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Phase 1 study of quizartinib in combination with induction and consolidation chemotherapy in patients with newly diagnosed acute myeloid leukemia

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Abstract

Novel therapies have potential to improve outcomes in patients with acute myeloid leukemia (AML) harboring FLT3-ITD mutations that have high risk of relapse and poor survival following standard of care (SOC) cytarabine/anthracycline-based induction/consolidation chemotherapy. Quizartinib is a selective and highly potent FLT3 inhibitor that has shown strong single-agent activity in relapsed or refractory (R/R) AML. This phase 1, open-label, sequential group dose-escalation trial (NCT 01390337) is the first evaluating safety and tolerability of quizartinib in combination with SOC chemotherapy in newly diagnosed AML (ndAML). Nineteen patients unselected for FLT3 mutational status received one of three quizartinib dihydrochloride dose levels (DL): 60 mg/d for 7 days (DL1; $n = 6$), 60 mg/d for 14 days (DL2; $n = 7$), and 40 mg/d for 14 days (DL-1; $n = 6$); administered orally starting on day 4 of chemotherapy. Median age was 43.8 years. Ten patients completed induction and consolidation. Three patients experienced dose-limiting toxicities (DLTs): 2 at DL2 (1 pericardial effusion; 1 febrile neutropenia, decreased platelet count, and QT prolongation); 1 at DL-1 (pericarditis). Maximum tolerated dose (MTD) was identified as DL-1. Most common grade 3/4 adverse events were febrile neutropenia (47%), neutropenia (42%), thrombocytopenia (32%), and anemia (26%). There were no apparent additional toxicities with addition of quizartinib to chemotherapy although grade ≤1 QT prolongation was observed at MTD. Sixteen patients (84%) achieved a response; 14 (74%) composite complete response; 2 (11%) morphologic leukemia-free state. The phase 3 QuANTUM-First trial (NCT02668653) is further evaluating the effect of quizartinib plus SOC chemotherapy in ndAML FLT3-ITD mutated patients.

1 | INTRODUCTION

One of the most common molecular alterations observed in patients with ndAML is internal tandem duplication (ITD) mutation of the FLT3 receptor, a driver mutation for disease progression that occurs in approximately 25% of cases.[1-6] FLT3-ITD mutation identifies a high-risk population of AML patients with an increased risk of early relapse, increased mortality following SOC chemotherapy, and a high need for improved treatment options.[2,7-9] Point mutations within the tyrosine kinase domain of FLT3 have been reported in approximately 7% of patients with AML but they have not been definitively linked to patient prognosis.[9,10]

Early clinical studies evaluating FLT3 inhibitors such as sunitinib and lestaurtinib in patients with AML demonstrated positive results.[11-14] However, these agents were limited by poor target

selectivity, suboptimal pharmacokinetic (PK)/pharmacodynamic (PD) properties, and off-target effects. In phase 2 trials, treatment with the multikinase inhibitor midostaurin demonstrated transient single-agent antileukemic activity in patients with ndAML or R/R AML.[15,16] In more recent phase 1/2 studies, midostaurin in combination with chemotherapy induced hematologic responses in patients with ndAML or R/R AML, albeit with increased rates of hematologic and other toxicities.[17-20] The phase 3 RATIFY study (NCT00651261) demonstrated significant overall survival benefit for midostaurin in combination with standard induction and consolidation chemotherapy in patients with FLT3-mutated ndAML.[21] Based on results of RATIFY, midostaurin was approved in combination with chemotherapy for treatment of adult patients with ndAML characterized by a FLT3 mutation.

An important limitation of first generation, less potent, FLT3 inhibitors is that while they are able to clear blasts from peripheral blood when used at their MTD, they often have little effect on bone marrow blasts.[22] Quizartinib is the first member of a next-generation class of FLT3 inhibitors that demonstrates more potent binding to FLT3, more selective inhibition of FLT3 kinase activity, and more potent growth inhibitory activity in AML cells harboring FLT3-ITD mutation vs multikinase inhibitors including midostaurin and lestaurtinib.[23–25] Importantly, quizartinib has demonstrated very high single agent activity in patients with FLT3-ITD mutated AML, with reported composite complete remission rates between 46% and 57%, overall response rates between 65% and 78%, and high rates (35%-42%) of successful bridging to allogeneic hematopoietic stem cell transplantation (allo-HSCT).[26–31] Here, we report results of the first phase 1 study to evaluate safety and tolerability of quizartinib in combination with standard cytarabine/daunorubicin induction and high-dose cytarabine (HiDAC) consolidation chemotherapy in patients with ndAML. The study was focused on safety and tolerability of quizartinib in combination with chemotherapy, and patients enrolled in the study were unselected for FLT3 mutation status. However, baseline assessment of FLT3 permitted analysis of responses by mutational status.

2 | METHODS

2.1 | Study design

Study 2689-CL-0005 (NCT01390337) was a 2-part, phase 1, multicenter, open-label, sequential group dose-escalation trial of quizartinib administered in combination with standard induction/consolidation chemotherapy in patients with ndAML. The study protocol was approved by Institutional Review Board (IRB)/Independent Ethics Committee (IEC) at participating centers. In accordance with applicable laws and regulations, all patients signed informed consent forms prior to screening.

Primary objectives of the study were determination of DLTs, definition of MTD, definition of recommended phase 2/3 dose, evaluation of safety and tolerability of quizartinib when combined with cytarabine/daunorubicin induction administered in a $7+3$ schedule and HiDAC consolidation therapy, and definition of DLTs and MTD of quizartinib given as maintenance following induction/consolidation chemotherapy. Secondary objectives were characterization of the PK of quizartinib and AC886 (the pharmacologically active metabolite of quizartinib) when quizartinib is given in combination with induction/consolidation therapy, and as maintenance monotherapy post consolidation in ndAML. Efficacy of quizartinib in combination with cytarabine/daunorubicin induction administered in a $7+3$ schedule was examined as an exploratory objective.

