CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-068

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Interdisciplinary Review Team for QT Studies
Response to a Request for Consultation: QT Study Review

NDA	22068
Brand Name	Tasigna
Generic Name	Nilotinib (AMN107)
Sponsor	Novartis
Indication	Treatment of chronic phase and accelerated phase Philadelphia chromosome positive chronic myelogenous leukemia (CML) in adult patients resistant to or intolerant to [redacted] prior therapy including imatinib
Dosage Form	200 mg hard capsules
Therapeutic Dose	400 mg BID
Duration of Therapeutic Use	To be used daily until disease progression or until no longer tolerated by the patient
Maximum Tolerated Dose	600 mg BID
Application Submission Date	2/29/06
Review Classification	S
Date Consult Received	11/1/06
Date Consult Due	2/16/07
Clinical Division	DDOP
PDUFA Date	7/29/07

1 SUMMARY

1.1 OVERALL SUMMARY OF FINDINGS

Based on preclinical data, nilotinib was likely to prolong QT, and study 2119 seems to confirm that (although it would still be useful to review the digital ECGs). The mean response to the therapeutic dose can be estimated based on 9 ms per 1000 ng/mL, but the uncertainty in the slope and inter-subject variability mean that many subjects will have larger effects.

The risks associated with QT prolongation can be mitigated by correcting electrolyte abnormalities, conditions that would be expected to raise exposure to nilotinib (here, food and CYP 3A4 inhibitors), and by prohibiting other QT-prolonging drugs. All of these seem like reasonable parts of a risk management strategy. Monitoring of QT is also a possibility, best performed around the time of Cmax. Patients and caregivers should also be alert to palpitations, dizziness, and syncope.

Dr. DeFelice in his review notes the possibility that QT prolongation without certain other features may not be ominous at all; there are not sufficient data to reach that conclusion. Assume some patients receiving nilotinib will, rarely, have torsades and sudden death.

1.2 REVIEWER'S COMMENTS

- The review team is unable to attest to the quality of the ECGs or the data extracted from them. There are no digital ECGs in the ECG Warehouse. This problem should be rectified by the sponsor before there is serious reliance upon the results of this study. A preliminary assessment of the findings follows.
- In the through QT study, the mean maximum concentration reached was 1669 ng/ml. The Cmax observed at steady state following the therapeutic dose i.e, 400 mg bid regimen, was 2260 ng/ml. Thus the extent of QT prolongation using the E14 analysis in the thorough QT study (maximum mean change in the ΔΔ QTcF at 1669 ng/ml: 17.7 msec) will underestimate the actual QT prolongation that will be observed at the therapeutic concentrations. Information about the slope of concentration-QT relationship can be used to predict the mean change in QTcF from the thorough QT study and should be incorporated. The label should include both, results of the thorough study as well as the concentration-QT analyses to predict the QT prolongation at therapeutic concentrations.
- There are additional factors that could increase nilotinib concentrations, such as co-administration of CYP3A4 inhibitors (2-fold increase in Cmax and 3-fold increase in AUC), administration of drug with food (2-fold increases in Cmax and AUC), and possibly administration to patients with hepatic impairment (although not yet evaluated), There would be expected to further prolong the QTc interval
- The QT study 2119 results of maximum mean placebo-subtracted treatment effect based on baseline adjusted mixed effects model are presented in Table 1. These results indicate nilotinib may associate with a QTcF prolongation at the therapeutic dose for failing to demonstrate that the upper bound of 2-sided 90% confidence interval for the largest time-matched mean effect to be $\leq 10ms$ per E14 Guidance. This reviewer also independently performed a mixed model and the findings are very similar with those reported from the sponsor.

Table 1. Maximum Mean Treatment Effect in QTcF Change since Baseline (Sponsor's Results Based on Mixed Effects Model)Treatment Comparison	Maximum Difference		
	At Hour	Mean	2-sided 90% C.I.
400 mg o.d. × 3d – placebo	12	1.5	(-5.98, 8.89)
400 mg b.i.d. × 3d – placebo	2	10.4	(2.85, 17.97)
400 mg o.d. × 8d – placebo	12	1.7	(-5.73, 9.12)
800 mg o.d. × 1d w/food – placebo w/food	5	17.7	(9.65, 25.84)
400 mg o.d. × 3d w/food – placebo w/food	5	15.7	(7.66, 23.82)

- Based on the results of time-matched placebo-subtracted change from baseline QTcF, a QT prolongation was indicated for 400 mg or 800 mg with a high fat food. The impact of using pooled placebo (placebo from cohorts 1, 2, and 4) on the estimation of treatment difference is not clear; test for homogeneity between placebo groups from 3 cohorts is not meaningful with only 5 subjects per placebo group. With only 5 subjects in the placebo group for comparison with 400 mg

b.i.d. × 8d dose cohort on Day 8, the placebo-subtracted mean change from baseline evaluations may be inaccurate.

2 PROPOSED LABEL

Labeling comments will be made at the request of the review division.

3 BACKGROUND

3.1 INDICATION

Treatment of chronic phase and accelerated phase Philadelphia chromosome positive chronic myelogenous leukemia (CML) in adult patients resistant to or intolerant to [redacted] prior therapy including imatinib.

3.2 DRUG CLASS

Nilotinib is an inhibitor of the Bcr-Abl kinase activity.

3.3 MARKET APPROVAL STATUS

Sponsor is currently seeking approval in the US. The drug is not approved in any other country.

3.4 CLINICAL PHARMACOLOGY

The following table summarizes the key features of nilotinib's clinical pharmacology.

Table 2. Highlights of Clinical Pharmacology

Therapeutic dose	400 mg BID	
Maximum tolerated dose	600 mg BID	
Principal adverse events	Rash, pruritus, nausea, headache, fatigue, diarrhea.	
Maximum dose tested	Single Dose	600 mg (in healthy volunteers)
	Multiple Dose	600 mg BID (in patients)
Exposures Achieved at Maximum Tested Dose	Single Dose	Mean (%CV) Cmax = 453 (45%) ng/mL Mean (%CV) AUC = 14576 (35%) ng.h/mL
	Multiple Dose (on day 15)	Mean (%CV) Cmax = 2210 (35%) ng/mL Mean (%CV) AUC = 16400 (42%) ng.h/mL
Range of linear PK	QD dosing: dose-proportional increase in Cmax and AUC from 50 to 400 mg, with no further increase in exposure up to 1200 mg. BID dosing: dose proportional increase in Cmax and AUC from 200 to 400 mg BID, with no further increase in exposure from 400 to 600 mg BID.	
Accumulation at steady state	~2-fold increase in Cmax and AUC following 400-1200 mg QD. 3.8-fold increase in Cmax and AUC following 400-600 mg BID.	
Metabolites	Two metabolites, P41.6 formed by hydroxylation of methyl group in the methyl imidazole ring, and P36.5 formed by further oxidation of the hydroxyl group to a carboxylic acid. These metabolites account for 4.7% and 6.1% of total drug exposure. Metabolism primarily by CYP3A4.	
Absorption	Absolute/Relative Bioavailability	Not reported

	Tmax	• Median (Range): 3.0 (2.0 – 12.0) hrs following 400 mg dose.
Distribution	Vss	Not reported
	% bound	98%
Elimination	Route	• Primary route: 90% of drug eliminated in feces, 69% as unchanged drug and 21% as metabolites • No renal elimination of drug or metabolites observed
	Terminal t½	• 17 hrs
	CL/F	Not reported
Intrinsic Factors	Age	No effect of age seen in population PK analysis
	Sex	20% higher exposures in females seen in population PK analysis
	Race	No effect of race seen in population PK analysis
	Hepatic & Renal Impairment	Effect of hepatic impairment not evaluated. No effect of renal impairment is expected given that the drug is not excreted renally.
Extrinsic Factors	Drug interactions	Coadministration of ketoconazole increased nilotinib Cmax by 84% and AUC by 3-fold on average. DDI study with CYP3A4 inducers not done.
	Food Effects	Following high-fat meal 30 min prior to dosing: AUC increased by 82% and Cmax increased by 112%. Following light breakfast 30 min prior to dosing: AUC increased by 29% and Cmax increased by 55%.
Expected High Clinical Exposure Scenario	Up to 2-fold increases in Cmax and 3-fold increase in AUC if co-administered with strong CYP3A4 inhibitors like ketoconazole. Up to 2-fold increases in Cmax and AUC if given after a high-fat meal.	

