A Single-Dose, Crossover, Placebo- and Moxifloxacin-Controlled Study to Assess the Effects of Neratinib (HKI-272) on Cardiac Repolarization in Healthy Adult Subjects

Bruce Hug, Richat Abbas, Cathie Leister, Jaime Burns, and Daryl Sonnichsen

Abstract

Purpose: Neratinib is an orally administered, small-molecule, irreversible pan-ErbB inhibitor in development for the treatment of ErbB2-positive breast cancer. This study assessed the effects of therapeutic and supratherapeutic neratinib concentrations on cardiac repolarization, in accordance with current regulatory guidance.

Experimental Design: This was a two-part study in healthy subjects. In part 1, subjects were randomized to receive placebo, 400 mg moxifloxacin, or 240 mg neratinib (therapeutic dose) following a high-fat meal. In part 2, after a washout period, subjects received placebo plus 400 mg ketoconazole or 240 mg neratinib plus ketoconazole (supratherapeutic dose). ANOVA was used to compare the baseline-adjusted QTc interval for neratinib with that of placebo (reference), and for neratinib plus ketoconazole with that of placebo plus ketoconazole (reference). Pharmacokinetic/pharmacodynamic analyses and categorical summaries of interval data were done. Assay sensitivity was evaluated by the effect of moxifloxacin on QTc compared with placebo.

Results: Sixty healthy subjects were enrolled in this study. The upper bounds of the 90% confidence interval for baseline-adjusted QTcN (population-specific corrected QT) were ≤ 10 milliseconds greater than the corresponding reference at all postdose time points under conditions of both therapeutic and supratherapeutic plasma concentrations of neratinib. Pharmacokinetic/pharmacodynamic analysis revealed no relationship between neratinib concentrations and QTc interval. No subjects had QTcI, QTcF, or QTcN intervals >450 milliseconds or change from baseline >30 milliseconds. Moxifloxacin produced a significant increase in QTcN compared with placebo (P < 0.05).

Conclusions: Therapeutic and supratherapeutic plasma concentrations of neratinib do not prolong the QTc interval in healthy subjects.
in cancer patients, supratherapeutic plasma concentrations are difficult to achieve without causing significant dropout due to toxicities (5, 6).

Neratinib (HKI-272) is an orally administered, small-molecule, irreversible pan-ErbB tyrosine kinase receptor inhibitor that blocks signal transduction through inhibition of ErbB-1, ErbB-2, and ErbB-4 (7–9), and is currently in phase 3 clinical development for the treatment of patients with ErbB-2 positive breast cancer. Neratinib is generally acceptably tolerated by cancer patients, although gastrointestinal adverse events are common and limit the investigational dose to 240 mg daily in ongoing phase 3 trials (10, 11).

Investigation of neratinib in single-dose clinical trials in healthy subjects provided information suggesting that supratherapeutic plasma concentrations supporting a TQT study could be achievable. In a single ascending dose study to evaluate neratinib from 120 to 800 mg, neratinib exposure [peak plasma concentration (Cmax) and area under the concentration-time curve (AUC)] reached a plateau at doses >400 mg when administered to fasted subjects (12). Nevertheless, gastrointestinal tolerability continued to decrease with doses >400 mg. The separation of tolerability from systemic plasma concentrations suggested that the gastrointestinal adverse events that limit dose escalation are determined, at least in part, by local effects as opposed to systemic effects.

The inferred mechanism of gastrointestinal toxicities suggested that supratherapeutic plasma concentrations of neratinib might be achievable if local gastrointestinal effects could be bypassed. We exploited the knowledge that neratinib is a CYP3A4 substrate and showed, in a drug interaction study, that supratherapeutic neratinib plasma concentrations were well tolerated if achieved by coadministration with ketoconazole, an inhibitor of neratinib metabolism (13). Supratherapeutic neratinib concentrations achieved through CYP3A4 inhibition would not provide information on effects of neratinib metabolites nor would it provide information on steady-state effects of the parent drug. Nevertheless, the available clinical data provided an opportunity to conduct a TQT study of neratinib in healthy subjects closely approximating the ICH E14 guidelines.