2.2 | Part 1: Quizartinib dose escalation

Quizartinib dose escalation was planned in successive cohorts of 5 or 6 patients (with minimum of 3 females in each cohort) to determine MTD in combination with cytarabine/daunorubicin-based chemotherapy.

A modified $3+3$ design was used, allowing for concurrent enrollment of 2 to 6 patients into a cohort based on number of evaluable patients enrolled, number experiencing DLTs, and number at risk for developing a DLT. Intrapatient dose escalations were not permitted.

Quizartinib dihydrochloride (hereafter referred to as quizartinib) was administered as an oral solution at least 1 hour before or 2 hours after a meal. The first dose level tested (DL1) was quizartinib 60 mg/d (equivalent to 53 mg quizartinib free base) administered on days 4 through 10 of induction therapy. The next dose tested was DL2, 60 mg/d administered on days 4 through 17 of induction. The third dose level tested was DL-1, quizartinib 40 mg/d (equivalent to 35.4 mg quizartinib free base) administered on days 4 through 17 of induction. Dose-escalation decisions were made by a dose-escalation committee consisting of sponsor's medical monitor and the principal investigator at each institution, based on DLTs that occurred during remission induction. If ≤ 1 of 6, or 0 of 5 patients experienced a DLT on DL1, dose was escalated to DL2. If DL2 was not tolerated (≥ 1 of 6 patients experienced a DLT), dose was reduced to DL-1. For consolidation, patients received the same quizartinib dose and schedule in consolidation that they received during induction.

During induction therapy cytarabine was administered as a continuous infusion at a dose of 200 mg/m^2/d on days 1 through 7, and daunorubicin was administered at a dose of 60 mg/m^2/d by intravenous (IV) infusion or IV push over approximately 15 minutes on days 1 through 3. Up to 2 cycles of induction therapy were allowed. For consolidation therapy administration of cytarabine was planned at a dose of 3 g/m^2/d IV over 3 hours every 12 hours on days 1, 3, and 5, with initiation of next cycle after blood count recovery but no earlier than 22 days after first dose of prior cycle. Patients could proceed directly to HSCT after achieving a response or continue to receive quizartinib as maintenance therapy after consolidation. For maintenance, patients received the same daily dose of quizartinib that they received during induction, administered daily in 28 day cycles for up to 12 cycles.

Quizartinib dosing was interrupted for patients experiencing asymptomatic grade 3 QT-interval prolongation (corrected according to Friderica's formula; QTcF) until QTcF was ≤ 30 ms above baseline (day 4 pre-quizartinib dose) with dose reduction during next induction/consolidation treatment cycle (reductions to <40 mg and re-escalation were not permitted). Patients discontinued quizartinib treatment if QTcF did not return to ≤ 30 ms above baseline within 7 days.

DLTs were defined as occurrence of any of the following events during the observation period and considered to be at least possibly related to study drug: a) any grade ≥ 3 nonhematologic toxicity occurring after first dose of quizartinib and by day 42 of last induction treatment cycle or before start of the first consolidation cycle, whichever was sooner, b) hematologic toxicities occurring after first dose of quizartinib that did not resolve by day 42 of last induction treatment cycle or before start of the first consolidation cycle, whichever was sooner, including peripheral absolute neutrophil count (ANC) <500/mm^3 (grade 4), non–transfusion-related platelet count $<20,000$/mm^3 due to documented bone marrow aplasia/hypoplasia (overall marrow cellularity $<20\%$), platelet count $<50,000$/mm^3 (grade ≥ 3) associated with bleeding, or platelet count $<25,000$/mm^3 (grade 4) requiring blood

transfusion, or c) any other study drug–related toxicity that occurred after first dose of quizartinib and caused cessation of study drug during remission induction or during maintenance. MTD was defined as highest dose of quizartinib associated with occurrence of a DLT in <33% of patients in a cohort. MTD was estimated to be dose level at which ≤1 of 6 or 0 of 5 patients experienced a DLT and one dose level below lowest dose level at which ≥2 out of 2 to 6 patients experienced a DLT.

2.3 | Part 2: MTD evaluation

The planned second part of the study consisted of evaluation of safety, efficacy, and PK of quizartinib administered at MTD determined in part 1 of the study in combination with induction remission or consolidation chemotherapy in expanded cohort of patients. MTD was defined as highest dose associated with occurrence of DLTs at the rate at which lower bound of 95% CI was <25% using the adjusted Wald method. An additional part of the study designed to be conducted in parallel to parts 1 and 2 consisted of evaluation of MTD for quizartinib when administered continuously in 28-day cycles for up to 12 cycles as maintenance therapy in patients who achieved composite complete remission (CRc) following induction or ≥1 cycle of consolidation chemotherapy. Quizartinib was to be dose-escalated in successive cohorts of 5 or 6 patients to determine maintenance MTD. The first dose level planned was quizartinib 60 mg, administered daily, and dose-escalation decisions were to be made based on DLTs that occurred during first 2 cycles (8 weeks) of maintenance therapy. Once maintenance MTD was determined, all remaining patients coming out of parts 1 and 2 of the study were to be treated at this dose. However, the study was terminated before the start of part 2 and prior to evaluation of maintenance MTD due to end of the collaboration agreement between the trial sponsors Ambit Biosciences and Astellas Pharma Inc., and total study enrollment consisted of 19 patients in part 1.