4 SPONSOR'S SUBMISSION

4.1 OVERVIEW/SUBMITTED DATA

The sponsor submitted data from a thorough QT study (study 2119). The sponsor also evaluated QT data collected in their phase 1 study in CML patients (study 2101) and phase 2 study in chronic phase-CML and accelerated phase-CML patients (study 2101E1 and study 2101E2).

4.2 QT STUDY 1

4.2.1 Title

A randomized, blinded, placebo and active controlled study to evaluate the cardiac safety of multiple doses of AMN107 in healthy volunteers

4.2.2 Protocol Number

CAMN107A2119

4.2.3 Objectives

The study was designed to be conducted in 2 parts: Part 1 of the study was a randomized, placebo-controlled, parallel group ascending dose cohort study conducted to establish the PK parameters of AMN107A (C_{max}, AUC) at doses that were safe and well tolerated in healthy volunteer subjects and to estimate associated changes in the QT interval. After the selection of the highest safe dose, the sponsor had planned to conduct Part II of the study which was designed as a thorough QT study with a placebo and moxifloxacin arm. However, the sponsor stopped the study after Part I of the study was completed.

Primary objectives (Part I)

- To determine the safety and tolerability of multiple doses of AMN107A in healthy adult volunteers.

Secondary objectives (Part I)

- To estimate the magnitude of the effect of multiple doses of AMN107A on the QTcF (Fridericia's correction) interval.
- To estimate the effect of AMN107A on cardiac conduction intervals (QT, QTcB (Bazett's correction), QTcI (individualized correction), QRS, RR and PR).

4.2.4 Design

4.2.4.1 Description

Part 1 was a single-blind, randomized, placebo-controlled parallel group study. Part 1 of the study involved 125 healthy male volunteers. Subjects were randomized to AMN107A or placebo administered in a fasting state or after a high fat meal.

There were 23 subjects, who received placebo medication on Day -1, who did not continue into the treatment period. A total of 102 subjects received AMN107A or placebo during the treatment period.

4.2.4.2 Sponsor's Justification for Design

Given the preclinical findings of hERG channel inhibition at concentrations below systemic exposure seen in patients, the sponsor had included intensive ECG monitoring in their phase 1 and phase 2 studies in CML patients. In addition, the sponsor was interested in further defining the potential for nilotinib to cause QTc prolongation under controlled conditions in healthy volunteers according to the ICH guidelines. However, nilotinib had only been given as single doses to healthy volunteers until that time. Given that the drug would have to be given as multiple doses to achieve near steady-state conditions, the first part of the study was defined as a dose-escalation safety study of QD and BID dosing of nilotinib. The sponsor had planned to conduct the thorough QT evaluation of nilotinib vs. placebo and moxifloxacin in Part II of the study.

4.2.4.3 Controls

In part 1 of study 2119, each cohort included 5 patients who received placebo in a manner identical to those that received the study drug.

4.2.4.4 Blinding

This was a single-blind study.

4.2.5 Population

The study enrolled one hundred and two (102) healthy male subjects, eighteen (18) to forty five (45) years of age, with a normal 12-lead ECG.

4.2.6 Study Treatments

4.2.6.1 Treatment Arms

Initially, three sequential dose-escalation cohorts were planned:
• Cohort 1: AMN107 400 mg QD (n=15) or placebo QD (n=5) for a total of 3 doses.
• Cohort 2: AMN107 400 mg BID (n=15) or placebo BID (n=5) for a total of 5 doses.
• Cohort 3: AMN107 600 mg BID (n=15) or placebo BID (n=5) for a total of 5 doses.

Dosing with 400 mg BID for 3 days was associated with reversible Grade 1 liver function test abnormalities in approximately 40% of the healthy volunteer subjects dosed. This was similar to the rate of Grade 1 liver function test abnormalities observed in patients with hematological malignancies after dosing to steady state at 400 mg BID. Based on this toxicity occurrence, this dose was not further investigated and the 600 mg BID dose (cohort 3) was not investigated.

The following cohorts were then evaluated with the goal of achieving concentrations of nilotinib that would be comparable to the steady-state levels seen in patients.
• Cohort 4: AMN107 400 mg QD (n=15) or placebo QD (n=5) for a total of 8 doses.
• Cohort 5 AMN107 800 mg QD (n=15) or placebo QD for a total 1 dose after a high fat meal.
• Cohort 6: AMN107 400 mg QD (n=15) or placebo QD for a total of 3 doses after a high fat meal.

4.2.6.2 Sponsor's Justification for Doses

The dose-escalation part of the study included the recommended clinical dose of 400 mg BID and a higher dose level to achieve concentrations that spanned the range of steady-state concentrations seen in the patients following the 400 mg BID dosing regimen. After the dose escalation phase ended, the remaining 3 cohorts were planned in an attempt to administer a lower dose for a longer duration (400 mg QD for 8 days) or with food (800 mg single dose with food and 400 mg BID for 3 days with food) to achieve concentrations similar to clinical concentrations seen in patients.

4.2.6.3 Instructions with regard to meals

As indicated above (section 4.2.6.1), cohorts 1, 2 and 4 were administered in the fasted state. Cohorts 5 and 6, by design, were administered the drug after a high fat meal.

4.2.6.4 Study Assessments

Table 3. Highlights of Schedule of Interventions for 3-day dosing regimens

Study Day	-1	1,2	3	4-7
Intervention	No treatment	Dosing QD or BID	Last dose	No treatment (washout)
12-Lead ECGs	Record ECGs[#] (Baseline)	Record ECGs[##]	Record ECGs[###]	None recorded
PK Samples for drug	None collected	Collected[##]	Collected[###]	None collected
Meal Instructions	None specified	Fasted	Fasted	None specified

#: On Day -1, ECGs at time 0 (pre-dose) and at 1, 2, 3, 4, 5, 6, 8, 12 h post-dose.
##: On Days 1 and 2, ECGs and PK at time 0 and at 3 h post-dose.
###: Starting on Day 3, ECGs and PK at time 0 and at 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, and 72 h post-dose.

Table 4. Highlights of Schedule of Interventions for 8-day dosing regimens

Study Day	-1	1-7	8	9-12
Intervention	No treatment	Dosing QD	Last dose	No treatment (washout)
12-Lead ECGs	Record ECGs[#] (Baseline)	Record ECGs[##]	Record ECGs[###]	None recorded
PK Samples for drug	None collected	Collected[##]	Collected[###]	None collected
Meal Instructions	None specified	Fasted	Fasted	None specified

#: On Day -1 and 1, ECGs at time 0 (pre-dose) and at 1, 2, 3, 4, 5, 6, 8, 12 h post-dose.
##: On Days 2, 3, 5 and 7, ECGs and PK at time 0 and at 3 h post-dose.
###: Starting on Day 8, ECGs and PK at time 0 and at 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, and 72 h post-dose.

