CD123, CD133, HLA-DR. Myeloid blasts will be characterized using this panel of antibodies to detect immunophenotypic variations compared with normal myeloid progenitors. This assay has been validated to a sensitivity level of 0.01 to 0.1% (1 cell in 1,000 to 10,000). The sensitivity may vary in individual patients due to a number of factors that are either intrinsic to the AML blasts, and/or bone marrow specimen quality. This requires an adequate specimen, with acquisition of at least 200,000 events, for a valid negative result. Note that positive results may be obtained with fewer events.

Post-Induction and Post First Consolidation Cycle MRD

FLT3-TKD (on FLT3-TKD patients)

Polymerase chain reaction (PCR)-based DNA analysis will be performed to detect codon 835/836 (tyrosine kinase domain, TKD) point mutation in FLT3. A multiplex PCR using fluorescently-labeled primers will be performed, followed by detection and sizing of PCR products

using capillary electrophoresis. For detecting point mutations in codons 835/836 (TKD), a restriction enzyme digestion of the PCR products will be performed prior to capillary electrophoresis. The lower limit of detection (analytical sensitivity) of this assay is approximately 1% of mutant DNA in a background of wild type DNA (equivalent to 10^{-2}). When a mutation is detected, the ratio of area under the peak of mutant over total (mutant+wild-type) FLT3 is reported for monitoring the mutant allele burden.

13.1.2.2 Invivoscribe: FLT3, NPM1 and FLT3 MRD

Prescreening FLT3 and Signal Ratio[46]

Primers flanking exons 14, 15 and the activation loop region of exon 20 of the FLT3 gene are used to amplify DNA extracted from a patient sample. The forward and reverse PCR primers are fluorescently labeled with different flurophores that serve to confirm the presence of sample signal.

The size of the FLT3 ITD PCR is determined by capillary electrophoresis and the signal ratio compares the signal intensity of the mutant to the wild type.

The FLT3-TKD PCR product is digested with EcoRV and the presence of the mutation is further assessed using capillary electrophoresis and the signal ratio compares the signal intensity of the mutant to the wild-type.

FLT3 and signal ratio results will be returned to sites.

Prescreening NPM1[47]

Primers targeting the area surrounding exon 12 of the NPM1 gene are used to amplify the patient's DNA. The size of the NPM1 PCR product is determined by capillary electrophoresis.

NPM1 results will be returned to sites.

Post-Induction and Post First Consolidation Cycle FLT3-ITD MRD Testing (on FLT3-ITD patients)[48]

The FLT3-ITD MRD test is an amplicon-based NGS assay. This assay can detect mutations with a mutant cell sensitivity of 10^{-4} (1 mutant cell in a background of ten thousand normal cells; equivalent to an allelic sensitivity of $5x10^{-5}$ when a single mutant allele is present).

The following three controls are included in every test:

1. A positive control with a FLT3 mutant allelic concentration of $5x10^{-5}$ (a mutant cell concentration of approximately 10^{-4}).
2. A negative control with a wild-type FLT3 gene.
3. A no template control with water in place of the DNA sample in the PCR reaction.

The sequencing output data is analyzed using an Invivoscribe developed proprietary FLT3-ITD MRD Data Analysis software.

13.1.3 Bone Marrow Sample Collection

Mailers and kits will be supplied. Instructions and shipping address will be provided. Refer to the Invivoscribe Lab Manual for the mailer and the PrE0905 Lab Manual for kit details.

- **Prescreening**
  - Invivoscribe
    - One 4 mL Sodium Heparin green top tube (need approximately .75 mL of bone marrow)
      - If bone marrow sample (preferred) is not available and patient has any circulating blasts, then one 4 mL Sodium Heparin green top tube of peripheral blood (need approximately 3 mL) will be allowed.
  - CBPF
    - Two 10 mL EDTA tube (request 6 mL (ideal amount) of bone marrow in each)
      - If diagnostic bone marrow was done prior to study enrollment or patient is inaspirable, then two 10 mL EDTA tubes of peripheral blood will be allowed if patient has any circulating blasts.
- **Post Induction (First Cycle and Second Cycle, 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)
    - Request 6 mL (ideal amount) of bone marrow in each
- **Post First Consolidation Cycle**
  - 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)
    - Request 6 mL (ideal amount) of bone marrow in each
- **Relapse (Optional)**
  - CBPF
    - Two 10 mL EDTA tubes (request 6 mL (ideal amount) of bone marrow in each)

Left-over derivatives from Invivoscribe will be shipped to the CBPF.