Materials and Methods

Study population

Healthy male (ages 18–50 years inclusive at screening) and female (ages 18–60 years) subjects of non-childbearing potential who met all the qualifying criteria were eligible for enrollment in this study. Body mass index and body weight required to be in the range of 18 to 30 kg/m2 and ≥50 kg, respectively. Subjects were ineligible to participate in this study if they had a history of long QT syndrome, syncope, or seizure or a family history of unexplained, sudden, cardiac-related death. Likewise, subjects were excluded if their Ca2+, Mg2+, and K+ levels were below the lower limit of normal or if their QTC duration was ≥450 milliseconds based on the machine-read tracing at screening or on study day –1. This study was conducted in accordance with the ICH guideline for Good Clinical Practice and the ethical principles that have their origins in the Declaration of Helsinki. Written informed consent was obtained from all subjects before enrollment in the study. The study protocol, amendments, and informed consent form were approved by an independent ethics committee or an institutional review board. Finally, the protocol underwent Special Protocol Assessment by the Food and Drug Administration before enrollment.

Study design and treatments

This was a single-center, two-part, five-period, randomized, single-dose, double-blind (with respect to neratinib), crossover, placebo- and open-label moxifloxacin-controlled study in healthy subjects. In part 1 (three periods), subjects were randomly administered single doses of 240 mg neratinib, 400 mg moxifloxacin, and placebo, one in each period, with a high-fat meal. In part 2 (two periods), subjects were administered singles doses of 240 mg neratinib and placebo, one in each period, in combination with 400 mg ketoconazole in a fasting state. Thus, parts 1 and 2 provide assessments of therapeutic and supratherapeutic neratinib plasma concentrations, respectively. Ketoconazole (400 mg) was administered 12 hours before neratinib on day –1, and was coadministered with neratinib on day 1, as previously reported (13). Treatments were administered in a crossover pattern. Subjects were randomly assigned to 1 of 12 dosage administration sequences, which consisted of a combination of each of the five treatment arms. Each period was separated by a 5-day washout. In addition, parts 1 and 2 were separated by a 9-day outpatient washout to ensure that each neratinib dose was separated by a minimum 14-day period.

Bioanalytic and pharmacokinetic analyses

Venous blood samples (5 mL each) for analysis were collected on study day 1 for each of the five periods at 2 hours predose and at 1.5, 3, 4, 5, 6, 8, 12, 24, and 48 hours following dose administration. The concentrations of

Translational Relevance

This study evaluated the effects of therapeutic and supratherapeutic concentrations of neratinib, an irreversible pan-ErbB inhibitor, as well as positive and negative controls, on cardiac repolarization in healthy subjects. By coadministering neratinib with a CYP3A4 inhibitor, it was possible to achieve higher plasma concentrations of parent drug than would have been tolerated following administration of neratinib alone. The robust evaluation of neratinib on the QT interval is particularly relevant given the cardiac repolarization liability of other small-molecule inhibitors of ErbB-2. This approach provides a novel alternative to the conventional QT evaluations of oncology drugs over more limited ranges of plasma concentrations.
neratinib and its metabolites, ketoconazole and moxi-
floxacin, in the plasma samples were measured by using
a validated liquid chromatography tandem mass spec-
trometry assay. The lower limit of quantitation for ner-
tatinib and its metabolites, ketoconazole and moxifloxacin,
was 3, 20, and 25 ng/mL, respectively. The plasma
neratinib, neratinib metabolites, ketoconazole and moxi-
floxacin concentration data for each subject were
analyzed by using a noncompartamental method (14)
with WinNonlin Enterprise application version 4.1
(Phar-
sight Corporation).

Electrocardiogram and QTc analyses
Triplicate 12-lead electrocardiogram (ECG) recordings
for each subject were obtained on study day 1 for each
of the five periods at −1, −0.5, and 0 hours (immediately
before dose administration), and at 1.5, 3, 4, 5, 6, 8, 12,
24, and 48 hours following dose administration in all
periods. ECG results, including rhythm, heart rate (HR),
PR, QRS, QT, standard Bazett correction (QTcB), and
standard Fridericia correction (QTcF) intervals were inter-
preted and measured by eRT. The QT intervals were
measured manually, and the superimposed median beat
and threshold methods were used. A population correction
(QTcN) and individual correction (QTcI) were computed
to assess the treatment differences in change from baseline
(numerical differences in the baseline-adjusted QTc, be-
tween neratinib versus placebo, and between neratinib
and placebo plus ketoconazole).