2.4 | Eligibility

Patients aged between 18 and 60 years with previously untreated ndAML as defined by the World Health Organization (WHO) criteria,[32] documented within 28 days prior to enrollment, and Eastern Cooperative Oncology Group (ECOG) performance status of ≤2 and meeting the following criteria were enrolled: adequate renal, hepatic, and coagulation parameters (alkaline phosphatase, aspartate aminotransferase [AST], and alanine aminotransferase [ALT] ≤2.5 × institutional upper limit of normal [ULN]; total bilirubin ≤1.5 × institutional ULN; serum creatinine ≤1.5 × institutional ULN or an estimated glomerular filtration rate (eGFR) of >50 mL/min as calculated by Modification of Diet in Renal Disease equation); use of a medically approved method of contraception; negative serum or urine pregnancy test (sensitivity ≤25 IU human chorionic gonadotropin [hCG]/L) within 72 hours prior to start of study drug administration in women of childbearing potential, ability to comply with study procedures and follow-up examinations.

Exclusion criteria included: diagnosis of acute promyelocytic leukemia (APL), French-American-British (FAB) classification M3 or WHO classification of APL with t(15;17)(q22;q12), BCR-ABL–positive chronic myelogenous leukemia in blast crisis, AML following an antecedent hematologic disorder (myelodysplasia or myeloproliferative neoplasm), acute bilineal/biphenotypic leukemia, or therapy-related AML; previous therapy for AML, with the exception of emergency leukapheresis, emergency treatment for hyperleukocytosis with hydroxyurea for ≤5 days, growth factor or cytokine support, or steroids for treatment of hypersensitivity or transfusion reactions; previous treatment with quizartinib; uncontrolled disseminated intravascular coagulation; central nervous system (CNS) leukemia; known positive test for human immunodeficiency virus, hepatitis C, or hepatitis B surface antigen; major surgery within 4 weeks prior to first dose of study drug; uncontrolled or significant cardiovascular disease; pre-existing disorder predisposing patient to a serious or life-threatening infection; active acute fungal, bacterial, viral, or other infection; concurrent disease that placed patient at undue risk to undergo induction therapy per protocol or that might obscure assessments of drug safety; requirement for treatment with concomitant drugs that prolong QT/QTc interval or strong CYP3A4 inhibitors or inducers, with the exception of antibiotics, antifungals, and antivirals that are used as SOC to prevent or treat infections and other such drugs that are considered absolutely essential for care of the patient; and requirement for treatment with anticoagulant therapy.

Standard FLT3 testing was conducted locally by investigators. FLT3 mutational status was confirmed for 12 patients by Navigate BioPharma (Carlsbad, CA) using PCR analysis. Results were not collected centrally and enrolled patients were unselected for FLT3 mutation status.

2.5 | Tolerability and safety assessments

Safety assessments were performed at baseline and throughout the study. Primary safety variables included adverse events (AEs), DLTs, physical examinations, vital signs, electrocardiograms (ECGs), complete blood counts (CBCs), chemistry evaluations, coagulation (PT, PTT, INR) evaluations, and urinalyses. All AEs were graded according to National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v. 4.0.[33] Routine laboratory assessments were collected and analyzed at local accredited laboratories within participating centers.

2.6 | Exploratory efficacy assessments

Bone marrow aspirates and/or biopsies were required for determination of AML disease response at each scheduled assessment (days 15 and 29 of induction cycles; day 21 of consolidation cycles). Bone marrow biopsies could be omitted at discretion of the investigator if aspirate was considered adequate. Use of a bone marrow evaluation performed prior to informed consent for diagnosis as baseline evaluation was permitted. Response was determined with consideration of hematology labs, bone marrow evaluation, transfusion status, and cytogenetics if appropriate and was based on adaptation of published criteria.[34,35] CRc rate was the primary efficacy variable. Assessment of minimal residual disease was not required.

2.7 | PK assessments

PK analyses were performed for patients with at least 1 measurable study drug concentration and exact date and time of blood sample

TABLE 1 Baseline patient characteristics

	60 mg/7-day (DL1) N = 6	60 mg/14-day (DL2) N = 7[a]	40 mg/14-day (DL-1) N = 6	TOTAL N = 19 n (%)
Age, median (min, max)	48.5 (25, 59)	43.0 (24, 59)	36.0 (22, 60)	43.0 (22, 60)
Sex				
Male	3	3	2	8 (42)
Female	3	4	4	11 (58)
Median time since diagnosis, days (min, max)	6.5 (1, 19)	4.0 (3, 7)	5.0 (2, 11)	5.0 (1, 19)
ECOG performance status				
0	2	1	3	6 (32)
1	3	4	3	10 (53)
2	1	2	0	3 (16)
Cytogenetic risk[b]				
Favorable	0	0	1	1 (5)
Intermediate	2	3	0	5 (26)
Unfavorable	1	0	0	1 (5)
Unknown	3	3	5	11 (58)
NPM1 mutation				
Yes	0	1	0	1 (5)
No	6	6	6	18 (95)
CEBPA mutation				
Yes	0	0	0	0
No	6	7	6	19 (100)
FLT3-ITD mutation				
Yes	3	4	2	9 (47)
No	3	3	4	10 (53)
AML-MRC				
Yes	0	1	1	2 (11)
No	6	6	5	17 (89)

AML, acute myeloid leukemia; CEBPA, CCAAT/enhancer binding protein alpha; DL, dose level; ECOG, Eastern Cooperative Oncology Group; FLT3, feline McDonough sarcoma (Fms)-like tyrosine kinase 3; ITD, internal tandem duplication; max, maximum; min, minimum; MRC, myelodysplastic-related changes; NPM1, nucleophosmin 1.
[a]One patient enrolled in the DL2 cohort discontinued on day 3 before receiving quizartinib.
[b]Cytogenetic risk was only collected locally. One patient in the DL2 cohort is not included.

collection and study drug dosing. PK parameters included area under the curve (AUC), maximum concentration (C_{max}), trough concentration (C_{trough}), and time to peak concentration (T_{max}).