4.2.7 Sponsor's Results

Originally planned as an initial ascending dose study followed by a positive-controlled (moxifloxacin) Thorough QT study, the second phase was not conducted. Thus, the study was a single-blind study with the following cohorts and randomized groups:

Cohorts	Groups	N
1	400 mg qd x 3 Placebo	15 5
2	400 mg bid x 5 Placebo	15 5
3	600 mg bid x 5 Placebo	Not done
4	400 mg qd x 8 Placebo	15 5
5/6	800 mg x 1 (fed) 400 mg qd x 3 (fed) Placebo	15 15 15

Cohort 3 was not done because of increases in LFTs observed with 400 mg bid. Subjects, normal males (predominantly Black; mean age of 30), were kept in hospital from baseline (day -1) through 7 days after the last dose. (12-lead) ECG triplicates and PK sampling were performed at times (h) 0, 1, 2, 3, 4, 5, 6, 8, and 12 after dosing at baseline and on the first day of dosing, at times 0 and 3 on intermediate days, and at 0, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, and 72 after the last dose. Digital ECGs were processed by [redacted]. Individualized QTc (QTcI) was based on a linear regression of each subject's baseline data.

4.2.7.1 Statistical Analyses

4.2.7.1.1 Primary Analysis

The ECG analyses were conducted in the ECG population, which included all subjects who received at least one dose of study drug and completed at least the Day –1 (baseline), and Day 3 (Day 8 for cohort 4) ECG sampling. Placebos from 3 cohorts (cohorts 1, 2, and 4) were pooled for a total of 15 placebo subjects for time-averaged comparison, and for 10 placebo subjects at Day 3 and 5 subjects at Day 8 for time point comparison with dose cohorts without food. Comparison for AMN treatment with food (cohorts 5 and 6) were relative to placebo with food with N=15 for each AMN treatment and placebo with food.

ECG measurements at each time point are an average of 3 ECG extractions or replicates. The measured ECG variables are the RR, PR, QRS, and QT intervals. Heart rate corrected variables are derived for QTcF by Friedericia's correction, QTcB by Bazett's correction. The individually corrected QT variable QTcI was derived by the equation log(QT)=a+b*log(RR) using the baseline QT-RR data. For each of the measured and corrected variables, the sponsor calculated baseline and change from baseline at each time point for each subject. The baseline values were calculated as either the average of ECGs performed on Day –1 at each specific time point or average of all time points 0 to 24 hr on Day –1.

The sponsor's primary analysis on ECG central tendency was based on a mixed effects model on change from baseline QTcF fitting terms for subject, treatment, time and treatment by time interaction, where subject was treated as a random effect variable. Baseline QTcF per time point was included as a covariate in the model. Results in Table 5 (study report Table 11-4) indicated a QTcF prolongation when the drug was administrated at 400 mg b.i.d. without food, or at 400 mg or 800 mg after a high fat meal.

Table 5. Placebo-subtracted mean difference from baseline (90% CIs) in QTcF by AMN107 treatments (Sponsor's results based on mixed effects model)

Hour (h)	400 mg o.d x 3 -Placebo	400 mg b.i.d x 3 -Placebo	400 mg o.d x 8 -Placebo	800 mg o.d. x 1 w/food -Placebo w/food	400 mg o.d. x 3 w/food -Placebo w/food
0	-8.4 (-15.80, -0.94)	5.9 (-1.65, 13.48)	-0.6 (-8.20, 6.65)	4.8 (-3.27, 12.93)	3.8 (-4.34, 11.85)
1	-6.9 (-14.29, 0.56)	6.4 (-1.17, 13.95)	-4.6 (-12.27, 2.59)	3.8 (-4.34, 11.88)	4.8 (-3.31, 12.90)
2	-4.9 (-12.32, 2.53)	10.4 (2.85, 17.97)	-5.4 (-12.83, 2.04)	6.2 (-1.87, 14.36)	10.1 (2.04, 18.23)
3	-4.0 (-11.42, 3.44)	4.8 (-2.79, 12.32)	-3.5 (-10.97, 3.89)	7.3 (-0.88, 15.46)	9.1 (0.99, 17.26)
4	-0.0 (-7.46, 7.39)	6.4 (-1.17, 13.94)	-3.2 (-10.58, 4.27)	17.0 (8.86, 25.04)	13.6 (5.47, 21.64)
5	-2.0 (-9.47, 5.39)	7.0 (-0.51, 14.60)	-7.1 (-14.51, 0.36)	17.7 (9.65, 25.64)	15.7 (7.66, 23.82)
6	-1.9 (-9.37, 5.50)	10.2 (2.61, 17.73)	-3.6 (-11.03, 3.83)	17.1 (9.01, 25.18)	10.3 (2.21, 18.37)
8	0.3 (-7.14, 7.71)	7.2 (-0.36, 14.74)	-3.4 (-10.87, 3.99)	12.8 (4.71, 20.91)	9.4 (1.34, 17.55)
12	1.5 (-5.96, 8.89)	2.6 (-4.93, 10.18)	1.7 (-5.73, 9.12)	15.9 (7.76, 23.95)	10.2 (2.07, 18.25)
24	-5.4 (-12.81, 2.05)	4.0 (-3.58, 11.54)	1.2 (-6.26, 8.60)	9.4 (1.30, 17.50)	7.0 (-1.08, 15.10)

Source: Table 14.2-1-2; Placebo is pooled from three cohorts (1, 2 and 4). The comparisons for AMN107 treatment with food (Cohorts 5 and 6) are relative to placebo with food.

Source: Study 2119 study report Table 11-4

The sponsor also presented the time-averaged results for the AMN treatments relative to placebo as shown in Table 5 (study report Table 11-7). The time-averaged placebo-subtracted QTcF change after food was 6.4 ms (90% CI: 2.1, 10.6) for the 400 mg dose and was 7.4 ms (90% CI: 3.2, 11.7) for the 800 mg dose.

Table 6. Statistical inference for Day 3 time averaged QTcF interval by treatment

Treatment Comparison	Estimate	90% Confidence Interval
400 mg o.d. x 3 days – placebo	-1.14	-5.29, 3.02
400 mg b.i.d. – placebo	6.06	1.84, 10.29
400 mg o.d. x 8 days – placebo	-5.06	-9.22, -0.09
800 mg x 1 with food - placebo with food	7.44	3.18, 11.71
400 mg o.d. x 3 days with food - placebo with food	6.37	2.12, 10.61

Placebo is pooled from three cohorts (1, 2 and 4). The comparisons for AMN107 treatment with food (Cohorts 5 and 6) are relative to placebo with food.

Source: Study 2119 study report Table 11-7

4.2.7.1.2 Categorical Analysis

The sponsor calculated the number and percentage of subjects in each treatment group who exceeded the upper limit values for change from baseline as suggested in the E14 guidance. The results for QTcF interval as shown in Table 7 (study report Table 11-10) do not indicate a good portion of individuals with extreme QTcF interval changes.