Primary end point
The primary objective of the analysis was the compari-
sion of baseline-adjusted QTc for neratinib versus placebo
(part 1, therapeutic plasma concentrations) and for ner-
tatinib plus ketoconazole versus placebo plus ketoconazole
(part 2, supratherapeutic plasma concentrations). The pri-
mary method for correcting the QTc interval for HR was
QTcN. A mixed analysis of covariance model was used
to assess the treatment differences in change from baseline
QTc. This model had fixed effects for sequence, treatment,
period, and time as well as a treatment by time interaction
term. Baseline was included as a covariate and subject as a
random effect. A two-sided 90% confidence interval (90%
CI) was computed for the baseline-adjusted difference in
QTc at each postdose time point between neratinib versus
placebo, and between neratinib plus ketoconazole versus
placebo plus ketoconazole.

QTc assay sensitivity
In our study, the difference in model-based baseline-ad-
justed QTcN between moxifloxacin and placebo
(ΔΔQTcN) was statistically compared using two-sided
90% CIs. If moxifloxacin showed a >5-millisecond in-
crease over placebo (lower bound of the 90% CI of the
QTcN difference was >5) for at least one time point, the
study would be deemed acceptable to evaluate the nerati-
nib effect on QTc.

Pharmacokinetic/pharmacodynamic analysis
The relationship between the placebo- and baseline-
adjusted QTcN (ΔΔQTcN) and the log-transformed con-
centrations of neratinib and moxifloxacin were evaluated
separately by using linear regression models. The plasma
centrations were log transformed to minimize the in-
fluence of high concentrations on the analysis. The phar-
macokinetic/pharmacodynamic (PK/PD) relationship of ketoconazole was not explored because determining its
QT effect was not an objective of the study. The following
model was used:

\[
(\Delta \Delta \text{QTcN}) = \beta_0 + \beta_1 (\log_{10} \text{neratinib or moxifloxacin plasma concentration}) + \beta_2 (\text{baseline QTcN})
\]

where \(\beta_0\) is the intercept, \(\beta_1\) is the coefficient for the
log concentration, and \(\beta_2\) is the coefficient for the
baseline QTcN.

Determination of the population sample size
For this study, data for ≥44 subjects were required to
achieve 83% power for obtaining 90% CIs for the dif-
ference in the baseline-adjusted QTc, between neratinib and
placebo, and between neratinib plus ketoconazole and
placebo plus ketoconazole, fully below 10 milliseconds
at each time point. The assumptions made in the sample
size calculations were as follows: (a) the intrasubject SD
was 8 milliseconds; (b) the true maximum QTc difference
in change from baseline versus placebo was ≤3 millisec-
onds and was done using the option for two one-sided
equivalence tests for a crossover design in nQuery Advisor
(Statistical Solutions).

Results
Demography and subject participation
Sixty healthy subjects were enrolled in this study [47
(78.0%) men and 13 (22.0%) women]. The median age
of enrolled subjects was 36 years (range, 18–57 years).
Forty-two (70.0%) subjects were white. In part 1 of the
study, 56 subjects received therapeutic doses of 240 mg
neratinib, 58 subjects received placebo, and 59 subjects
received 400 mg moxifloxacin. In part 2 of the study, 54
subjects received the supratherapeutic dose of 240 mg ner-
tatinib plus ketoconazole, and 53 subjects received placebo
plus ketoconazole.

Safety
Data for all 60 enrolled subjects were included in the
safety analysis. The most commonly reported adverse
events (AE) were gastrointestinal disorders (57% subjects),


including diarrhea (42%), nausea (28%), and vomiting (13%), consistent with the previously reported tolerability profile of neratinib in healthy subjects (10, 11). The frequency of gastrointestinal AEs in subjects who were administered 240 mg neratinib and 240 mg neratinib plus ketoconazole was 34% and 48%, respectively. No cardiovascular AEs related to administration of neratinib were reported. No torsade de pointes, sudden death, ventricular tachycardia, ventricular fibrillation, ventricular flutter, syncope, or seizure was observed. No QTc intervals were >450 milliseconds or increased from baseline by 30 milliseconds following administration of neratinib or neratinib plus ketoconazole. Other interval data were within limits typical for studies in healthy subjects. All AEs were considered by the principal investigator to be mild in severity, with the exception of moderate ventricular extrasystoles (one subject), which occurred after administration of moxifloxacin. No serious AEs or deaths were reported during this study.