2.8 | Statistical analyses

Planned study enrollment was up to 52 patients, with up to 18 patients in part 1 and up to 34 patients in part 2, allowing for treatment of minimum 20 patients at the MTD. Demographic information was summarized using descriptive statistics. All data processing, summarization, and analyses were performed using SAS® Version 9.2.

Safety analyses consisted of data summaries for AEs, DLTs, physical examinations, vital signs, ECGs, complete blood counts, chemistry evaluations, coagulation evaluations, and urinalyses and were conducted on all patients receiving at least 1 dose of any study drug. Treatment-emergent AEs were coded to system organ class and preferred term using MedDRA terminology. Number and percent of patients experiencing 1 or more AE(s), relationship to study drug and severity of AEs were summarized by cohort. AEs leading to permanent

discontinuation of study drug, and SAEs were summarized by NCI-CTCAE grade and relationship to study drug. Changes in laboratory values, including hematology, urinalysis, serum chemistry, and coagulation (PT, PTT, INR), vital signs, and ECG parameters were summarized by cohort using descriptive statistics.

Efficacy data analyses were conducted on all patients who received at least 1 dose of any study drug and had at least 1 postbaseline efficacy measurement. Response was assessed at each visit, and best overall response was assigned at end of study by the investigator. Response rate was estimated as number of responders divided by number of patients in the analysis population. Patients with unknown, missing, or no information on response at end of study were treated as nonresponders. Number and percent of patients were summarized by category. 95% CIs were produced for best overall response rate based on Fisher's exact method.

Plasma concentrations and PK parameters were summarized by cohort using descriptive statistics, including number of patients, mean, standard deviation, minimum, median, maximum, geometric mean, and coefficient of variation (CV) of the mean and geometric mean. PK data

TABLE 2 Most common (≥20%) treatment-emergent adverse events by dose cohort

Preferred Term	60 mg/7-day (DL1) N = 6 n (%)	60 mg/14-day (DL2) N = 7[a] n (%)	40 mg/14-day (DL-1) N = 6 n (%)	TOTAL N = 19 n (%)
Nausea	5 (83)	6 (86)	4 (67)	15 (79)
Diarrhea	3 (50)	4 (57)	5 (83)	12 (63)
Constipation	3 (50)	5 (71)	3 (50)	11 (58)
Hypokalemia	4 (67)	4 (57)	2 (33)	10 (53)
Hypomagnesemia	3 (50)	5 (71)	2 (33)	10 (53)
Neutropenia	5 (83)	3 (43)	2 (33)	10 (53)
Febrile neutropenia	3 (50)	3 (43)	3 (50)	9 (47)
Vomiting	4 (67)	3 (43)	2 (33)	9 (47)
Fatigue	2 (33)	3 (43)	2 (33)	7 (37)
Headache	2 (33)	3 (43)	2 (33)	7 (37)
Hypophosphatemia	4 (67)	3 (43)	0	7 (37)
Hypotension	2 (33)	2 (29)	3 (50)	7 (37)
Pyrexia	3 (50)	2 (29)	2 (33)	7 (37)
Rash	2 (33)	2 (29)	3 (50)	7 (37)
Anemia	3 (50)	2 (29)	1 (17)	6 (32)
Anxiety	2 (33)	3 (43)	1 (17)	6 (32)
Hemorrhoids	2 (33)	1 (14)	3 (50)	6 (32)
Hypocalcemia	3 (50)	1 (14)	2 (33)	6 (32)
Thrombocytopenia	4 (67)	1 (14)	1 (17)	6 (32)
Abdominal pain	2 (33)	1 (14)	2 (33)	5 (26)
Drug eruption	1 (17)	4 (57)	0	5 (26)
Dyspepsia	2 (33)	2 (29)	1 (17)	5 (26)
Dyspnea	2 (33)	3 (43)	0	5 (26)
Hypertension	4 (67)	1 (14)	0	5 (26)
Hypoalbuminemia	1 (17)	2 (29)	1 (17)	4 (21)
Mucosal inflammation	2 (33)	2 (29)	0	4 (21)
Edema, peripheral	2 (33)	0	2 (33)	4 (21)

DL, dose level.

[a]One patient enrolled in the DL2 cohort discontinued on day 3 before receiving quizartinib.

for quizartinib and AC886 were summarized for cohorts with sufficient data available on day 4 or 10.

3 | RESULTS

3.1 | Patient demographics

A total of 19 patients were enrolled between November 2011 and July 2013. A CONSORT diagram is included as Supporting Information Figure S1. Six patients were treated with quizartinib DL1, 7 with DL2, and 6 with DL-1. Eighteen patients received treatment with quizartinib. One patient assigned to DL2 had a serious AE (SAE; cerebrovascular

accident) on day 1 and was discontinued from study on day 3 before receiving any quizartinib. Patient baseline characteristics were generally similar between the dose levels (Table 1). Median age was 43 years. Sixteen patients (84%) had ECOG performance status of 0 or 1. Nine patients (47%) were positive for FLT3-ITD mutation, defined as FLT3-ITD/WT FLT3 allelic ratio ≥10% (3 at DL1, 4 at DL2, and 2 at DL-1).

