ORIGINAL ARTICLE

Effects of CYP3A inhibitors on the pharmacokinetics of quizartinib, a potent and selective FLT3 inhibitor, and its active metabolite

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Aims: Quizartinib is an oral, highly potent and selective next-generation FMS-like tyrosine kinase 3 (FLT3) inhibitor under investigation in patients with *FLT3*-internal tandem duplication-mutated acute myeloid leukaemia. This drug–drug interaction study assessed the pharmacokinetics (PK) of quizartinib when coadministered with strong or moderate cytochrome P450 3A (CYP3A) inhibitors.

Methods: In this parallel-group study, subjects were randomised to receive: (i) quizartinib + ketoconazole; (ii) quizartinib + fluconazole; or (iii) quizartinib alone. On Days 1–28, subjects received ketoconazole 200 mg or fluconazole 200 mg twice daily, and on Day 8, all subjects received a single 30-mg quizartinib dose. Blood samples were collected for PK analyses, steady-state PK parameters were simulated by superpositioning, and safety was assessed.

Results: Ninety-three healthy subjects were randomised; 86 completed the study. When administered with ketoconazole, geometric mean ratios (90% confidence interval) for quizartinib maximum observed plasma concentration (C_{max}) and area under the plasma concentration–time curve (AUC) from time 0 extrapolated to infinity were 117% (105%, 130%) and 194% (169%, 223%), respectively, *vs* quizartinib alone. Steady-state PK simulation demonstrated ~2-fold increase of both steady-state C_{max} and AUC from time 0 to the end of the dosing interval when quizartinib was administered with ketoconazole due to accumulation of quizartinib at steady state. When administered with fluconazole, geometric mean ratios (90% confidence interval) for quizartinib C_{max} and AUC from time 0 extrapolated to infinity were 111% (100%, 124%) and 120% (104%, 138%), respectively, *vs* quizartinib alone. Overall, 5.4% of subjects experienced quizartinib-related adverse events; no serious adverse events or deaths occurred.

Conclusions: These results suggest reducing the dose of quizartinib when coadministered with a strong CYP3A inhibitor, but not with a moderate or weak

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CYP3A inhibitor. This dose reduction was implemented in phase 3 evaluation of quizartinib.

1 | INTRODUCTION

Acute myeloid leukaemia (AML) is a heterogeneous disease characterised by clonal evolution with poor prognosis and limited treatment options for patients.[1-3] The most common driver mutations in AML include mutations in FMS-like tyrosine kinase 3 (*FLT3*; primarily internal tandem duplication [ITD]), and are reported in ~25% of patients with newly diagnosed disease.[4] Patients with *FLT3*-ITD mutated AML have a particularly poor prognosis (i.e. increased risk of relapse and shorter overall survival) following standard-of-care chemotherapy,[3,4] making FLT3 an attractive therapeutic target. Quizartinib is an orally administered, highly potent and selective next-generation FLT3 inhibitor in phase 3 development in patients with *FLT3*-ITD mutated AML (QuANTUM-R: NCT02039726; QuANTUM-First: NCT02668653). In phase 2 studies, quizartinib treatment resulted in a composite complete remission of disease in patients with relapsed or refractory *FLT3*-ITD mutated AML (composite complete remission rates of 44% to 47%) and successfully bridged 34% to 42% to potentially curative hematopoietic stem cell transplantation.[5,6] Quizartinib is generally well tolerated and is known to be associated with QT interval prolongation (corrected according to Fridericia's formula; QTcF),[7,8] which was dependent on quizartinib plasma concentrations in modelling analyses.[6,7]

Previous pharmacokinetic (PK) studies indicate that quizartinib demonstrates dose-proportional increases in exposure, is extensively metabolised, is predominantly eliminated in faeces, has a median effective half-life of 73 hours (data on file, Daiichi Sankyo, Inc), and accumulates approximately 5-fold (data on file, Daiichi Sankyo, Inc) after continuous once-daily dosing.[9] Quizartinib is primarily metabolised by cytochrome P450 3A (CYP3A), and 1 of the metabolites is the mono-oxidative product AC886, which is biologically active. AC886 is also the only other major species, besides quizartinib, detected in plasma,[9] with a relative metabolite-to-parent ratio of approximately 0.6 following repeated daily dosing in a phase 2 trial of quizartinib in patients with AML (data on file, Daiichi Sankyo, Inc). AC886 is also a substrate for CYP3A and can be further metabolised by the enzyme (data on file, Daiichi Sankyo, Inc).

Patients with AML are at high risk for invasive fungal infections,[10,11] and some antifungal agents, such as azoles, that are used to prevent and treat invasive fungal infections in patients with AML are known to inhibit CYP3A.[11,12] Because quizartinib is metabolised by CYP3A enzymes, coadministration of such therapies could alter overall exposure to quizartinib. Therefore, understanding the effect of drugs that inhibit CYP3A on the PK of quizartinib and AC886 is important for clinically safe and effective use of quizartinib. This

What is already known about this subject

- Quizartinib dihydrochloride is an oral selective next-generation FMS-like tyrosine kinase 3 (FLT3) inhibitor under investigation for *FLT3*-internal tandem duplication mutated acute myeloid leukaemia.

- Quizartinib is metabolised by cytochrome P450 3A (CYP3A); understanding the effect of antifungal drugs that inhibit CYP3A enzymes on the pharmacokinetics of quizartinib and its active metabolite, AC886, is important to guide dose adjustments to ensure safety and clinical efficacy.