Of the 60 subjects who enrolled, 52 (87%) completed the study and 8 (13%) discontinued participation before completing all five periods of the study. Only one subject discontinued due to an AE (ventricular extrasystoles following the administration of moxifloxacin).

Pharmacokinetics for neratinib

Blood samples from 59 subjects were available for PK analysis. Following administration of single-dose (240 mg) neratinib with food, the mean (%CV) for C\text{max} and AUC was 68 ng/mL (40%) and 1,236 ng·h/mL (39%), respectively, consistent with published data for both healthy subjects and cancer patients (10, 12). Following coadministration of 240 mg neratinib with ketoconazole, the mean (%CV) for C\text{max} was 163 ng/mL (46%) and AUC was 3,801 ng·h/mL (49%), a 2.4-fold and 3-fold increase, respectively, compared with single-dose neratinib alone. Neratinib data are consistent with previous observations (13).

In this study, two major circulating metabolites of neratinib, neratinib pyridine N-oxide (M3) and neratinib dimethylamine N-oxide (M7), were analyzed (data not shown). Following administration of 240 mg neratinib alone, the C\text{max} and AUC of M3 were 17% and 7% of the neratinib, respectively, and the C\text{max} and AUC of M7 were 16% and 9% of the neratinib, respectively. Following neratinib coadministration with ketoconazole, M3 exposure (AUC\text{c}) was decreased by ∼10-fold, from 7% to 0.7%, due to ketoconazole inhibition of the CYP3A4 metabolic pathway, which is responsible for the generation of M3. However, there was no significant change in M7 exposure (AUC\text{c} 13%) following coadministration of neratinib with ketoconazole because M7 is mainly produced by flavin-containing monoxygenases.

Plasma neratinib concentrations peaked at 5 and 6 hours for the therapeutic and supratherapeutic dose conditions, respectively (Fig. 1), and the elevated plasma neratinib concentrations represented meaningful extremes compared with those observed in cancer patients [2.2-fold increase over the mean observed in cancer patients who received 240 mg neratinib daily at steady-state with food (mean C\text{max}\text{,} 74 ng/mL)]. Furthermore, 13 subjects achieved neratinib mean C\text{max} > 219 ng/mL, a 3-fold increase compared with the mean C\text{max} observed in cancer patients (10). The highest C\text{max} achieved in an individual subject in the supratherapeutic dose treatment was 327 ng/mL. By comparison, the highest neratinib plasma concentration observed to date in any individual cancer patient is 247 ng/mL.1 These results suggest that neratinib exposures following coadministration with ketoconazole are representative of supratherapeutic neratinib exposures under clinical conditions.

1 Unpublished data.
Pharmacokinetics for ketoconazole

Because ketoconazole can independently increase the QT interval (15), the pharmacokinetics of ketoconazole were evaluated when neratinib was coadministered with ketoconazole and when placebo was coadministered with ketoconazole. Following oral administration of multiple doses of ketoconazole in combination with a single dose of 240 mg neratinib or placebo, the ketoconazole $C_{\text{max}}$ was 9,140 ng/mL (CV = 35%) and 9,446 ng/mL (CV = 42%), respectively, and the median $t_{\text{max}}$ was 3 hours for both treatment groups. For the ketoconazole plus neratinib and ketoconazole plus placebo groups, ketoconazole AUC was 77,292 ng·h/mL (CV = 41%) and 72,967 ng·h/mL (CV = 48%), respectively. The least-square geometric mean ratio (and the 90% CI around the ratios) of ketoconazole plus neratinib versus ketoconazole plus placebo and for $C_{\text{max}}$ and AUC were 101.33% (90% CI, 91.75–111.92) and 112.20% (90% CI, 100.77–124.93), respectively. Overall, the data suggest that ketoconazole exposures following multiple oral doses of ketoconazole concomitantly administered with a single oral dose of neratinib (period 4) were equivalent to exposures following multiple oral doses of ketoconazole with a placebo (period 5). Therefore, the relative effect on the QT interval of supratherapeutic concentrations was calculated as the difference in baseline-adjusted QTcN for neratinib compared with placebo (reference) and the effect of supratherapeutic concentrations was calculated as the difference in baseline-adjusted QTcN for neratinib plus ketoconazole compared with placebo.