3.2 | Treatment exposure and patient disposition

Ten patients (53%) completed treatment, defined as having completed consolidation therapy, having responded to treatment and proceeded to HSCT, or having continued to maintenance therapy (Supporting

Information Figure). Two patients treated at DL2 ended treatment due to study drug–related AEs: 1 patient had grade 3 palmar-plantar erythrodys-esthesia syndrome on day 23 and 1 patient had grade 3 nausea and grade 3 pericarditis, both classified as SAEs, starting on day 7. One patient ended treatment due to fatal cardiac arrest not considered to be related to study drug on day 53. No patients had dose reductions of quizartinib. Median duration of quizartinib induction therapy was 14.0 days (7.0 days for DL1, 16.5 days for DL2, and 14.0 days for DL-1). Six patients had a second cycle of induction chemotherapy plus quizartinib (2 at DL1, 3 at DL2, and 1 at DL-1). Nine patients (47%) received consolidation therapy (3 at DL1, 2 at DL2, and 4 at DL-1). Median duration of quizartinib consolidation therapy was 14.0 days (7.0 days for DL1, 20.5 days for DL2, and 14.0 days for DL-1). Nine patients (47%) proceeded to HSCT. One patient (5%) treated at DL1 completed 3 cycles of consolidation therapy on day 121 and continued to maintenance therapy with quizartinib through day 497.

Duration of cytarabine was generally similar across cohorts during induction and consolidation therapy. Patients in the DL-1 cohort received cytarabine for an average of 6 days vs 9 days for the DL1 cohort and 10 days for the DL2 cohort. Patients in the DL2 cohort received slightly higher doses of cytarabine than those in DL1 and DL-1 cohorts only during cycle 1 of consolidation therapy. One patient treated at DL-1 had cytarabine dose interrupted due to oropharyngeal pain. Duration and doses of daunorubicin during induction therapy were generally similar across cohorts. No patients had dose reductions of daunorubicin during the study. One patient treated at DL-1 had had interruption of daunorubicin and cytarabine due to oropharyngeal pain.

3.3 | Safety

A total of 3 patients had reports of DLTs. No DLTs were observed at DL1, which led to dose escalation to DL2 cohort where DLTs were observed in 2 of 6 patients (1 patient with grade 4 pericardial effusion [not study drug related]; 1 patient with febrile neutropenia [possibly study drug related], platelet count decreased, and grade 3 QT prolongation). Therefore, dose was reduced to DL-1 where only 1 patient had a DLT (grade 3 pericardi-tis). DL-1 was identified as the MTD. The most common treatment-emergent AEs (all grades) reported included nausea (79%), diarrhea (63%), constipation (58%), hypokalemia (53%), hypomagnesemia (53%), neutro-penia (53%), febrile neutropenia (47%), and vomiting (47%) (Table 2).

A total of 11 cardiac AEs were reported in a total of 5 patients. Five cardiac events (45%) were considered related to study drug. Of these 5 drug-related cardiac events, grade ≥3 events were in 2 patients (DLT of grade 3 pericarditis and grade 4 cardiac tamponade). No car-diac AEs were reported in patients undergoing consolidation therapy but ECG QT prolongation was reported in 1 patient treated at MTD.

Seventy-nine percent of patients experienced grade ≥3 AEs. The most common grade 3/4 AEs were predominantly hematologic, and included febrile neutropenia (47%), neutropenia (42%), thrombocytopenia (32%), anemia (26%), and leukopenia (16%) (Table 3). Median time to neu-trophil recovery for patients with serious neutropenia was 42.5 days for DL1, 52.0 days for DL2, and 50.0 days for DL-1. Median time to platelet recovery for patients with serious thrombocytopenia was 24.5 days for DL1, 19.0 days for DL2, and 17.0 days for DL-1. Nonhematologic grade

TABLE 3 Treatment-emergent grade ≥3 adverse events (≥10%)

| Preferred Term[b] | Maximum CTCAE Grade[a] | | | TOTAL N = 19 n (%) |
	Grade 3 n (%)	Grade 4 n (%)	Grade 5 n (%)	
Overall	5 (26)	9 (47)	1 (5)[c]	15 (79)
Febrile neutropenia	9 (47)	0	0	9 (47)
Neutropenia	0	8 (42)	0	8 (42)
Thrombocytopenia	0	6 (32)	0	6 (32)
Anemia	5 (26)	0	0	5 (26)
Hypophosphatemia	4 (21)	0	0	4 (21)
Leukopenia	0	3 (16)	0	3 (16)
Nausea	2 (11)	0	0	2 (11)
Esophagitis	2 (11)	0	0	2 (11)
Drug eruption	2 (11)	0	0	2 (11)
Decreased appetite	2 (11)	0	0	2 (11)
Hypotension	2 (11)	0	0	2 (11)

AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events; DL, dose level.
[a]CTCAE grades: 3 = severe; 4 = life-threatening; 5 = death.
[b]At the preferred term level, patients reporting more than 1 AE were counted only once using the highest severity.
[c]One grade 5 treatment-emergent AE was reported at DL2 (cardiac arrest). The AE was not considered to be related to study drug.

3/4 AEs reported in ≥10% of patients included hypophosphatemia, nau-sea, esophagitis, decreased appetite, drug eruption, and hypotension. Twelve patients (63%) experienced a maximum change in QTcF interval >30 ms and two patients (11%) had change >60 ms, both treated at DL2. Nine patients (47%) had QTcF interval >450 ms. Two patients (11%) experienced grade 3 QTcF prolongation; both were treated at DL2 and symptoms resolved with dose interruption. There were no cases of grade 2/3 QTcF prolongation in patients treated at DL1 and DL-1, and no cases of grade 4 QTcF prolongation reported at any dose tested.