13.1.4 Bone Marrow Sample Processing and Shipment

All samples collected will be labeled with a unique numeric identifier that will be coded for patient privacy protection.

Invivoscribe

Ship one 4 mL Sodium Heparin green top tube to Invivoscribe at Prescreening (bone marrow or blood).

Post Induction (first cycle and second cycle, as applicable) and Post First Consolidation Cycle ship one 4 mL EDTA tube to Invivoscribe (bone marrow).

CBPF

Ship two 10 mL EDTA tubes to CBPF at Prescreening.

Post Induction, Post First Consolidation Cycle and Relapse (as applicable) bone marrow samples will be shipped to CBPF.

Samples should be shipped the same day they are obtained except as noted in the Invivoscribe Lab Manual and PrE0905 Lab Manual (i.e., samples cannot be received on Sunday). Samples will be shipped on a cold pack via overnight courier.

14 Administrative

14.1 Protocol Compliance

The study shall be conducted as described in this protocol. All revisions to the protocol must be discussed with, and be prepared by PrECOG and/or representatives. The Investigator should not implement any deviation or change to the protocol or consent without prior review and documented approval from PrECOG and/or representatives and the Institutional Review Board (IRB) of an amendment, except where necessary to eliminate an immediate hazard(s) to study patients.

If a deviation or change to the approved protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB approval, notification will be submitted to the IRB for review and approval as soon as possible afterward. Documentation of approval signed by the chairperson or designee of the IRB(s) should be in the study records. If PrECOG and/or representatives provides an amendment that substantially alters the study design or increases the potential risk to the patient; the consent form must be revised and submitted to the IRB(s) for review and approval; the revised form must be used to obtain consent from patients currently enrolled in the study if they are affected by the Amendment; and the new form must be used to obtain consent from new patients prior to study entry. Information as to who investigators should send correspondence will be provided in additional study documents.

14.2 Institutional Review Board (IRB)

Before study initiation, the Investigator must have written and dated approval from their respective IRB for the protocol, consent form, patient recruitment materials/process and any other written information to be provided to patients. The Investigator should also provide the IRB with a copy of the Investigator Brochure or product labeling, and any updates.

The Investigator should provide the IRB with reports, updates, and other information (e.g., Safety Updates, amendments, and administrative letters) according to regulatory requirements, IRB or study site procedures.

14.3 Informed Consent Procedures

Investigators must ensure that patients who volunteer for clinical trials or their legally acceptable representative are clearly and fully informed about the purpose, potential risks and other information.

A protocol specific informed consent form (ICF) template will be provided to sites. Preparation of the site-specific consent form is the responsibility of the site Investigator and must include all applicable regulatory and IRB requirements, and must adhere to Good Clinical Practices (GCP) and to the ethical principles that have their origin in the Declaration of Helsinki. All changes to the ICF template will be approved by PrECOG and/or their representatives prior to implementation.

In accordance with the Health Information Portability and Accountability Act (HIPAA), the consent process will also include written authorization by patients to release medical information to allow PrECOG and/or its agents, regulatory authorities, and the IRB of record at the study site for access to patient records and medical information relevant to the study, including the medical history. This will be documented in the informed consent form or other approved form obtained at the time of informed consent per institutional policies. This form should also be submitted to PrECOG and/or its agents for review prior to its implementation.

The Investigator must provide the patient or legally acceptable representative with a copy of the consent form and written information about the study in the language in which the patient is most proficient. The language must be non-technical and easily understood. The Investigator should allow time necessary for patient or patient's legally acceptable representative to inquire about the details of the study, then informed consent must be signed and personally dated by

the patient or the patient's legally acceptable representative and by the person who conducted the informed consent discussion. The patient or legally acceptable representative should receive a copy of the signed informed consent and any other written information provided to study patients prior to patient's participation in the trial. The investigator is responsible for assuring adequate documentation of this process and for storage and maintenance of the original signed consent form for each patient/subject.

The informed consent and any other information provided to patients or the patient's legally acceptable representative, should be revised whenever important new information becomes available that is relevant to the patient's consent, and should receive IRB approval prior to use. The Investigator, or a person designated by the Investigator should inform the patient or the patient's legally acceptable representative of all pertinent aspects of the study and of any new information relevant to the patient's willingness to continue participation in the study. This communication should be documented in the patient record. During a patient's participation in the trial, any updates to the consent form and any updates to the written information will be provided to the patient.