Plasma concentrations of moxifloxacin

After a single oral dose of 400 mg moxifloxacin, the moxifloxacin $C_{\text{max}}$ was reached with a median $t_{\text{max}}$ of 3 hours (range 1.5–6 hours). The mean moxifloxacin $C_{\text{max}}$ was 2,012 ng/mL (CV = 41%). The moxifloxacin $C_{\text{max}}$ observed in this study was consistent with the reported $C_{\text{max}}$ of this drug (16).

QT correction

The QT interval, highly dependent on HR, was corrected to produce measures not related to HR. Four different QT
corrections, QTcB, QTcF, QTcI, and QTcN, were applied. The correction factor for QTcN was 0.204 with a 95% CI of 0.186 to 0.221. The average correction factor for QTcI was 0.201 with a 95% CI of 0.171 to 0.231. Correlation coefficients for QTcB, QTcF, QTcN, and QTcI were calculated to assess the relationship of each correction to HR. The Pearson correlations for QTcB, QTcF, QTcN, and QTcI were 0.528, 0.170, –0.173, and –0.173, respectively. QTcN, the prespecified primary end point of the study, was not related to HR. It should be noted that all HR readings were between 45 and 120 bpm throughout the study, and there were no changes from baseline HR >15 bpm at any time during the study. In addition, change in baseline-adjusted HR following neratinib administration was not significantly different from that following placebo administration at any of the postdose time points, thus suggesting that neratinib does not affect HR.

### Table 1. Change from baseline-adjusted QTcN for moxifloxacin versus placebo

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>LSM</th>
<th>P</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>3.631</td>
<td>0.0058</td>
<td>(1.47 to 5.80)</td>
</tr>
<tr>
<td>3</td>
<td>6.487</td>
<td>&lt;0.0001</td>
<td>(4.33 to 8.65)</td>
</tr>
<tr>
<td>4</td>
<td>8.591</td>
<td>&lt;0.0001</td>
<td>(6.43 to 10.75)</td>
</tr>
<tr>
<td>5</td>
<td>6.537</td>
<td>&lt;0.0001</td>
<td>(4.38 to 8.69)</td>
</tr>
<tr>
<td>6</td>
<td>6.773</td>
<td>&lt;0.0001</td>
<td>(4.62 to 8.93)</td>
</tr>
<tr>
<td>8</td>
<td>7.954</td>
<td>&lt;0.0001</td>
<td>(5.80 to 10.11)</td>
</tr>
<tr>
<td>12</td>
<td>6.777</td>
<td>&lt;0.0001</td>
<td>(4.62 to 8.93)</td>
</tr>
<tr>
<td>24</td>
<td>3.9073</td>
<td>0.003</td>
<td>(1.74 to 6.07)</td>
</tr>
<tr>
<td>48</td>
<td>–0.298</td>
<td>0.8209</td>
<td>(–2.46 to 1.87)</td>
</tr>
</tbody>
</table>

Abbreviations: LSM, least-squares mean; QTcN, corrected QT based on a population-specific correction formula.

### Table 2. Change from baseline-adjusted QTcN for 240 mg neratinib versus placebo

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>LSM</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>0.1452</td>
<td>(–2.04 to 2.33)</td>
</tr>
<tr>
<td>3</td>
<td>–1.437</td>
<td>(–3.62 to 0.75)</td>
</tr>
<tr>
<td>4</td>
<td>1.1333</td>
<td>(–1.07 to 3.30)</td>
</tr>
<tr>
<td>5</td>
<td>–1.4828</td>
<td>(–3.67 to 0.70)</td>
</tr>
<tr>
<td>6</td>
<td>–0.9811</td>
<td>(–3.17 to 1.20)</td>
</tr>
<tr>
<td>8</td>
<td>–0.7891</td>
<td>(–2.98 to 1.40)</td>
</tr>
<tr>
<td>12</td>
<td>0.8197</td>
<td>(–1.37 to 3.01)</td>
</tr>
<tr>
<td>24</td>
<td>–1.4612</td>
<td>(–3.65 to 0.72)</td>
</tr>
<tr>
<td>48</td>
<td>–0.9237</td>
<td>(–3.11 to 1.26)</td>
</tr>
</tbody>
</table>