3.4 | Exploratory efficacy

Overall, 16 patients (84%) achieved a response to therapy (Table 4). Fourteen patients (74%) achieved CRc (9 CR, 2 CRp, 3 CRi) and 2 patients (11%) were leukemia-free based on morphologic criteria. No recurrences or treatment failures were reported while on study. Four of 6 patients (67%) treated at the MTD of DL-1 had complete remis-sions. Five patients (26%) required a second induction cycle to achieve a response. Six of 9 patients with FLT3-ITD–mutations (67%) achieved CRc and 2 (22%) achieved MLFS. Eight of 10 patients (80%) with no FLT3-ITD–mutation achieved CRc.

3.5 | Pharmacokinetics

The analyses of PK was limited by the small number of patients enrolled and the limited availability of PK samples. Results of the PK analyses are presented in the Supporting Information content.

TABLE 4 Best overall response (intent-to-treat analysis set)

	60 mg/7-day (DL1) N = 6 n (%)	60 mg/14-day (DL2) N = 7[a] n (%)	40 mg/14-day (DL-1) N = 6 n (%)	TOTAL N = 19 n (%)
Best Overall Response				
Morphologic leukemia-free state[b]	1 (17)	1 (14)	0	2 (11)
CRc[c]	5 (83)	5 (71)	4 (67)	14 (74)
CR[d]	4 (67)	3 (43)	2 (33)	9 (47)
CRp[e]	1 (17)	0	1 (17)	2 (11)
CRi[f]	0	2 (29)	1 (17)	3 (16)
Treatment failure[g]	0	0	0	0
No response	0	0	1 (17)	1 (5)
Not evaluable	0	1 (14)	1 (17)	2 (11)
Recurrence[h]	0	0	0	0

CR, complete remission; CRc, composite complete remission; CRi, complete remission with incomplete hematologic recovery; CRp, complete remission with incomplete platelet recovery; DL, dose level.

[a]One patient enrolled in the DL2 cohort discontinued on day 3 before receiving quizartinib.

[b]Defined as <5% bone marrow blasts in an aspirate sample with marrow spicules and with a count of ≥200 nucleated cells, no blasts with Auer rods, and no persistence of extramedullary disease.

[c]Defined as rates of CR + CRp + CRi.

[d]Defined as morphologic leukemia-free state and absolute neutrophil count ≥1000/mm^3, platelet count ≥100,000/mm^3, patient independent of transfusions.

[e]Defined as CR except for platelet count <100,000/mm^3.

[f]Defined as CR except for absolute neutrophil count <1000/mm^3 with or without platelet count <100,000/mm^3. Transfusion independence not required.

[g]Defined as failure to achieve a CR, CRp, or CRi.

[h]Defined as relapse after CR, new dysplastic changes, reappearance or development of cytologically proven extramedullary disease, or reappearance of cytogenetic or molecular abnormality.

4 | DISCUSSION

Quizartinib is a novel FLT3 inhibitor with greater selectivity and potency compared to other broad-acting multikinase inhibitors,[23,24,36–38] and has demonstrated robust single-agent activity in clinical studies in heavily pretreated patients with R/R AML.[26,28,39–41] The importance of FLT3-ITD mutation as a negative prognostic factor in patients with AML has led to the hypothesis that targeted inhibition of this receptor may improve therapeutic outcomes in these patients. Key goals of this study were to examine safety and tolerability of quizartinib in combination with standard 7 + 3 cytarabine/daunorubicin-based induction and HiDAC consolidation chemotherapy in patients with ndAML and identification of an MTD for future clinical studies.

Based on our findings, the DL-1 dose and schedule (40 mg/d of quizartinib for 14 days) was tolerated in combination with intensive induction chemotherapy. DL1 was also tolerated; however, DL-1 was selected for future studies based on it being associated with DLTs in ≤1 out of 6 patients as well as leading to a higher median total dose of quizartinib administered versus DL1. The selection of the 40 mg dose is supported by prior monotherapy/combination studies showing complete inhibition of FLT3 in plasma inhibitory activity assay, in peripheral blood and bone marrow,[40,42] and by pharmacodynamic assays showing complete inhibition of FLT3-ITD signaling with daily 30 mg dosing.[29]

Toxicities associated with quizartinib plus chemotherapy induction were manageable. DLTs were pericardial effusion (grade 4), febrile neutropenia, decreased platelet count, QT prolongation (grade 3), and pericarditis (grade 3). As anticipated, the majority of AEs reported were hematologic. Importantly, no significant additional or unexpected toxicities were observed with the combination regimen. Prolongation of QTcF following administration of quizartinib in combination with or just after standard cytotoxic chemotherapy did not result in serious clinical manifestations. No grade 4 QTcF prolongation was reported and no grade 3 QTcF prolongation was reported at the dose of 40 mg/d for 14 days. Notably, rates of grade ≥2 QTcF prolongation observed in this trial with the combination regimen were consistent with low rates of QTcF prolongation observed in a phase 2b trial in patients with R/R AML treated with quizartinib monotherapy at 30 or 60 mg/d.[39]

A key finding of the study was early evidence of antileukemic activity of quizartinib in combination with standard chemotherapy. Responses were observed in 84% of patients, with a notable CRc rate of 74%, and full hematologic/platelet recovery in 75% of responders. Two-thirds of patients treated at MTD had a CRc. Additionally, no relapses were observed over the course of the study. Forty-seven percent of patients enrolled on trial underwent HSCT. One patient who completed induction and consolidation therapy with the combination regimen proceeded to receive maintenance therapy with single-agent

quizartinib at 60 mg/d through day 497 and was still in CR at completion of 12 cycles of therapy.