14.4 Safety Communication

Investigators will be notified of all AEs that are serious, unexpected, and definitely, probably, or possibly related to the investigational product. Upon receiving such notices, the Investigator must review and retain the notice with the Investigator Brochure and submit a copy of this information to the IRB according to local regulations. The Investigator and IRB will determine if the informed consent requires revision. The Investigator should also comply with the IRB procedures for reporting any other safety information. All revisions should be submitted to PrECOG and/or agents for review.

14.5 Monitoring

Representatives and agents of PrECOG and, as applicable to the study, the manufacturer of investigational product must be allowed to visit all study site locations periodically to assess the data, quality and study integrity. The purpose of this visit is to review study records and directly compare them with source documents and discuss the conduct of the study with the Investigator, and verify that the facilities remain acceptable. Monitoring of drug accountability will also occur.

The study may be evaluated by other auditors and government inspectors who must be allowed access to electronic Case Report Forms (eCRFs), source documents and other study files. The Investigator must notify PrECOG of any scheduled visits by regulatory authorities, and submit copies of all reports. Information as to who investigators should notify of an audit or where to address questions will be provided in additional study materials.

14.6 Study Records

An Investigator is required to maintain adequate regulatory files with corresponding communication and approvals, accurate histories, observations and other data on each individual treated. Full details of required regulatory documents will be provided in additional study materials. Data reported on the eCRFs must be consistent with the source documents as part of the patient record.

The confidentiality of records that could identify patients must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

A study specific signature record will be maintained to document signatures and initials of all persons at a study site who are authorized to make entries and/or corrections on eCRFs as well as document other study-specific roles.

14.7 Electronic Case Report Form (eCRF) Information

Additional information regarding eCRF instructions, timelines for data entry/ submission and query completion can be found in supplemental materials provided to the site. Sites will be expected to complete eCRFs as per the schedule provided and submit all relevant data as per the specified timelines. All items recorded on eCRFs must be found in source documents.

The completed eCRF must be promptly reviewed, electronically signed, and dated by the Principal Investigator.

Instructions for management of patients who do not receive any protocol therapy:

If a patient is registered and does not receive any assigned protocol treatment, baseline, Serious Adverse Event and follow-up data will still be entered and must be submitted according to the eCRF instructions. Document the reason for not starting protocol treatment on the appropriate electronic end of treatment form.

14.8 Records Retention

FDA Regulations (21CFR 312.62) require clinical investigators to retain all trial-related documentation, including source documents for the periods described below for studies performed under a US Investigational New Drug (IND):

- two years after the FDA approves the marketing application, or
- two years after the FDA disapproves the application for the indication being studied, or
- two years after the FDA is notified by the sponsor of the discontinuation of trials and that an application will not be submitted.

The Investigator must retain investigational product disposition records, copies of eCRFs (or electronic files), and source documents for the maximum period required by applicable regulations and guidelines, or Institution procedures, whichever is longer. The Investigator must contact PrECOG and/or representatives prior to destroying any records associated with the study.

Information as to who investigators should contact for questions will be provided in additional study documents. PrECOG and/or representatives will notify the Investigator when the trial records for this study are no longer needed.

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Appendix I: ECOG Performance Status

PS 0	Fully active, able to carry on all pre-disease performance without restriction
PS 1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature e.g. light house work, office work.
PS 2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
PS 3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
PS 4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

Source: Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair

Appendix II: Calculation of Creatinine Clearance Using the Cockcroft-Gault Formula

$$\text{Creatinine clearance for males} = \frac{(140 - \text{age [years]})\ (\text{body wt [kg]})}{(72)\ (\text{serum creatinine [mg/dL]})}$$

$$\text{Creatinine clearance for females} = \frac{(140 - \text{age [years]})\ (\text{body wt [kg]})}{(72)\ (\text{serum creatinine [mg/dL]})} \times 0.85$$

NOTE: Actual body weight (wt) in kg.

Source: Gault MH, Longerich LL, Harnett JD, et al. Predicting glomerular function from adjusted serum creatinine (editorial). Nephron 1992; 62:249

Appendix III: Contraceptive Requirements

Women of Childbearing Potential (WOCBP) participants may choose complete abstinence and have pregnancy test, as specified in Schedule of Assessments.

WOCBP DEFINITIONS AND METHODS OF CONTRACEPTION DEFINITIONS

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile.

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following (at least 1 month prior to screening):
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy
- Post-menopausal

Documentation of any of these categories can come from the site personnel's review of the female subject's medical records, medical examination, or medical history interview.