Table 7. Number (%) of subjects with notable QTcF values

Parameter	AMN107 400 mg o.d. n=15	AMN107 400 mg b.i.d x 3 D n=14	AMN107 400 mg o.d. x 8 D n=15	Placebo n=15	AMN107 800 mg o.d. x 1 D w/food n=14	AMN107 400 mg o.d. x 3 D w/food n=14	Placebo w/food n=15	Total n=102
Increase >30 (ms)	0	4 (28.6)	1 (6.7)	1 (6.7)	2 (14.3)	1 (7.1)	0	9 (8.8)
Increase >60 (ms)	None observed							
Abs. value >450 (ms)	None observed							
Abs. value >480 (ms)	None observed							
Abs. value >500 (ms)	None observed							

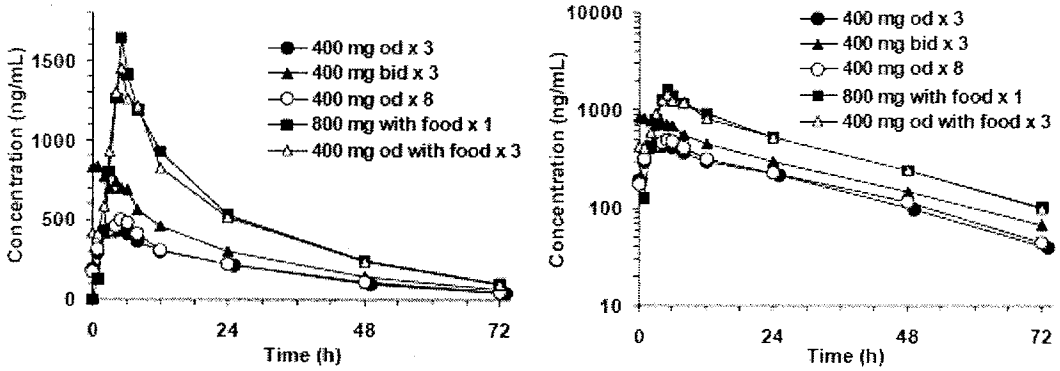
Source: Study 2119 study report Table 11-10

4.2.7.2 Clinical Pharmacology

4.2.7.2.1 Pharmacokinetic Analysis

The following figure shows the mean concentration-time profiles for the cohorts. The highest Cmax was seen in the 800 mg x1d with food cohort. However, the concentrations achieved were lower than the steady-state levels of nilotinib obtained in the phase 1 study of nilotinib in CML patients. Based on the PK analysis of patient data in the phase 1 study, the mean (± SD) Cmax at steady-state for the 400 mg BID regimen was 2260 (± 800) ng/ml.

Figure 1. Mean nilotinib concentration-time profiles (Left panel: linear scale, Right panel: log scale).



(sponsor's figure 11-8 on page 77 of study 2119 study report)

4.2.7.2.2 Exposure-Response Analysis

The concentration-QT relationship was evaluated for the data from study 2119 using linear mixed-effects modeling using SAS (version 9.0). The dependant variable was the placebo-corrected and (time-matched) baseline corrected QTcF (ΔΔQTcF). Since this was not a crossover study, the placebo correction was done using the average QTcF obtained in the placebo group. The sponsor conducted the concentration-QTcF analysis and the Agency repeated the analysis to verify the accuracy of the analysis.

To look for hysteresis in the relationship between QT and plasma levels of nilotinib, the reviewers examined plots of concentration-response (in this case, QTcI) for the 15 subjects who received the 800-mg dose. The four largest responses are shown in Figure 2 (QTcI) and Figure 3 (change from baseline in QTcI). At the upper end of the concentration range, there is no consistent pattern.

Figure 2. Examination of hysteresis in exposure-response (QTcI)

These four subjects had the largest absolute values in QTcI in the 800-mg group. Points are connected in the order the data were acquired. All of the curves start with a zero concentration. Data are from the dataset derived\a_ecg3pk.xpt.

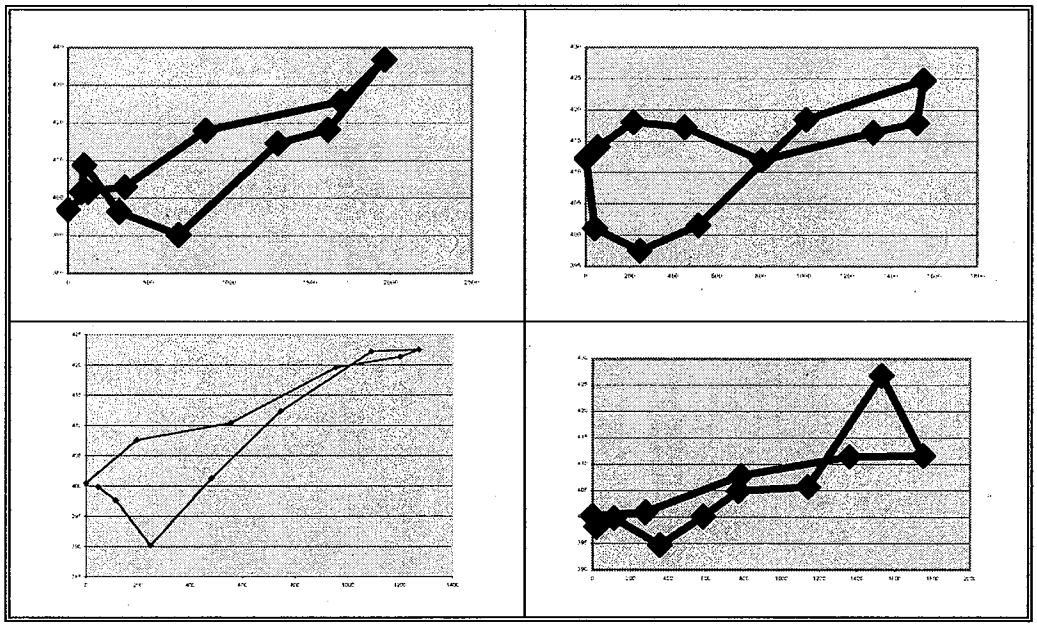
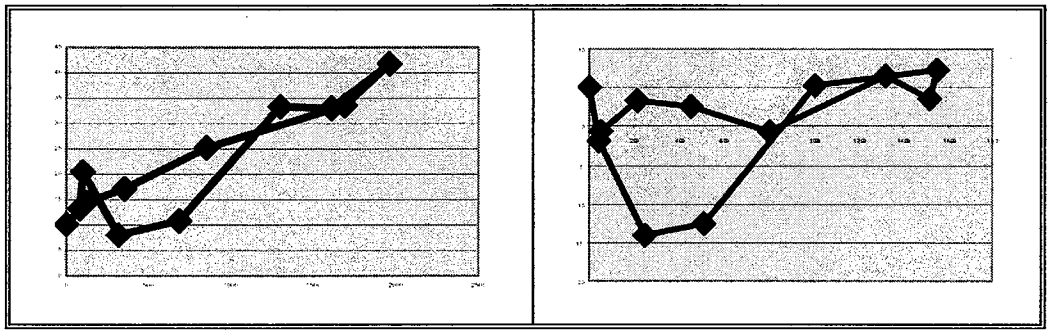
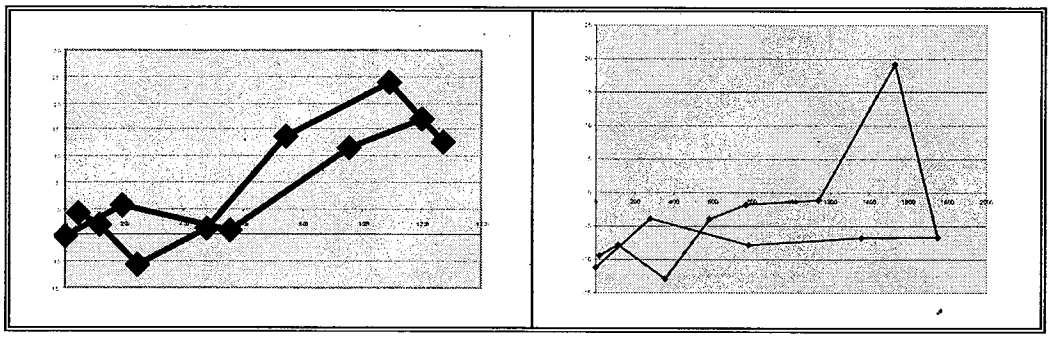