Primary end point (QTcN)

The primary end point was the effect of therapeutic and supratherapeutic plasma neratinib concentrations on QTcN at all postdose time points. The effect of therapeutic concentrations was calculated as the difference in baseline-adjusted QTcN for neratinib compared with placebo (reference) and the effect of supratherapeutic concentrations was calculated as the difference in baseline-adjusted QTcN for neratinib plus ketoconazole compared with placebo.
plus ketoconazole (reference). The upper bounds of the 90% CI for ΔΔQTcN were <10 milliseconds greater than the respective reference, at all postdose time points, for both the therapeutic and the supratherapeutic dose comparisons (Tables 2 and 3). This finding confirms that neratinib does not produce an effect on the QT interval. In addition, at no time point was the lower bound of the 90% CI greater than 0, thus indicating that the effect of neratinib was, statistically, not different from placebo. Similar results were obtained for QTcB, QTcF, and QTcI (data not shown).

**PK/PD analysis**

The PK/PD relationship between QTcN versus neratinib and moxifloxacin, separately, were examined graphically and statistically. Scatter plots of the individual QTcN change from baseline versus neratinib plasma concentrations following administration of 240 mg neratinib, and coadministration of 240 mg neratinib with ketoconazole revealed that the QTcN change was randomly distributed with no clear trend relative to neratinib plasma concentrations (Fig. 2). Multiple linear regression models on placebo- and baseline-adjusted QTcN (ΔΔQTcN) versus log-transformed concentrations were fitted with postdose data for all subjects included in the statistical analysis for each analyte separately. A summary of the results for the models for each analyte is presented in Table 4. The slope coefficient on log-transformed neratinib concentrations of 0.33 was not significantly different from 0 (P = 0.3029), and the 95% CI (−0.29 to 0.94) contained 0, thus suggesting no relationship between plasma neratinib concentrations and change from baseline QTcN.

**Discussion**

The conduct of a healthy subject TQT trial circumvents many of the obstacles to cardiac repolarization evaluations in cancer patients. Importantly, the collection of robust ECG data standardized for operator, interval measurements, and critical variables, including time of day, is possible. Administration of placebo and positive controls to healthy subjects is routine. Finally, recruitment of subjects for participation in a multiperiod, inpatient, procedure-intensive, nontherapeutic clinical trial is more readily accomplished for healthy populations than cancer patient populations. Because 240 mg neratinib has an acceptable safety and tolerability profile in healthy subjects when administered as a single dose, alone or in combination with ketoconazole, it was feasible to conduct the study in a noncancer patient population (13).

In this study, we evaluated the effects of neratinib on cardiac repolarization in healthy subjects while adhering to the recommended study design of the ICH E14. Our study fulfilled the requirements for a TQT study, including evaluations of both therapeutic and supratherapeutic neratinib concentrations and the appropriate negative and positive controls. Although many drugs in the oncology

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**Table 3. Change from baseline-adjusted QTcN for neratinib plus ketoconazole versus placebo plus ketoconazole**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>LSM</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>−1.9094</td>
<td>(−4.29 to 0.47)</td>
</tr>
<tr>
<td>3</td>
<td>−4.9378</td>
<td>(−7.31 to −2.56)</td>
</tr>
<tr>
<td>4</td>
<td>−2.9459</td>
<td>(−5.32 to −0.57)</td>
</tr>
<tr>
<td>5</td>
<td>−0.6031</td>
<td>(−2.98 to 1.77)</td>
</tr>
<tr>
<td>6</td>
<td>0.2536</td>
<td>(−2.12 to 2.63)</td>
</tr>
<tr>
<td>8</td>
<td>1.3272</td>
<td>(−1.05 to 3.70)</td>
</tr>
<tr>
<td>12</td>
<td>−2.9177</td>
<td>(−5.29 to −0.54)</td>
</tr>
<tr>
<td>24</td>
<td>−3.235</td>
<td>(−5.61 to −0.86)</td>
</tr>
<tr>
<td>48</td>
<td>−0.895</td>
<td>(−3.28 to 1.49)</td>
</tr>
</tbody>
</table>