What this study adds

- The results of this study provide information regarding dose adjustments for quizartinib in combination with CYP3A inhibitors for clinically safe and effective use of quizartinib.

- When administered with a strong CYP3A inhibitor, reducing quizartinib doses from 30 to 20 mg or from 60 to 30 mg is recommended.

- No dose adjustment is needed when quizartinib is coadministered with a moderate or weak CYP3A inhibitor.

drug–drug interaction study was performed to provide dosing guidance for quizartinib when it is coadministered with strong or moderate CYP3A inhibitors.

2 | METHODS

2.1 | Study design

This open-label, randomised, parallel-group (3 treatment groups) study was conducted at 2 sites in the USA (Madison, WI [principal investigator, C. Hale] and Overland Park, KS [principal investigator, M. Kankam]) from February to May 2013 to evaluate the effect of CYP3A inhibition by ketoconazole, a strong inhibitor, or fluconazole, a moderate inhibitor, on the PK of quizartinib and its active metabolite, AC886, in healthy subjects. The tolerability and safety of quizartinib when coadministered with ketoconazole or fluconazole were also assessed. This study was performed prior to the October 2013 US

Food and Drug Administration advisory recommending against the use of ketoconazole as a strong CYP3A inhibitor in drug–drug interaction studies due to potential liver injury and adrenal gland toxicity. A parallel-group design was selected based on safety considerations associated with multiple dosing of quizartinib in healthy volunteers and the long effective half-life of quizartinib (73 hours). The study was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonisation/Good Clinical Practice (ICH/GCP), as well as all applicable state, local and federal regulations. The study protocol, amendments and informed consent forms were reviewed and approved by the institutional review board at each site. All subjects provided written informed consent before any study-related procedure took place. A schematic of the study design, including timeline of treatments and sample collection, is shown in Figure 1.

2.2 | Eligibility

Healthy males and females, aged 18–55 years, were eligible for enrolment into the study. Key inclusion criteria were body mass index of 18–32 kg/m^2; serum potassium, magnesium and calcium within normal limits; adequate hepatic and coagulation parameters; and adequate renal function, as defined by serum creatinine $\leq 1.5 \times$ upper limit of normal and estimated creatinine clearance at screening ≥ 80 mL/min according to the Cockcroft–Gault equation. Main exclusion criteria were history of clinically significant disease, abnormality or drug allergy; treatment with any investigational product in a clinical study within 30 days (or 5 drug-half-lives, whichever is longer); and use or anticipated use of prescription medications including hormonal contraceptives, over-the-counter medications, herbal products or dietary supplements.

2.3 | Randomisation and treatments

Subjects were randomised at a ratio of 1:1:1 to 1 of 3 treatment arms: ketoconazole + quizartinib arm, fluconazole + quizartinib arm, or quizartinib arm as shown in Figure 1. On Days 1 through 28, each subject randomised into the ketoconazole + quizartinib arm received 200 mg oral ketoconazole (Teva Generics, Sellersville, PA, USA) twice

daily, and each subject randomised into the fluconazole + quizartinib arm received 200 mg oral fluconazole (Teva Generics, Sellersville, PA, USA) twice daily. Ketoconazole and fluconazole were administered for 8 days prior to quizartinib to ensure maximum inhibition of CYP3A.[13,14] On Day 8, all subjects received a single oral dose of quizartinib (administered as 30 mg quizartinib dihydrochloride, equivalent to 26.5 mg quizartinib in free-base form), as a tablet with 240 mL water, in the morning, following a 10-hour fast; subjects continued to fast for 4 hours after dosing. The 30-mg dose of quizartinib is a clinically relevant dose that was chosen because it would not be expected to result in clinically significant QT prolongation even in the presence of a drug–drug interaction that substantially increases exposure to quizartinib.[7,8]

Patients were admitted to the study site on Day −1 and were discharged after study procedures on Day 1 with study medication to take on an outpatient basis; patients were readmitted to the study site on Day 5 until discharge on Day 17. On Days 18 to 28, patients took study medications on an outpatient basis, with visits to study site for collection of blood samples and adverse event (AE) assessments, returning for the final visit on Day 29.

2.4 | Sample collection and analytic methodology

Blood samples for measurement of plasma quizartinib and AC886 concentrations were collected from all subjects before quizartinib dosing on Day 8 and at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 288, 360, 432 and 504 hours after quizartinib dihydrochloride administration. Plasma concentrations of quizartinib and AC886 were measured by BASi (West Lafayette, IN, USA) using a validated liquid chromatography–tandem mass spectrometry method. The analytical range validated was from 2.00 to 2000 ng/mL for both quizartinib and AC886. Selectivity towards 6 different batches of plasma was proved. Interference of 14 different compounds, including ketoconazole and fluconazole, was evaluated and no interference from these compounds was observed. For quizartinib at the lower limit of quantitation (LLOQ), precision was 5.1 (% coefficient of variation [CV]) and accuracy ranged from 101.5 to 98.5%. For quizartinib at the upper limit of quantitation, precision
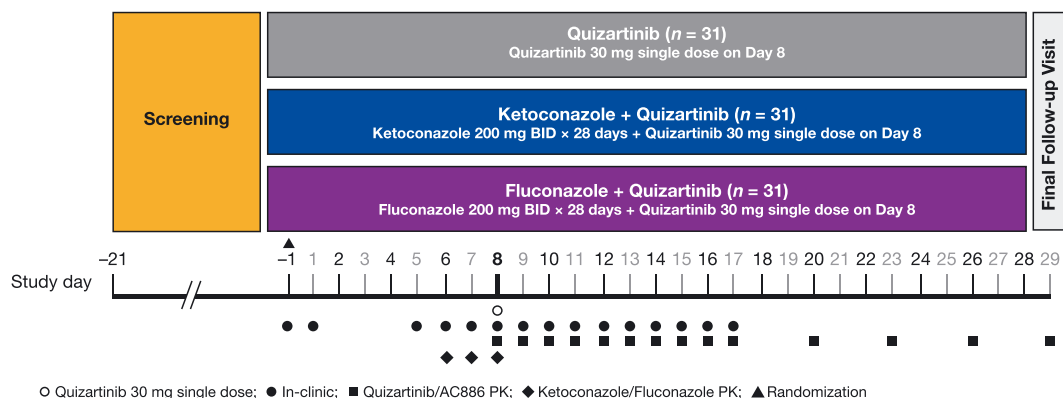