Figure 3. Examination of hysteresis in exposure-response (delta QTcI)

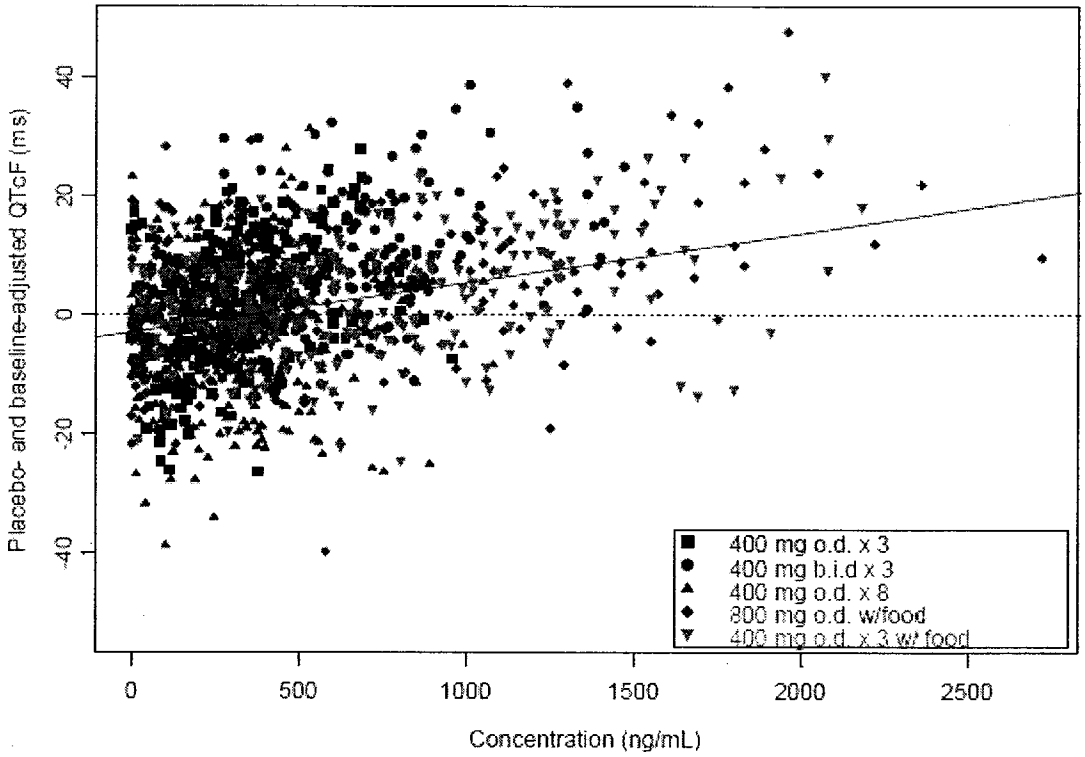
These are the same four subjects as in Figure 2. Data are from the dataset derived\a_ecg3pk.xpt.





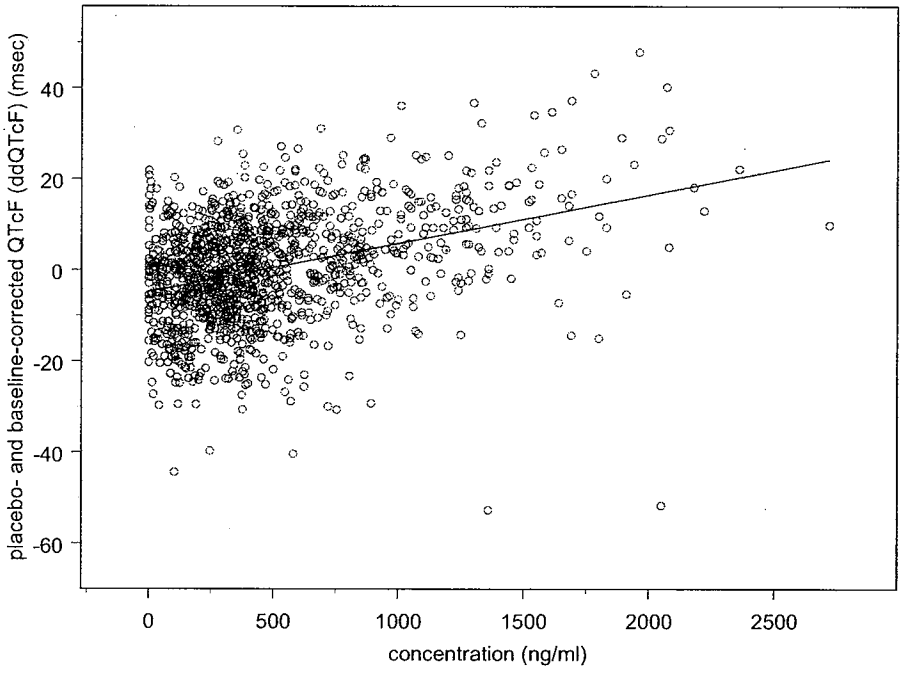
Results of the mixed-effects modeling showed a significant effect of concentration on the ΔΔQTcF. The following figures show the result of the sponsor's analysis and the Agency's analysis. Both analyses yielded nearly identical estimates for the slope of the ΔΔQTcF vs. concentration relationship:
Sponsor's estimate: 0.0084 ms per ng/mL of nilotinib.
Agency's estimate: 0.0085 ms per ng/mL of nilotinib (90% CI: 0.0064, 0.0107).

Figure 4. *AMN107 serum concentration (ng/mL) vs. placebo- and baseline adjusted QTcF (ΔΔQTcF) (ms) – Study 2119 – Sponsor's Analysis.*



(Sponsor's figure 11-5 page 72 of study 2119 study report)

Figure 5. *AMN107 serum concentration (ng/mL) vs. placebo- and baseline adjusted QTcF (ΔΔQTcF) (ms) – Study 2119 – Agency Analysis.*



4.3 EXPOSURE-RESPONSE ANALYSIS OF PHASE 1 AND PHASE 2 DATA

4.3.1 Exposure-Response Analysis of Phase 1 study data

The nilotinib concentration data and QTcF data derived from serial ECGs obtained from the patients in the phase 1 study (study 2101) in CML patients were examined for concentration-QT relationships. The following table summarizes the number of patients and dose levels evaluated in this study.

Table 8. Number of patients and dose levels evaluated in the phase 1 study 2101.

	QD dosing		BID dosing		Total
Dose Groups	50 mg/day 100 mg/day 300 mg/day 400 mg/day	600 mg/day 1200 mg/day	400 mg BID	600 mg BID	
Number of Patients	34	35	32	18	119

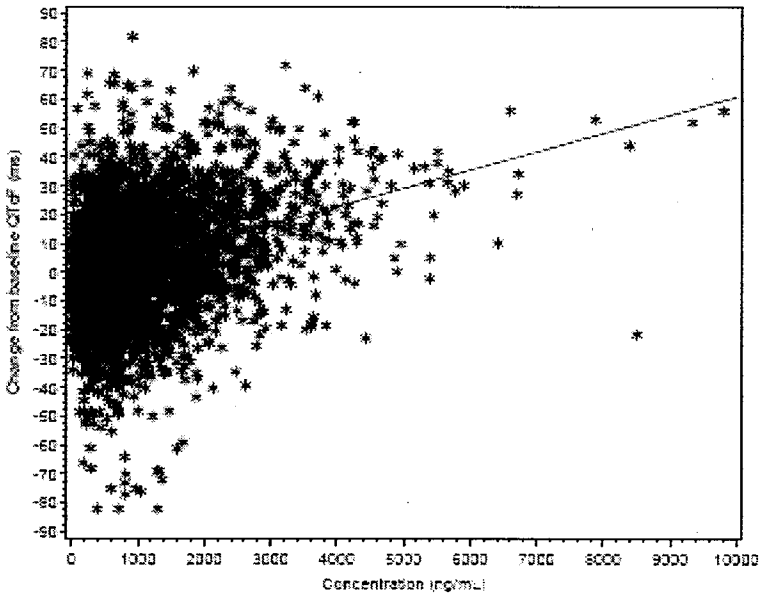
ECG measurements were made at the following times:
For once-daily dosing: Days 1 and 15: pre-dose and 1, 2, 3, 4, 7, 10 and 24 hrs, Days 8 and 22: pre-dose, and Day 28: pre-dose and 1, 3, 5, 9 and 24 hrs.
For twice-daily dosing: Days 1, 8 and 15: pre-dose, 1, 2, 3, 5, 8 and 12 hrs, Days 22 and 28: pre-dose.