A postmenopausal state is defined as at least 12 months after last regular menstrual bleeding without an alternative medical cause.

- In case the last regular menstrual bleeding cannot be clearly determined, confirmation with repeated follicle-stimulating hormone (FSH) measurements of at least >40 IU/L (or higher per local institutional guidelines), is required.

Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status by repeated FSH measurements before study enrollment.

CONTRACEPTION GUIDANCE FOR FEMALE PARTICIPANTS OF CHILD BEARING POTENTIAL

One of the highly effective methods of contraception listed below is required at the time of informed consent and until the end of relevant systemic exposure, defined as 180 days after the final study drug administration.[a]

Highly Effective Contraceptive Methods (Failure rate of <1% per year when used consistently and correctly.)[b]

Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation

- oral
- intravaginal
- transdermal

Progestogen-only hormonal contraception associated with inhibition of ovulation

- oral
- injectable
- implantable

Hormonal methods of contraception containing a combination of estrogen and progesterone, vaginal ring, injectables, implants and intrauterine hormone-releasing system (IUS)

- intrauterine device (IUD)
- bilateral tubal occlusion

Male is sterile due to a bilateral orchiectomy.

Vasectomized partner: *(A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.)*

Sexual abstinence: *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant. It is not necessary to use any other method of contraception when complete abstinence is elected.*

[a] Local laws and regulations may require use of alternative and/or additional contraception methods.

[b] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies.

CONTRACEPTION GUIDANCE FOR MALE PARTICIPANTS WITH PARTNER(S) OF CHILD BEARING POTENTIAL

Male participants with female partners of childbearing potential are eligible to participate if they agree to the following during treatment and until the end of relevant systemic exposure defined as 120 days after final drug administration.

- Inform any and all partner(s) of their participation in a clinical drug study and the need to comply with contraception instructions as directed by the investigator.
- Male participants are required to remain abstinent use a condom during treatment and until end of relevant systemic exposure defined as 120 days after final drug administration.
- Female partners of male participants who have not undergone a vasectomy with the absence of sperm confirmed or a bilateral orchiectomy should consider use of effective methods of contraception until the end of relevant systemic exposure, defined as 120 days after final drug administration.

Appendix IV: NYHA Classification

Class	Symptoms
Class I	Patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath), or anginal pain.
Class II	Patients with cardiac disease resulting in slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea or anginal pain.
Class III	Patients with cardiac disease resulting in marked limitation of physical activity. Comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea or anginal pain.
Class IV	Patients with cardiac disease resulting in inability to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.
Source: Oxford Textbook of Internal Medicine. Vol. 2, pp 2228. Oxford University Press. 1997	

Appendix V: Additional Excluded and Cautionary Medications

The following list describes medications and foods that are common strong inhibitors of CYP3A. This list should not be considered all inclusive. Consult individual drug labels for specific information on a compound's propensity to inhibit CYP3A.

NOTE: Treatment with concomitant drugs that are strong inhibitors of CYP3A should be avoided with the exception of antibiotics, antifungals and antivirals that are used as standard of care to prevent or treat infections.

Strong CYP3A Inhibitors	
Drug Type	**Generic Drug Name**
Human Immunodeficiency Virus Protease Inhibitors	Indinavir Nelfinavir Lopinavir/ritonavir Ritonavir Saquinavir
Food/Juice	Grapefruit juice
Others	Boceprevir Telaprevir Clarithromycin Telithromycin Conivaptan Itraconazole Ketoconazole Posaconazole Voriconazole Nefazodone

CYP: Cytochrome P450.

Source: Table 3 in FDA Draft Guidance for Industry – Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Recommendations:

http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf

Treatment with concomitant drugs that are strong inducers of CYP3A are prohibited. The following list describes medications and foods that are common strong inducers of CYP3A. This list should not be considered all inclusive. Consult individual drug labels for specific information on a compound's propensity to induce CYP3A.

Strong CYP3A Inducers	
Drug Type	**Generic Drug Name**
Antiepileptic, Anticonvulsant	Carbamazepine Phenytoin
Antibiotic	Rifampicin
Food/Juice Supplement	St. John's Wort

CYP: Cytochrome P450.

Source: Table 4 in FDA Draft Guidance for Industry – Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Recommendations:

http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf

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The following list describes medications that target serotonin receptors. This list should not be considered all inclusive. Consult individual drug labels for specific information on whether a compound targets serotonin receptors.