FIGURE 1 Study design. BID, twice daily; PK, pharmacokinetics

was 3.1 (%CV) and accuracy ranged between 106.9 and 93.1%. For AC886 at the LLOQ, precision was 7.6 (%CV) and accuracy ranged from 101.0 to 99.0%. For AC886 at the upper limit of quantitation, precision was 4.3 (%CV) and accuracy ranged between 106.1 and 93.9% between runs.

To confirm steady-state concentrations of ketoconazole and fluconazole, blood samples were collected before the morning doses on Days 6–8. Blood samples were also collected on Day 8 after the morning doses: at 2 and 12 hours after the morning dose for ketoconazole, and at 3 and 12 hours after the morning dose for fluconazole. Plasma concentrations of ketoconazole and fluconazole were measured by BASi using a validated liquid chromatography–tandem mass spectrometry method. The analytical range validated was from 10.0 to 10 000 ng/mL for both ketoconazole and fluconazole. The LLOQ validated was 10.0 ng/mL. Selectivity towards 6 different batches of plasma was proved. Interference of quizartinib and AC886 (at 500 and 100 ng/mL, respectively) was evaluated, and no interference from these compounds was observed. For ketoconazole at the LLOQ, precision was 8.6 (%CV) and accuracy ranged from 110.2 to 89.8%. For ketoconazole at 8000 ng/mL, precision was 2.3 (%CV) and accuracy ranged between 103.9 and 96.1%. For fluconazole at the LLOQ, precision was 9.5 (%CV) and accuracy ranged from 107.1 to 93.0%. For fluconazole at the upper limit of quantitation, precision was 1.9 (%CV) and accuracy ranged between 102.4 and 97.6% between runs.

2.5 | Safety

All randomised subjects who received quizartinib or at least 1 dose of CYP3A inhibitor were included in the safety analysis population. Safety was assessed with physical examinations, vital signs, 12-lead electrocardiograms, AE evaluations, and clinical laboratory tests. Haematology, chemistry and urinalysis determinations were performed by a local laboratory. AEs were evaluated during the study according to National Cancer Institute Common Terminology Criteria for Adverse Events v4.03 and assessed for severity, relation to study drugs and clinical significance.

2.6 | PK analyses

The PK analysis population consisted of all subjects who received the quizartinib dose and had evaluable maximum observed plasma concentration (C_{max}) and area under the plasma concentration–time curve from time 0 to the last quantifiable plasma concentration (AUC_{last}) or from time 0 extrapolated to infinity (AUC_{inf}) for quizartinib or AC886. For ketoconazole and fluconazole, trough concentrations on Days 6, 7 and 8 allowed for assessment of achieving steady-state; together with concentrations around time to C_{max} (T_{max}) on Day 8, they also allowed for assessment of consistency with published data. For quizartinib, time points on day 1 (0, 0.25, 0.5, 1, 2, 3, 4, 5, 6 and 8 hours) were included to capture C_{max}. Time points extending to 48 hours (0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 36 and 48 hours) were included to capture C_{max} for AC886. Blood samples were collected

over 504 hours to capture the distribution and terminal elimination half-lives of quizartinib and AC886 (i.e. over 2 times the half-life, which was anticipated to be extended in the presence of the strong CYP3A inhibitor ketoconazole). PK parameters in plasma, including C_{max}, AUC_{last}, AUC_{inf}, apparent terminal elimination half-life ($T_{1/2}$), T_{max} and apparent systemic clearance (CL/F) were calculated using WinNonlin® Professional version 5.2 (Certara USA, Inc., Princeton, NJ, USA) and standard noncompartmental methods.[15] AUC_{last} was calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations. AUC_{inf} was calculated as $AUC_{last} + C_{last}/\lambda Z$, where C_{last} is the last quantifiable plasma concentration and λZ is the terminal elimination rate constant, which was calculated using least squares (LS) regression of logarithmically transformed data points of plasma concentration in the terminal elimination phase. The actual time of blood sample collection and the free-base equivalent dose of quizartinib dihydrochloride were used for calculation of PK parameters. The sum and ratio for PK parameters were calculated with R v.2.15 (R Foundation for Statistical Computing, Vienna, Austria).