The concentration data and QTcF data were time-matched, in that ECG measurements were taken within 60 minutes of the corresponding nilotinib pharmacokinetic sampling. There was no time-matched baseline data and no placebo data, so each individual's QTcF data were subtracted from the individual's averaged baseline ECG measurements. The baseline assessment included 6 sequential 12-lead ECGs, separated by at least 5-10 minutes, performed on day 1 of Cycle 1 prior to the first administration of nilotinib.

A linear mixed-effect model was employed with the nilotinib serum concentration and QTcF data pairs. The sponsor conducted the concentration-QTcF analysis and the Agency repeated the analysis to verify the accuracy of the analysis.

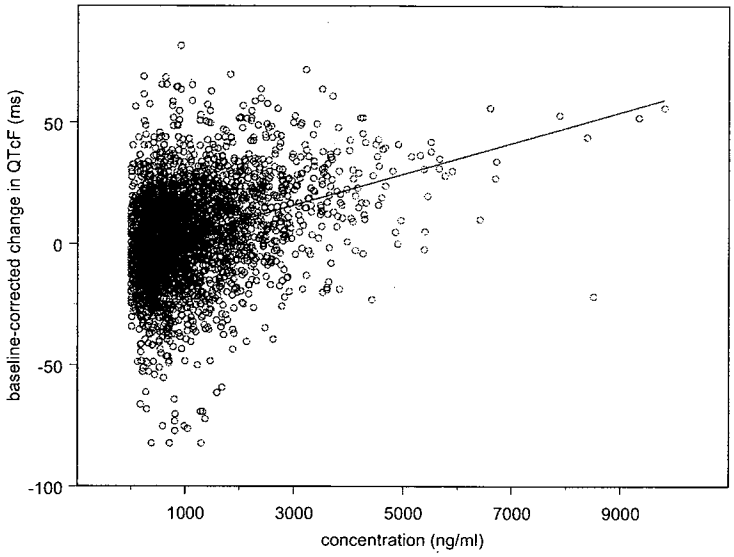
Results of the mixed-effects modeling showed a significant effect of concentration on the ΔQTcF. The following figures show the result of the sponsor's analysis and the Agency's analysis. Both analyses yielded nearly identical estimates for the slope of the ΔQTcF vs. concentration relationship:
Sponsor's estimate: 0.0064 ms per ng/mL of nilotinib.
Agency's estimate: 0.0064 ms per ng/mL of nilotinib (90% CI: 0.005387, 0.007413).

Figure 6. *AMN107 serum concentration (ng/mL) vs. baseline adjusted QTcF (ΔQTcF) (ms) – Study 2101 – Sponsor's Analysis.*



(Sponsor's figure 11-12 page 61 of study 2101 Phase 1 study report)

Figure 7. *AMN107 serum concentration (ng/mL) vs. baseline adjusted QTcF (ΔQTcF) (ms) – Study 2101 – Agency Analysis.*



4.3.2 Exposure-Response Analysis of Phase 2 study data

The ECG and PK data obtained in the phase 2 study of nilotinib in CML patients (in accelerated phase and in chronic phase) were also evaluated using linear mixed effects analysis. In both these studies, the dose was 400 mg BID.

In both phase 2 studies, four PK samples were collected on day 8 or later (at steady-state) during the first cycle. ECG measurements were obtained on day 1 (pre-dose, 1, 3 and 5 hrs) and day 8 (pre-dose, 1-3 hrs, 5-7 hrs and 9-12 hrs) during the first cycle.
The concentration data and QTcF data included in the analysis were time-matched, in that ECG measurements were taken within 60 minutes of the corresponding nilotinib pharmacokinetic sampling. This resulted in available data from 39 (of 89) patients in the AP cohort, and 152 (of 282) patients in the CP cohort.

There was no time-matched baseline data and no placebo data, so each individual's QTcF data were subtracted from the individual's averaged baseline ECG measurements. The baseline assessment included 3 sequential 12-lead ECGs, separated by at least 5-10 minutes, performed on day 1 of Cycle 1 prior to the first administration of nilotinib.

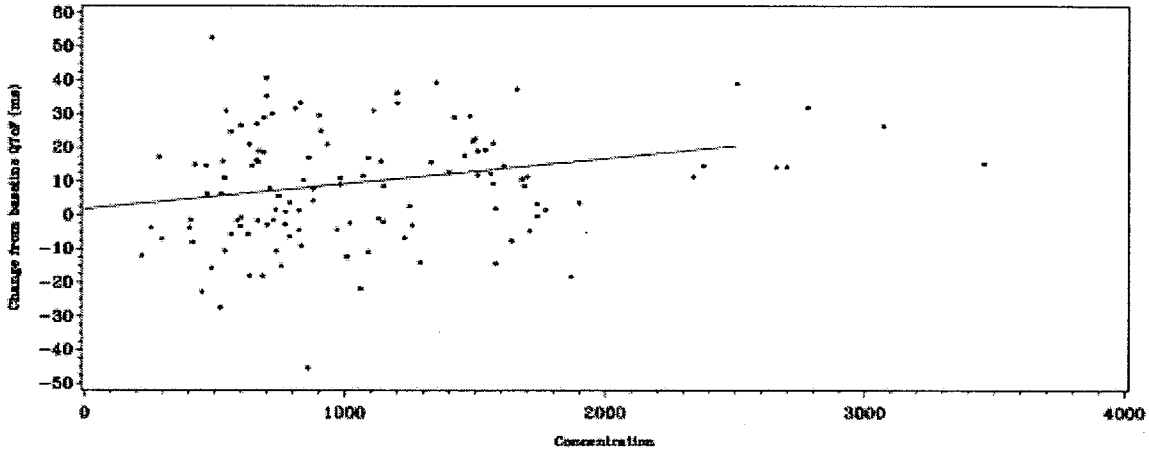
The following figures show the results of the sponsor's analysis of these data. There was a significant association between concentration and change in QTcF for both datasets. The baseline QTcF was included in the sponsor's model as a covariate.

The slope of the relationship for the accelerated phase CML patients was 0.0075 ms per ng/ml of nilotinib.

The slope of the relationship for the chronic phase CML patients was 0.0040 ms per ng/ml of nilotinib.