NOTE: Treatment with concomitant drugs that target serotonin $5HT_{2B}R$ or sigma nonspecific receptor should be avoided with the exception of drugs that are considered absolutely essential for the care of the patient.

Drugs Targeting Serotonin Receptors	
Drug Type	**Generic Drug Name**
Affinity or function to $5HT_{2B}R$	Eletriptan Hydrobromide Escitalopram Fluoxetine Sertraline

$5HT_{2B}R$: 5-Hydroxytryptamine Receptor 2B.

The following list describes medications and foods that are common inhibitors or inducers of P-gp. This list should not be considered all inclusive. Consult individual drug labels for specific information on a compound's propensity to inhibit or induce P-gp.

NOTE: Treatment with concomitant drugs that are strong inhibitors or inducers of P-gp should be avoided with the exception of drugs that are considered absolutely essential for the care of the patient.

P-gp Inhibitors or Inducers			
Transporter	**Gene**	**Inhibitor**	**Inducer**
P-gp	*ABCB1*	Amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, dronedarone, erythromycin, felodipine, itraconazole, ketoconazole, lopinavir and ritonavir, quercetin, quinidine, ranolazine, verapamil	Avasimibe, carbamazepine, phenytoin, rifampin, St. John's wort, tipranavir/ritonavir

P-gp: P-glycoprotein.

Source:https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers

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The following list describes drugs that are known to prolong QT or QTc. This list should not be considered all inclusive. Consult individual drug labels for specific information on whether a compound is known to prolong QT or QTc.

Drugs That May Prolong QT or QTc	
Drug Type	**Generic Drug Name**
Class IA antiarrhythmics	Quinidine Procainamide Disopyramide
Class IC antiarrhythmics	Flecainide Propafenone Moricizine
Class III antiarrhythmics	Amiodarone Sotalol Bretylium Ibutilide Dofetilide
Antipsychotics	Thioridazine Mesoridazine Chlorpromazine Prochlorperazine Trifluoperazine Fluphenazine Perphenazine Pimozide Risperidone Ziprasadone Lithium Haloperidol
Tricyclic/tetracyclic antidepressants	Amitriptyline Desipramine Doxepin Dosulepin hydrochloride Imipramine Maprotiline
Selective serotonin and norepinephrine reuptake inhibitors (SSNRIs) antidepressants	Venlafaxine
Macrolide antibiotics	Azithromycin Erythromycin Clarithromycin Dirithromycin Roxithromycin Tulathromycin
Fluoroquinolone antibiotics	Moxifloxacin Gatifloxacin

Drugs That May Prolong QT or QTc	
Drug Type	**Generic Drug Name**
Azole antifungals	Ketoconazole Fluconazole Itraconazole Posaconazole Voriconazole
Antimalarials	Amodiaquine Atovaquone Chloroquine Doxycycline Halofantrine Mefloquine Proguanil Primaquine Pyrimethamine Quinine Sulphadoxine
Antiprotozoals	Pentamidine
Antiemetics	Droperidol Dolasetron Granisetron Ondansetron
Antiestrogens	Tamoxifen
Immunosuppressants	Tacrolimus

Appendix VI: Investigator's Statement

1. I have carefully read this protocol entitled **"Randomized Trial of Gilteritinib vs Midostaurin in FLT3 Mutated Acute Myeloid Leukemia (AML)", Version 3.0 dated 10/5/2022 (Protocol Number PrE0905)** and agree that it contains all the necessary information required to conduct the study. I agree to conduct the study as outlined in the protocol.
2. I agree to conduct this study according to the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, the principles of Good Clinical Practice (GCP) as described in 21 Code of Federal Regulations (CFR) and any applicable local requirements.
3. I understand that this trial and any subsequent changes to the trial will not be initiated without approval of the appropriate Institutional Review Board, and that all administrative requirements of the governing body of the institution will be complied with fully.
4. Informed written consent will be obtained from all participating patients in accordance with institutional and Food and Drug Administration (FDA) requirements as specified in Title 21, CFR, Part 50.
5. I understand that my signature on the electronic Case Report Form (eCRF) indicates that I have carefully reviewed each page and accept full responsibility for the contents thereof.
6. I understand that the information presented in this study protocol is confidential, and I hereby assure that no information based on the conduct of the study will be released without prior consent from PrECOG, LLC unless this requirement is superseded by the FDA.

Principal Investigator (PI):

PI Name: _____

Site Name: _____

Signature of PI: _____

Date of Signature: _______________

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