2.7 | Statistics

A sample size of 25 subjects per group was determined to yield ≤10% relative standard error of the mean, based on the observed intersubject CV of approximately 60% for PK parameters in a prior drug–drug interaction study in healthy volunteers (data on file, Daiichi Sankyo, Inc). Descriptive statistics were used to summarise plasma concentrations and PK parameters of the PK analysis population using SAS® software version 9.3 (SAS Institute Inc., Cary, NC, USA). Inferential statistics were used for drug–drug interaction assessment. An analysis of variance (ANOVA) was performed to assess the effect of ketoconazole and fluconazole on PK parameters of quizartinib and AC886. The ANOVA model included treatment as the fixed effect and subject as the random effect. Exposure parameters (C_{max}, AUC_{last}, AUC_{inf}) were natural logarithm (ln)-transformed prior to the analyses in the ANOVA. The ln-transformed PK parameters were used in the ANOVA for calculation of LS means and standard error and LS mean differences between treatments. Geometric LS mean ratios were calculated along with 90% confidence intervals (CI). Absence of a drug–drug interaction was concluded if the 90% CIs for the test vs reference ratios of the geometric LS means were completely contained within the interval between 80 and 125% for AUCs and C_{max}. Safety parameters were summarised in the safety analysis population using descriptive statistics and SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA).

2.8 | Simulation analysis

Given the linear PK of quizartinib, predictions of PK parameters, including steady-state C_{max} ($C_{max,ss}$), AUC from time 0 to the end of the dosing interval (AUC_τ) and T_{max} at steady-state ($T_{max,ss}$), were simulated by superpositioning using Phoenix 6.3 (Certara USA, Inc.,

Princeton, NJ, USA). Statistical analysis and determination of drug–drug interaction for predicted PK parameters, AUC_τ and $C_{max,ss}$, were performed as described above for exposure parameters (AUC_{inf}, AUC_{last}, C_{max}).

2.9 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[16] and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18.[17]

3 | RESULTS

3.1 | Demographics and baseline characteristics

A total of 93 subjects were enrolled in the study, with 31 randomised into each treatment arm; 89 subjects received quizartinib and 86 subjects completed all study procedures (Figure 2). Overall, 7 subjects discontinued from the study: 4 prior to receiving quizartinib and 3 after receiving quizartinib. Of the subjects who withdrew before receiving quizartinib, 2 withdrew consent, 1 discontinued for AE (bacterial vaginitis) and 1 was withdrawn by the sponsor. Of the

3 subjects who discontinued after receiving quizartinib treatment, 1 discontinued for AE (animal bite) and 2 were lost to follow-up. Demographics and baseline characteristics were generally similar between the treatment groups (Table 1).

3.2 | PK results

Mean (± standard deviation) plasma concentrations of ketoconazole were 1.5 ± 0.8, 1.4 ± 0.7 and 1.4 ± 0.8 μg/mL at predose on Days 6, 7 and 8, respectively. Mean (± standard deviation) plasma concentrations of fluconazole were 13.2 ± 4.1, 13.8 ± 3.8 and 14.5 ± 3.7 μg/mL on Days 6, 7 and 8, respectively. The consistency of ketoconazole and fluconazole concentrations over these 3 days shows that ketoconazole and fluconazole had reached steady-state by Day 8, the day when quizartinib was administered.

Plasma concentration–time profiles of quizartinib were well characterised, with median T_{max} occurring 4 hours after dosing in all treatment groups (Figure 3, Table 2). The median T_{max} of AC886 occurred at 48.0 hours and 5.0 hours postdose in the ketoconazole + quizartinib and fluconazole + quizartinib arms, respectively, compared with 5.1 hours postdose in the quizartinib arm (Table S1; Figure S1). The ratio of the geometric LS means of C_{max} of AC886 to the C_{max} of quizartinib was 0.13, 0.04 and 0.12 in the quizartinib, ketoconazole + quizartinib and fluconazole + quizartinib treatment arms, respectively.
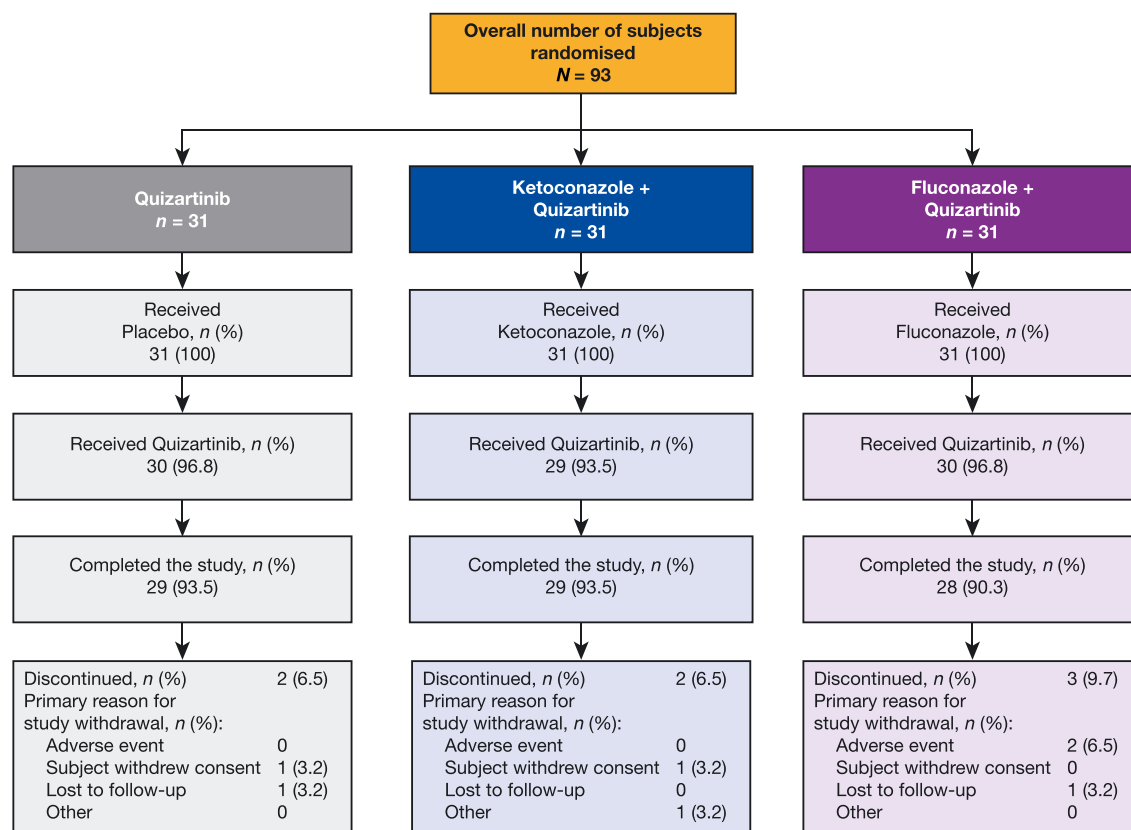