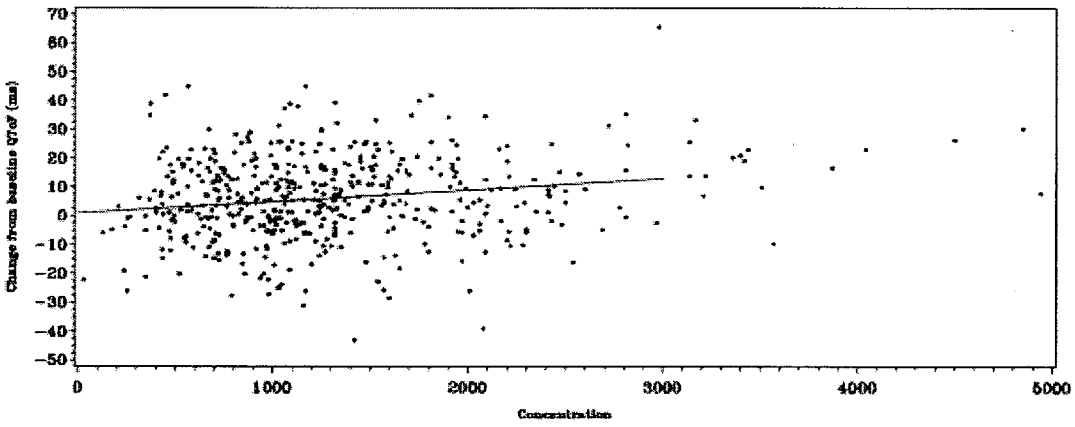
These estimates are consistent with the findings of the study in healthy volunteers as well as the phase 1 study in patients.

Figure 8. *AMN107 serum concentration (ng/mL) vs. baseline adjusted QTcF (ΔQTcF) (ms) in Accelerated Phase CML patients – Phase 2 Study 2101E1 – Sponsor's Analysis.*



(Sponsor's figure 12-2 page 140 of study 2101E1 Phase 2 study report)

Figure 9. *AMN107 serum concentration (ng/mL) vs. baseline corrected QTcF (ms) in Chronic Phase CML patients – – Phase 2 Study 2101E2 – Sponsor's Analysis.*



(Sponsor's figure 12-2 page 144 of study 2101E2 Phase 2 study report)

The concentration-QTc relationship was also analyzed by the Agency using linear mixed effects modeling. The results of the analysis are consistent with the sponsor's analysis with significant associations seen between concentration and change in QTcF from baseline.

Figure 10. *AMN107 serum concentration (ng/mL) vs. baseline corrected QTcF (ms) in Accelerated Phase CML patients – Phase 2 Study 2101E1 – Agency's Analysis.*

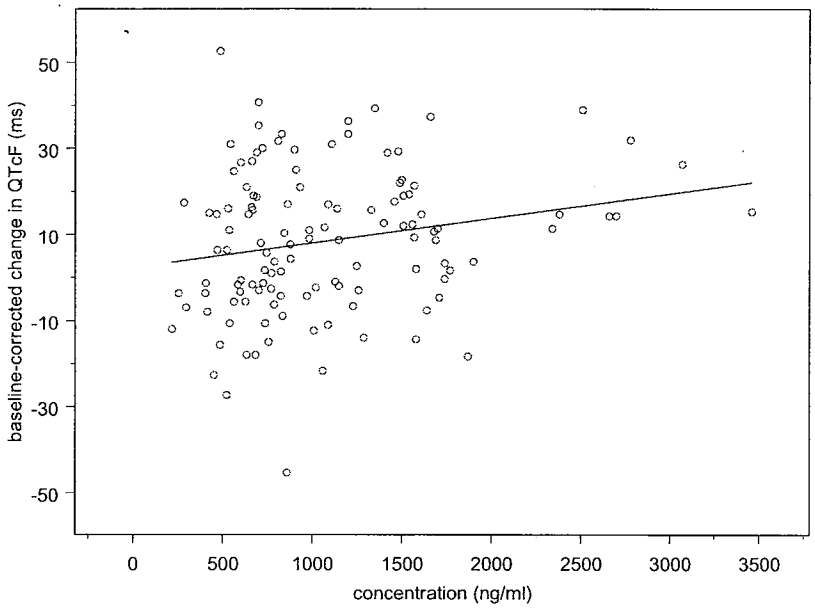
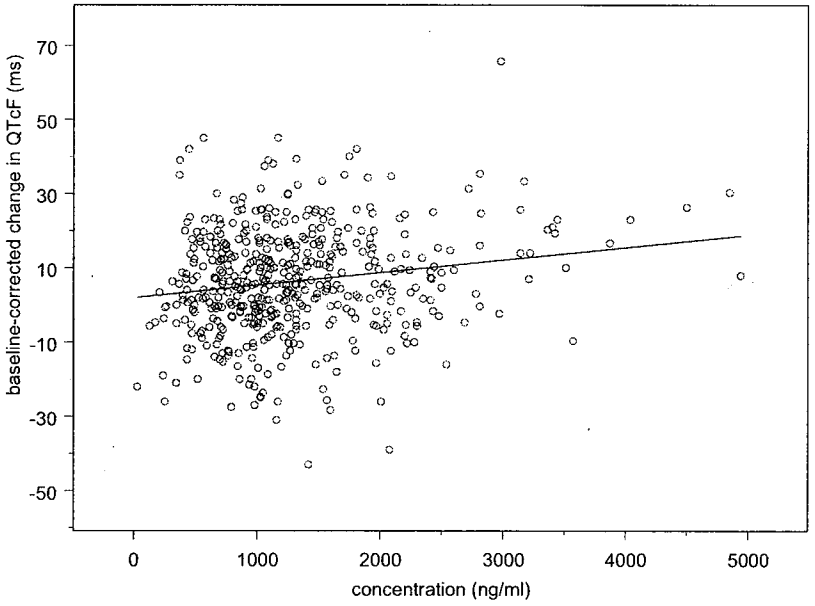


Figure 11. *AMN107 serum concentration (ng/mL) vs. baseline corrected QTcF (ms) in Chronic Phase CML patients – Phase 2 Study 2101E2 – Agency's Analysis.*



The slope of the relationship for the accelerated phase CML patients was 0.0046 ms per ng/ml of nilotinib (90% CI: 0.0028, 0.0064).

The slope of the relationship for the chronic phase CML patients was 0.0038 ms per ng/ml of nilotinib (90% CI: 0.0021, 0.0054).

5 REVIEWERS' ASSESSMENT

5.1 STATISTICAL

In comparison to the model based treatment difference (active-placebo), the reviewer calculated the mean and 90% confidence interval for difference between treatment and placebo based on observed mean and standard deviation of change from baseline QTcF by dose cohort and placebo as reported by the sponsor in study report Table 14.2-2.2. The results as shown in Table 12 suggest a QT prolongation in all dose cohorts with the 90% upper confidence limit >10 ms at least one post-dose time point. However, theses observed treatment differences do not further adjust for baseline values as the mixed model does.