An important limitation of this study is the small size of the patient population. Total study enrollment was limited to 19 patients because of early study termination. This resulted in more limited readouts on safety, PK, and preliminary efficacy. However, the study provided strong evidence that quizartinib can be safely administered in combination with SOC induction/consolidation chemotherapy. Additionally, we observed early evidence of activity and potential to elicit strong responses with this combination regimen. This work supports further clinical evaluation of this regimen in patients with ndAML.

The phase 3 RATIFY study demonstrated significantly improved OS in FLT3 mutated ndAML with standard induction/consolidation chemotherapy plus midostaurin vs placebo.[21] However, it is unclear if the benefit of midostaurin in these patients is related to inhibition of FLT3 activity or other actions of the drug. Given that FLT3-ITD is a driver mutation in AML,[4] it is possible that an agent such as quizartinib that has greater potency and selectivity toward FLT3-ITD may have a more pronounced effect on the clinical course of disease. The phase 3 QuANTUM-First trial (NCT02668653) is currently evaluating quizartinib 40 mg vs placebo in combination with SOC induction/consolidation chemotherapy and as maintenance therapy in patients with FLT3-ITD mutated ndAML. Patients enrolled in QuANTUM-First are undergoing cardiac monitoring to gain a better understanding of effects of quizartinib on risk of QTc prolongation as well as overall cardiac effects in a larger patient population. Additionally, the phase 3 QuANTUM-R trial (NCT02039726) compares quizartinib vs salvage chemotherapy in patients with FLT3-ITD–mutated R/R AML. These studies will provide important information regarding clinical benefit of sustained inhibition of ITD-mutated FLT3 AML with a selective and highly potent inhibitor of FLT3.

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REFERENCES

[1] Kiyoi H, Naoe T, Nakano Y, et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. *Blood.* 1999; 93(9):3074–3080.

[2] Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood.* 2001;98(6):1752–1759.

[3] Schnittger S, Schoch C, Dugas M, et al. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood.* 2002;100(1):59–66.

[4] Testa U, Pelosi E. The impact of FLT3 mutations on the development of acute myeloid leukemias. *Leuk Res Treatment.* 2013;2013: 275760. http://dx.doi.org/10.1155/2013/275760.

[5] Thiede C, Steudel C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood.* 2002;99(12):4326–4335.

[6] Whitman SP, Archer KJ, Feng L, et al. Absence of the wild-type allele predicts poor prognosis in adult *de novo* acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of *FLT3*: a cancer and leukemia group B study. *Cancer Res.* 2001;61 (19):7233–7239.

[7] Boissel N, Cayuela JM, Preudhomme C, et al. Prognostic significance of FLT3 internal tandem repeat in patients with *de novo* acute myeloid leukemia treated with reinforced courses of chemotherapy. *Leukemia.* 2002;16(9):1699–1704.

[8] Fey MF, Buske C. on behalf of ESMO Guidelines Working Group. Acute myeloblastic leukaemias in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2013;24(suppl 6):vi138–vi143.

[9] Natonal Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Acute Myeloid Leukemia. Version 3.2017. Available online at: https://www.nccn.org. Accessed November 7, 2017.

[10] Levis M. FLT3 mutations in acute myeloid leukemia: what is the best approach in 2013?. *Hematol Am Soc Hematol Educ Progr.* 2013; 2013:220–226.

[11] Fiedler W, Serve H, Döhner H, et al. A phase 1 study of SU11248 in the treatment of patients with refractory or resistant acute myeloid leukemia (AML) or not amenable to conventional therapy for the disease. *Blood.* 2005;105(3):986–993.

[12] Knapper S, Burnett AK, Littlewood T, et al. A phase 2 trial of the FLT3 inhibitor lestaurtinib (CEP701) as first-line treatment for older patients with acute myeloid leukemia not considered fit for intensive chemotherapy. *Blood.* 2006;108(10):3262–3270.

[13] O'Farrell AM, Foran JM, Fiedler W, et al. An innovative phase I clinical study demonstrates inhibition of FLT3 phosphorylation by SU11248 in acute myeloid leukemia patients. *Clin Cancer Res.* 2003; 9(15):5465–5476.

[14] Smith BD, Levis M, Beran M, et al. Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with

relapsed or refractory acute myeloid leukemia. *Blood.* 2004;103(10): 3669–3676.

[15] Fischer T, Stone RM, DeAngelo DJ, et al. Phase IIB trial of oral midostaurin (PKC412), the FMS-like tyrosine kinase 3 receptor (FLT3) and multi-targeted kinase inhibitor, in patients with acute myeloid leukemia and high-risk myelodysplastic syndrome with either wild-type or mutated *FLT3. J Clin Oncol.* 2010;28(28):4339–4345.

[16] Stone RM, DeAngelo DJ, Klimek V, et al. Patients with acute myeloid leukemia and an activating mutation in *FLT3* respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412. *Blood.* 2005;105(1):54–60.

[17] Cooper BW, Kindwall-Keller TL, Craig MD, et al. A phase I study of midostaurin and azacitidine in relapsed and elderly AML patients. *Clin Lymphoma Myeloma Leuk.* 2015;15(7):428–432.e2.

[18] Stone RM, Fischer T, Paquette R, et al. Phase IB study of the FLT3 kinase inhibitor midostaurin with chemotherapy in younger newly diagnosed adult patients with acute myeloid leukemia. *Leukemia.* 2012;26(9):2061–2068.

[19] Strati P, Kantarjian H, Ravandi F, et al. Phase I/II trial of the combination of midostaurin (PKC412) and 5-azacytidine for patients with acute myeloid leukemia and myelodysplastic syndrome. *Am J Hematol.* 2015;90(4):276–281.