FIGURE 2 CONSORT study flowchart

TABLE 1 Demographics and baseline characteristics of study subjects

	Quizartinib (n = 31)	Ketoconazole + quizartinib (n = 31)	Fluconazole + quizartinib (n = 31)	Overall (n = 93)
Age, y				
Mean (SD)	30.5 (8.1)	34.7 (10.1)	36.8 (8.7)	34.0 (9.3)
Sex, n (%)				
Female	8 (25.8)	7 (22.6)	8 (25.8)	23 (24.7)
Male	23 (74.2)	24 (77.4)	23 (74.2)	70 (75.3)
Race, n (%)				
White	16 (51.6)	19 (61.3)	23 (74.2)	58 (62.4)
Black or African American	14 (45.2)	10 (32.3)	6 (19.4)	30 (32.3)
Asian	0	0	1 (3.2)	1 (1.1)
Other[a]	1 (3.2)	2 (6.5)	1 (3.2)	4 (4.3)
Weight, kg				
Mean (SD)	78.2 (10.2)	79.1 (12.0)	79.8 (13.8)	79.0 (12.0)
Body mass index, kg/m^2				
Mean (SD)	26.3 (2.9)	26.6 (2.9)	25.7 (3.6)	26.2 (3.1)

SD, standard deviation.

[a]Includes classifications of Black/Native Hawaiian/Pacific Islander; Other: Italian American; and White/Black or African American.
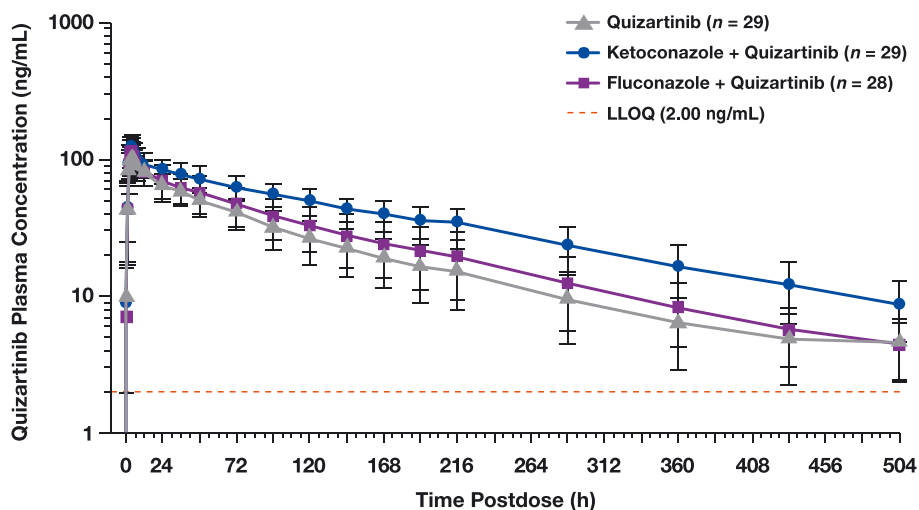
FIGURE 3 Mean (± standard deviation) concentration–time profiles of quizartinib in plasma after administration of single 30-mg dose of quizartinib alone or with ketoconazole or fluconazole (semi-log scale). LLOQ, lower limit of quantitation

3.2.1 | Effect of ketoconazole on quizartinib and AC886 PK

Quizartinib exposure increased with ketoconazole coadministration (Table 2). Geometric LS mean C_{max}, AUC_{last} and AUC_{inf} values for quizartinib increased by 17, 86 and 94%, respectively, with ketoconazole coadministration. Exposure to the metabolite AC886 was decreased with geometric LS means of C_{max}, AUC_{last} and AUC_{inf}, decreasing to 40, 67 and 85%, respectively, with ketoconazole coadministration (Table S1). However, as parent quizartinib is the major component in plasma, total quizartinib + AC886 displayed similar increases in these PK parameters as parent quizartinib, albeit of

slightly smaller magnitude, with geometric LS mean C_{max}, AUC_{last} and AUC_{inf} values of 5, 59 and 82%, respectively, with ketoconazole coadministration (Table S2). As expected due to the potential interaction with CYP3A4 inhibitors, the 90% CIs for the geometric mean ratios for C_{max}, AUC_{last} and AUC_{inf} for both quizartinib and AC886 were outside the bioequivalence interval of 80–125% (Table 2 and Table S1).