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![Fig. 2. Scatter plots of individual changes from baseline-adjusted QTcN difference from placebo versus plasma neratinib concentration for healthy subjects.](chart.png)
therapeutic area are not suitable for such an analysis, we were able to exploit the knowledge that supratherapeutic plasma concentrations of neratinib could be achieved by coadministration of well-tolerated doses of neratinib with ketoconazole.

A neratinib dose of 240 mg has shown activity in phase 2 trials of patients with ErbB-2 positive breast cancer (11, 17) and is currently in phase 3 development for breast cancer. As anticipated from previous experience with single 240 mg doses of neratinib in healthy subjects, the tolerability profile was only modestly diminished when plasma neratinib exposures (C\text{max} and AUC) were increased ∼2.4- and 3-fold by coadministration of an inhibitor of neratinib metabolism. The plasma neratinib concentrations achieved exceeded those observed in cancer patients, and were well above those that could be tolerated by healthy subjects following administration of single neratinib doses without ketoconazole. All AEs observed during the period of neratinib administration were mild in severity and resolved without the need for medical intervention.

The evaluation of both therapeutic and supratherapeutic doses of neratinib produced no detectable change in baseline-adjusted QTc interval compared with the relevant comparator. QTcN was prespecified as the primary end point and method of correction, and no relevant differences were observed for QTcF or other corrections, including QTcB, QTcF, or QTcE. Likewise, an evaluation of the concentration-effect relationship between neratinib and QTcN failed to reveal any effect of neratinib on cardiac repolarization. Importantly, the positive control, moxifloxacin, produced a significant effect on QTcN, thus verifying that the study conditions were appropriately sensitive for detecting the effects on cardiac repolarization.

Table 4. Results from PK/PD analysis of placebo- and baseline-adjusted QTcN on log-transformed neratinib and moxifloxacin concentrations

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Model</th>
<th>Analysis on coefficient for log(concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neratinib</td>
<td>ΔΔQTcN = 24.61 + 0.33·log(neratinib)−0.07·(baseline QTcN)</td>
<td>0.3029 (-0.29 to 0.94)</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>ΔΔQTcN = −2.28 + 2.63·log(moxifloxacin)−0.03·(baseline QTcN)</td>
<td>&lt;0.0001 (1.92 to 3.35)</td>
</tr>
</tbody>
</table>

Abbreviation: ΔΔQTcN, placebo- and baseline-adjusted QTcN change.

Although this study provides valuable information related to the effect of neratinib on cardiac repolarization, it has several limitations. First, the use of the CYP3A4 inhibitor ketoconazole to achieve supratherapeutic plasma neratinib concentrations eliminates the utility of the study for assessments of effects of neratinib metabolites. Indeed, several active neratinib metabolites are produced by CYP3A4.2 If any of these metabolites have an effect on the QT interval that is distinct from neratinib, then this effect would not be captured by our supratherapeutic assessment. Second, 78% of the subjects in this study were healthy men. Although pharmacodynamic effects of neratinib were not observed, for some agents, such as quinidine, the concentration-QT effects have been more pronounced in women than in men (20). Finally, in this study, only single doses of neratinib were administered. Therefore, any theoretical cardiac accumulation of neratinib that might occur at steady state would not be captured.

The cardiac repolarization effect of investigational drugs is a component of risk-benefit assessment by patients, physicians, and regulatory agencies. Many approved anticancer pharmaceutical agents can prolong the QT interval, and the knowledge is valuable for the individualized selection of treatments and safe management of patients. Our study provides an approach to optimally characterize the cardiac repolarization liability of an investigational signal transduction inhibitor.

Disclosure of Potential Conflicts of Interest

All authors were employees of Wyeth Research at the time of the study and may have held stock in Wyeth, which was acquired by Pfizer Inc., in October 2009.

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2 Unpublished data.
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