[20] Williams CB, Kambhampati S, Fiskus W, et al. Preclinical and phase I results of decitabine in combination with midostaurin (PKC412) for newly diagnosed elderly or relapsed/refractory adult patients with acute myeloid leukemia. *Pharmacotherapy.* 2013;33(12):1341–1352.

[21] Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a *FLT3* mutation. *N Engl J Med.* 2017;377(5):454–464.

[22] Pratz KW, Levis MJ. Bench to bedside targeting of FLT3 in acute leukemia. *Curr Drug Targets.* 2010;11(7):781–789.

[23] Chao Q, Sprankle KG, Grotzfeld RM, et al. Identification of *N*-(5-*tert*-butyl-isoxazol-3-yl)-*N'*-{4-[7-(2-morpholin-4-yl-ethoxy)imidazo [2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea dihydrochloride (AC220), a uniquely potent, selective, and efficacious FMS-like tyrosine kinase-3 (FLT3) inhibitor. *J Med Chem.* 2009;52(23):7808–7816.

[24] Zarrinkar PP, Gunawardane RN, Cramer MD, et al. AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML). *Blood.* 2009;114(14):2984–2992.

[25] Zorn JA, Wang Q, Fujimura E, Barros T, Kuriyan J. Crystal structure of the FLT3 kinase domain bound to the inhibitor quizartinib (AC220). *PLoS One.* 2015;10(4):e0121177. doi:10.1371/journal.pone.0121177.

[26] Cortes JE, Perl AE, Dombret H, et al. Response rate and bridging to hematopoietic stem cell transplantation (HSCT) with quizartinib (AC220) in patients with FLT3-ITD positive or negative relapsed/refractory AML after second-line chemotherapy or previous bone marrow transplant. *J Clin Oncol.* 2013;31(suppl):7012.

[27] Döhner H, Perl A, Rousselot P, et al. Efficacy and safety of quizartinib (AC220) in patients age ≥60 years with FLT3-ITD–positive relapsed/refractory acute myeloid leukemia (AML). *Haematologica.* 2013;98(suppl 1):233.

[28] Levis M, Martinelli G, Perl AE, et al. The benefit of treatment with quizartinib and subsequent bridging to HSCT for FLT3-ITD(+) patients with AML. *J Clin Oncol.* 2014;32(5)(suppl):7093.

[29] Levis MJ, Cortes JE, Gammon GM, et al. Laboratory and clinical investigations to identify the optimal dosing strategy for quizartinib (AC220) monotherapy in FLT3-ITD-positive (+) relapsed/refractory (R/R) acute myeloid leukemia (AML). *Blood.* 2016;128(22):4042.

[30] Russel N, Tallman MS, Goldberg S, et al. Quizartinib (AC220) in patients with FLT3-ITD(+) relapsed or refractory acute myeloid leukemia: final results of a randomized phase 2 study. *Haematologica.* 2014;99(suppl 1):36.

[31] Schiller GJ, Tallman MS, Goldberg SL, et al. Final results of a randomized phase 2 study showing the clinical benefit of quizartinib (AC220) in patients with FLT3-ITD positive relapsed or refractory acute myeloid leukemia. *J Clin Oncol.* 2014;32(5)(suppl):7100.

[32] Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* 4th ed. Lyon, France: IARC/WHO Press; 2008.

[33] National Institutes of Health. Cancer Therapy Evaluation Program (CTEP): Common Terminology Criteria for Adverse Events (CTCAE) v4.0. Available online at: https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf. Accessed November 7, 2017.

[34] Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. *J Clin Oncol.* 2003;21(24):4642–4649.

[35] Sievers EL, Larson RA, Stadtmauer EA, for Mylotarg Study Group, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J Clin Oncol.* 2001;19(13):3244–3254.

[36] Gunawardane RN, Nepomuceno RR, Rooks AM, et al. Transient exposure to quizartinib mediates sustained inhibition of FLT3 signaling while specifically inducing apoptosis in FLT3-activated leukemia cells. *Mol Cancer Ther.* 2013;12(4):438–447.

[37] Levis M. Quizartinib in acute myeloid leukemia. *Clin Adv Hematol Oncol.* 2013;11(9):586–588.

[38] Wander SA, Levis MJ, Fathi AT. The evolving role of FLT3 inhibitors in acute myeloid leukemia: quizartinib and beyond. *Ther Adv Hematol.* 2014;5(3):65–77.

[39] Cortes J, Tallman MS, Schiller G, et al. Results of a phase 2 randomized, open-label, study of lower doses of quizartinib (AC220; ASP2689) in subjects with FLT3-ITD positive relapsed or refractory acute myeloid leukemia (AML). *Blood.* 2013;122(21):494.

[40] Cortes JE, Kantarjian H, Foran JM, et al. Phase I study of quizartinib administered daily to patients with relapsed or refractory acute myeloid leukemia irrespective of FMS-like tyrosine kinase 3–internal tandem duplication status. *J Clin Oncol.* 2013;31(29):3681–3687.

[41] Cortes JE, Perl AE, Dombret H, et al. Final results of a phase 2 open-label, monotherapy efficacy and safety study of quizartinib (AC220) in patients ≥ 60 years of age with FLT3 ITD positive or negative relapsed/refractory acute myeloid leukemia. *Blood.* 2012; 120(21):48.

[42] Burnett AK, Bowen D, Russell N, et al. AC220 (quizartinib) can be safely combined with conventional chemotherapy in older patients with newly diagnosed acute myeloid leukaemia: experience from the AML18 pilot trial. *Blood.* 2013;122(21):622.

SUPPORTING INFORMATION

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