The $T_{1/2}$ of quizartinib and AC886 increased by 46 and 96%, respectively, when quizartinib was coadministered with ketoconazole. The CL/F of quizartinib decreased by 50% when ketoconazole was coadministered with quizartinib.

Simulation of steady-state PK predicted significant increases in quizartinib exposure after repeated daily dosing with coadministration

TABLE 2 Statistical comparisons (ANOVA) of quizartinib pharmacokinetic (PK) parameters after a single 30-mg dose of quizartinib alone or with ketoconazole or fluconazole

PK parameter	Quizartinib Geometric LS mean (SE) [n]	Ketoconazole + quizartinib		Fluconazole + quizartinib	
		Geometric LS mean (SE) [n]	Ratio of geometric LS mean,[a] % (90% CI)	Geometric LS mean (SE) [n]	Ratio of geometric LS mean,[b] % (90% CI)
C_{max} (ng/mL)	103.9 (4.6) [n = 29]	121.4 (5.4) [n = 29]	116.9 (105.2, 129.8)	115.7 (5.3) [n = 28]	111.4 (100.2, 123.9)
AUC_{last} (ng•h/mL)	9,098.0 (506.7) [n = 29]	16,959.0 (944.6) [n = 29]	186.4 (163.5, 212.5)	10,903.0 (618.0) [n = 28]	119.8 (105.0, 136.8)
AUC_{inf} (ng•h/mL)	9,626.0 (564.4) [n = 29]	18,706.0 (1,116.0) [n = 28]	194.3 (169.1, 223.4)	11,549 (689.2) [n = 28]	120.0 (104.4, 137.9)
Mean $T_{1/2}$, h (CV%)	102 (26.8) [n = 29]	149 (23.0) [n = 28]		112 (27.7) [n = 28]	
Geometric mean CL/F, L/h (%CV)	2.75 (35.9) [n = 29]	1.42 (26.1) [n = 28]		2.29 (34.3) [n = 28]	
Median T_{max}, h (min, max)	4.0 (2.0, 8.0) [n = 29]	4.0 (3.0, 6.0) [n = 29]		4.0 (2.0, 5.0) [n = 28]	

[a](Ketoconazole + quizartinib)/(quizartinib);

[b](fluconazole + quizartinib)/(quizartinib). C_{max}, maximum observed concentration; AUC_{last}, area under the concentration–time curve from time 0 to the time of the last quantifiable concentration; AUC_{inf}, area under the concentration–time curve from time 0 to infinity; $T_{1/2}$, apparent terminal phase elimination half-life; CL/F, apparent systemic clearance; T_{max}, actual sampling time to reach maximum observed concentration; LS, least squares; SE, standard error; CI, confidence interval; CV, coefficient of variation; SD, standard deviation; min, minimum; max, maximum; n, number of subjects used for analysis.

TABLE 3 Statistical comparisons (ANOVA) of simulated quizartinib steady-state pharmacokinetic parameters after daily administration of 30 mg quizartinib alone or with ketoconazole

PK parameter	Quizartinib Geometric LS mean (SE) [n]	Ketoconazole + quizartinib	
		Geometric LS mean (SE) [n]	Ratio of geometric LS mean,[a] % (90% CI)
$C_{max,ss}$ (ng/mL)	451.1 (25.4) [n = 29]	841.0 (48.2) [n = 28]	186.4 (163.1, 213.1)
AUC_τ (ng•h/mL)	9,582.0 (562.0) [n = 29]	18,751.0 (1119.0) [n = 28]	195.7 (170.3, 224.9)
Median $T_{max,ss}$, h (min, max)	3.9 (2.0, 6.0) [n = 29]	4.0 (3.0, 6.0) [n = 29]	

[a](Ketoconazole + quizartinib)/(quizartinib).

$C_{max,ss}$, predicted steady-state peak concentration; AUC_τ, area under the plasma concentration–time curve from time 0 to the end of the dosing interval; $T_{max,ss}$, predicted actual sampling time to reach maximum observed concentration; LS, least square; SE, standard error; CI, confidence interval; CV, coefficient of variation; SD, standard deviation; min, minimum; max, maximum; n, number of subjects used for simulation.

of ketoconazole (Table 3). The $C_{max,ss}$ and AUC_τ were predicted to increase by 86% and 96%, respectively. The $T_{max,ss}$ was similar when quizartinib was dosed alone or with ketoconazole (Table 3). Exposure to the metabolite AC886 at steady-state was decreased by 14 and 18% for geometric LS means of AUC_τ and C_{max}, respectively, with ketoconazole coadministration compared with quizartinib alone (Table S3). In addition, the metabolite:parent ratio of concentrations was found to be consistent over 21 days for each of the treatment arms. As expected from the concentration–time profiles, subjects receiving ketoconazole had the lowest ratios of metabolite:parent followed by the group that received fluconazole. The group that received quizartinib alone had the highest ratios.

3.2.2 | Effect of fluconazole on quizartinib PK

The geometric LS means for quizartinib C_{max}, AUC_{last} and AUC_{inf} increased by 11, 20 and 20%, respectively, with fluconazole coadministration. The 90% CI of the geometric LS mean for C_{max} was entirely within the 80–125% prespecified interval, while the upper limits of the

90% CIs for AUC_{last} and AUC_{inf} were 137 and 138%, respectively, falling outside the upper 125% limit (Table 2). Mean $T_{1/2}$ and CL/F of quizartinib had modest changes of ~10 and 17%, respectively, with fluconazole coadministration. The trends in plasma PK parameters of AC886 and quizartinib + AC886 were similar to those observed for parent quizartinib (Tables S1 and S2).

Consistent with the single-dose results, simulation of steady-state concentration of quizartinib in plasma after repeated daily dosing of quizartinib with fluconazole vs alone resulted in minor changes to AUC_τ and $C_{max,ss}$ (data not shown).

3.3 | Safety

Overall, coadministration of ketoconazole or fluconazole with quizartinib was well tolerated in healthy subjects.

The proportions of subjects with AEs were similar across groups. Thirty-six subjects experienced at least 1 AE during the study: 13 (41.9%) subjects in the quizartinib arm, 9 (29.0%) subjects in the quizartinib + ketoconazole arm and 14 (45.2%) subjects in the

fluconazole + quizartinib arm. Of the 36 subjects who experienced AEs, a large majority of subjects (32 subjects, 88.9%) experienced mild/Grade 1 AEs. Three subjects (8.3%) experienced moderate/Grade 2 AEs and 1 subject (2.8%) experienced a severe/Grade 3 AE. The severe AE was musculoskeletal stiffness of the neck, which was not considered related to any of the study medications, and resolved in 15 days. There were no life-threatening/Grade 4 AEs or deaths. There were no serious AEs observed in the study.

Only 5 (5.4%) subjects overall experienced AEs considered to be possibly related to quizartinib. These 5 subjects were distributed throughout the treatment groups as follows: 3 subjects in the quizartinib arm, 1 in the ketoconazole + quizartinib arm, and 1 in the fluconazole + quizartinib arm. The most common AEs related to quizartinib were gastrointestinal disorders and were experienced by 1 subject in each treatment arm.

No significant abnormalities in vital signs or changes to QTcF (Table S4), haematology or clinical chemistry were observed during the study.

4 | DISCUSSION

There is substantial risk for infections, including invasive fungal infections, in patients with AML.[10,11] As a result, concomitant use of antifungals is common in these patients. Some antifungals (such as azoles) that are used to prevent and treat invasive fungal infections in patients with AML can inhibit CYP3A.[11,12] As quizartinib is primarily metabolised by CYP3A,[9] coadministration of quizartinib with some therapies used to prevent invasive fungal infections can potentially lead to drug–drug interactions and increased AEs from quizartinib treatment. This study assessed the effect of strong and moderate CYP3A inhibitors on the PK and safety of quizartinib to better inform dosing recommendations for phase 3 evaluation of quizartinib. These recommendations aim to ensure consistent, clinically efficacious and safe exposure to quizartinib.

To ensure maximal inhibition of CYP3A, this study was designed to evaluate the PK of quizartinib and AC886, the main and biologically active metabolite of quizartinib, once ketoconazole and fluconazole had reached steady-state. Prior studies showed that by Day 8 of treatment, the strong CYP3A inhibitor, ketoconazole, and the moderate CYP3A inhibitor, fluconazole, reached steady-state concentrations.[13,14] Concentrations of ketoconazole and fluconazole were consistent between Days 6 through 8 in our study, and support that steady-state concentrations of ketoconazole and fluconazole were reached by Day 8 when quizartinib was administered, ensuring maximal inhibition of the enzyme.

Consistent with *in vitro* observations that quizartinib is a substrate for CYP3A, increases in quizartinib exposure were observed when quizartinib was coadministered with a strong or moderate CYP3A inhibitor. In addition, moderate inhibition of CYP3A by fluconazole had no significant effect on the PK of AC886, the main and biologically active metabolite of quizartinib. However, strong inhibition of CYP3A by ketoconazole decreased C_{max} and increased the $T_{1/2}$ and

T_{max} of AC886. This resulted in relatively flat plasma concentration–time profiles of AC886 in the presence of ketoconazole, preventing reliable estimation of $T_{1/2}$ of AC886 in several subjects (Table S1). These changes are consistent with previous data demonstrating that AC886 is produced when quizartinib is metabolised by CYP3A[9] and is, itself, also a substrate for CYP3A (data on file, Daiichi Sankyo, Inc).

After administration of an oral dose, quizartinib is the major moiety in circulation, and AC886 is a smaller component. The observed increase in exposure to the total circulating active moiety (quizartinib + AC886) because of CYP3A inhibition was similar in trend and magnitude to the increased exposure for quizartinib alone. There was an approximate 2-fold increase in quizartinib AUC when quizartinib was coadministered with a strong CYP3A inhibitor, ketoconazole. Despite increases in quizartinib exposure when administered with CYP3A inhibitors, there were no differences observed in AEs possibly related to quizartinib following a single dose in healthy subjects across the 3 treatment arms.

As a result of the long half-life of quizartinib, the drug exhibits an approximate 5-fold accumulation with repeated dosing. Thus, the single-dose C_{max} may not be representative of $C_{max,ss}$. When a single dose of quizartinib was administered with ketoconazole, terminal $T_{1/2}$ increased from 98 to 145 hours, supporting the inhibitory effects of ketoconazole on CYP3A metabolism. The effect of this prolonged terminal $T_{1/2}$ on steady-state quizartinib exposure ($C_{max,ss}$ and AUC_{inf}) was simulated using nonparametric superposition because the PK is time-independent and linear with increasing dose (data not shown). Although this method may result in biased estimations of variability, the simulations were used to provide an estimate of $C_{max,ss}$, which is relevant for the predication of QT prolongation. The increase in $C_{max,ss}$ was 86% for quizartinib with a strong CYP3A inhibitor, ketoconazole, *vs* quizartinib alone and consistent with the observed increase in AUC_{inf}. Coadministration of quizartinib with a moderate CYP3A inhibitor, fluconazole, resulted in small ($\leq$20%) increases in quizartinib exposure.

This drug interaction study was conducted in healthy subjects, while quizartinib is used to treat patients with AML. There are reports that intestinal mucositis and neutropenia, experienced by AML patients, may impact oral absorption of posaconazole[18] or clearance of vancomycin,[19] respectively. An exploratory exposure–response analysis for febrile neutropenia, a common AE in AML patients, was performed in the phase 2 study. No clear correlation between PK parameters and the frequency of febrile neutropenia was apparent. In a population PK analysis for AML patients who received quizartinib with or without strong CYP3A inhibitors, a similar degree of increase in quizartinib exposure was estimated to that observed in this study (data on file, Daiichi Sankyo, Inc). Furthermore, the dose reduction for strong CYP3A inhibitors based on this study was implemented in a recently completed phase 3 study (QuANTUM-R), which showed a positive study outcome.[20]

The results of this study suggest reducing the dose of quizartinib when administered concomitantly with strong CYP3A inhibitors that are medically necessary for the care of the patient. Modeling of the concentration/QT relationship in a prior phase 2 study indicated that

quizartinib prolongs QT intervals in a concentration-dependent manner.[7] A robust exposure-response model for concentration-QTc (C-QTc) was conducted using phase 2 study data. The model predicted a geometric mean change in QTcF (ΔQTcF) of 19.3 ms at the observed mean $C_{max,ss}$ for quizartinib 60 mg QD, and the upper 90% CI of ΔQTcF exceeded the 20-ms threshold (data on file, Daiichi Sankyo, Inc). Quizartinib demonstrated a robust relationship, while the metabolite AC886 had a 10-fold lower effect on QTcF. Therefore, given the 1.9-fold increase in quizartinib $C_{max,ss}$ observed in this study, the quizartinib dose was reduced from 60 to 30 mg with concomitant strong CYP3A inhibitors to reduce the risk of QTcF >500 ms, a clinically significant threshold. Although there were no clinically relevant QT prolongations with concomitant administration of ketoconazole or fluconazole with a single 30-mg dose of quizartinib in the current study, clinically relevant and dose-dependent QT prolongations have been observed following repeat daily dosing of quizartinib in previous clinical studies in patients with AML.[5-7,21] The increased exposure to quizartinib with ketoconazole supports reducing quizartinib doses in patients receiving a strong CYP3A inhibitor. Based on the fluconazole arm data, no dose reduction is needed when quizartinib is coadministered with a moderate or weak CYP3A inhibitor. This approach to dose adjustment aims to minimise variability in drug exposure and reduce the risk of QT prolongation in patients receiving quizartinib in conjunction with a drug that inhibits its metabolism via CYP3A. In the QuANTUM-R phase 3 study that evaluated patients with relapsed/refractory *FLT3*-ITD-mutated AML, the quizartinib dosage regimen was a 30-mg starting dose followed by escalation to 60 mg; the study showed prolonged overall survival *vs* standard chemotherapy.[20] In the ongoing QuANTUM-First phase 3 study evaluating patients with newly diagnosed *FLT3*-ITD-mutated AML, the quizartinib group is receiving a 40-mg dose in combination with standard induction and consolidation chemotherapy, then a 30-mg starting dose followed by 60 mg as the target dose.[22] The findings of the current study support dosage reductions in patients receiving a strong CYP3A inhibitor. In scenarios wherein patients would normally receive 30 mg (or 40 mg; administered as quizartinib dihydrochloride, equivalent to 26.5 or 35.3 mg, respectively, in free-base form) or 60 mg of quizartinib (administered as quizartinib dihydrochloride, equivalent to 53 mg in free-base form), doses of quizartinib should be reduced to 20 or 30 mg, respectively (administered as quizartinib dihydrochloride, equivalent to 17.7 or 26.5 mg, respectively, in free-base form), in patients receiving a strong CYP3A inhibitor.[23] This guidance was implemented in the 2 phase 3 trials, and additional population PK analysis from QuANTUM-R will further define the effects of dose modification on quizartinib PK when administered with strong CYP3A inhibitors.

COMPETING INTERESTS

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. Relationships may not relate to the subject matter of this manuscript.

J.L. was an employee of Daiichi Sankyo at the time this study was conducted. M.K. has no competing interests to declare. D.T. was an employee of Daiichi Sankyo and Ambit Biosciences at the time this study was conducted.

G.G. was an employee of Daiichi Sankyo at the time this study was conducted, with stock or other ownership with the company. Travel, accommodations, and expenses was funded by Daiichi Sankyo..

CONTRIBUTORS

All authors contributed to the conception and design of the work, analysis and interpretation of data, drafting or revising the manuscript, and final approval of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.