Table 9. Placebo-subtracted mean difference from baseline (90% CIs) in QTcF by AMN107 treatments (Reviewer's results based on observed group mean and standard deviation)

Hour (h)	400 mg o.d. × 3 (n=15) -placebo (n=10)	400 mg b.i.d × 3 (n=14) -placebo (n=10)	400 mg o.d. × 8 (n=15) -placebo (n=5)	800 mg o.d. × 1 w/food (n=14) -placebo w/food (n=15)	400 mg o.d. × 3 w/food (n=14) -placebo w/food (n=15)
0	-5.44 (-12.3, 1.44)	11.46 (4.55, 18.37)	2.03 (-7.98, 12.04)	-2.63 (-8.09, 2.83)	-3.25 (-8.41, 1.91)
1	-5.85 (-14.4, 2.67)	5.95 (-1.97, 13.87)	-5.16 (-18.2, 7.91)	-1.89 (-8.55, 4.77)	-0.44 (-6.52, 5.64)
2	-7.61 (-15.3, 0.09)	5.28 (-3.67, 14.23)	-5.07 (-14.5, 4.34)	0.36 (-5.70, 6.42)	5.98 (0.91, 11.05)
3	-1.24 (-10.3, 7.79)	4.05 (-5.12, 13.22)	-1.86 (-13.4, 9.68)	-2.29 (-9.54, 4.96)	1.43 (-5.27, 8.13)
4	-0.84 (-8.98, 7.30)	2.97 (-5.00, 10.94)	-1.02 (-14.3, 12.23)	13.65 (4.65, 22.65)	10.32 (3.43, 17.21)
5	-0.87 (-8.36, 6.62)	5.64 (-2.46, 13.74)	-8.31 (-18.7, 2.10)	13.64 (6.68, 20.60)	14.06 (7.65, 20.47)
6	-0.20 (-6.02, 5.62)	7.15 (1.71, 12.59)	-1.49 (-15.9, 12.97)	14.42 (7.91, 20.93)	8.31 (-1.80, 18.42)
8	1.32 (-5.63, 8.27)	2.60 (-4.05, 9.25)	-3.98 (-19.6, 11.68)	8.38 (0.20, 16.56)	4.38 (-3.02, 11.78)
12	5.84 (-0.90, 12.58)	0.31 (-7.33, 7.95)	0.71 (-4.57, 5.99)	11.76 (6.54, 16.98)	6.90 (-2.36, 16.16)
24	0.61 (-6.46, 7.68)	5.87 (-1.00, 12.74)	-4.74 (-14.9, 5.43)	4.74 (-2.50, 11.98)	3.86 (-2.85, 10.57)

Source Data: Study 2119 study report Table 14.2-2.2

Hour represents the post-dose assessment time point on Day 3 all dose cohorts except 400 mg o.d. × 8d cohort, which started post-dose assessment on Day 8.

Placebo without food is pooled from cohorts 1 and 2 for Day 3, and placebo without food is from cohort 4 for Day 8. The comparisons for AMN107 treatment with food (Cohorts 5 and 6) are relative to placebo with food.

5.2 CLINICAL PHARMACOLOGY

The following table summarizes the slope estimates obtained from the Agency's analysis of the study in healthy volunteers, the phase 1 study and the two phase 2 study groups. The table also includes the predicted prolongation at the mean Cmax of 2260 ng/ml seen for the clinically recommended dosing regimen of 400 mg BID.

Table 10. Estimates of slope of concentration-QT relationships for Agency's Analysis of data in healthy volunteers, phase 1 and phase 2 study groups.

Study/Sample	Intercept of conc-QT relationship	Slope of conc-QT relationship using time matched BL correction and placebo corrected QTcF (Estimate, 90% CI)	Predicted mean QT prolongation based on slope estimate	Predicted QT prolongation based on 90% confidence limit of slope
2119: Healthy volunteers	-3.44	0.0086 (0.0063, 0.0107)	15.8	(11.2 to 20.5)
Study/Sample	Intercept of conc-QT relationship (Estimate, 90% CI)	Slope of conc-QT relationship using within-day BL corrected QTcF (Estimate, 90% CI	Predicted mean QT prolongation based on slope estimate	Predicted QT prolongation based on 90% confidence limits of slope
2101 phase 1: CML patients	-3.17	0.0065 (0.0054, 0.0075)	11.4	(8.7 to 14.1)
2101E1 phase2: CML-AP patients	3.88	0.0046 (0.0028, 0.0064)	14.3	(10.2 to 18.3)
2101E2 phase 2: CML-CP patients	1.09	0.0038 (0.0021, 0.0054)	9.7	(5.8 to 13.3)
2101 phase 2: combined	1.56	0.0040 (0.0024, 0.0056)	10.6	(8.5 to 12.8)

The predictions obtained in the phase 1 and phase 2 are slightly lower than the predictions based on the relationship in healthy volunteers, and may be due to differences between the two studies with regard to the populations studied, lack of placebo control in the phase 1 and phase 2 studies, use of pre-dose baseline correction in the phase 1 and phase 2 studies rather than time-matched baseline correction used in study 2119.

Despite the differences, the results of analysis of data across studies indicate a significant prolongation of the QTc interval at peak steady-state concentrations corresponding to the clinically recommended dosing regimen of 400 mg BID.

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Memorandum

FROM: Albert DeFelice, Ph.D, Pharmacology Team leader
Division of Cardiovascular and Renal Products

THROUGH: Norman L. Stockbridge, M.D., Ph.D., Director
Division of Cardiovascular and Renal Products

TO: Karen A. Hicks, M.D., Medical Officer
HFD-110, Division of Cardiovascular and Renal Products

Christy Cottrell
Consumer safety Officer
HFD-150, Division of Drug Oncology Products

SUBJECT: Consult to QTGroup: NDA 22-068: Tasigna (nilotinib); Malignancy; Serial No. 000, Submitted by Novartis Sept. 29, 2006

DUE DATE: February 16,, 2006

COMPLETED: January 24, 2007

This consult - a brief overview ancillary to the medical consultative review of the thorough QTc clinical trial (2119) of this oncolytic, and to other CV safety deliberations as well - focuses on: *in vitro* effects of AMN107(nilotinib) and its metabolite BJA783 on I_{Kr} current through hERG potassium channels in transfected human embryonic kidney cells (**studies 0380166 and 0616144);** on electrophysiology of isolated rabbit

heart (studies **0618514** and **0350152**); GLP-compliant cardiovascular safety study in the dog (single dose, telemetry: **study 0380165**); in vitro effects on platelet and coagulation biomarkers (study no. **0680135**); and vasoactivity (human coronary and subcutaneous arteries: study no. **0680132**)

Summary:

Based on sponsors assertion that the therapeutic blood level range is 1-10 μg /ml, the concentration- related decrease in hERG current by up to 90% ($IC_{50\%}$ = 0.07 μg/ml); the prolongation of isolated rabbit heart APD at ≥ 1.6 μg/ml; and the concentration related (1-10 μg/ml) adverse effects on human platelet "aggregability" and expression of platelet surface receptors has cardiovascular safety implications *prima facie* and *a priori.* However, in a 4-week study in conscious trained dogs, there were no adverse cardiac electrophysiological effects , including QTc and QRS interval parameters, at up to 45 mg/Kg p.o.which afforded blood levels up to 1-2 μg/ml. Cardiac electrophysiological effects were also absent in a telemetered conscious dog study at up to 300 mg/Kg po (blood levels not monitored). A positive QTc-prolonging control was not included in any of the studies overviewed with the exception of the hERG assay where terfenadine behaved as expected.

A. Effect on hERG current in transfected human embryonic kidney cells (HEK293).

1. AMN107: **Study no. 0380166**. Performed by [redacted]

Methods/test system comments:

The mammalian cells used are preferred over *Xenopus* oocytes. The hERG channel was deliberately expressed in a human embryonic kidney cell line lacking endogenous hERG channels. Expression in a mammalian cell line is preferred to transient expression in *Xenopus* oocytes which consistently show 10-100 fold lower sensitivity to hERG channel blockers (Rampe *et al,* 1997)

The hERG current (I_{Kr}) was recorded in the context of HEPES –buffered PSS containing 0.3% DMSO, and both at room temp. and at 35° C. The DMSO ([redacted]) used at 0.3% as vehicle is asserted to have no effect on such current at ≤ 0.3% concentrations (DMSO data said to be on file at [redacted]). The study report is silent regarding limits of solubility, and stability of test compound in this medium/temperature.

Samples of each concentration of test article were obtained and frozen. However they were subsequently discarded without analysis to confirm targeted strength.

Certificate of analysis indicates [redacted] drug content, and necessity to protect from light.

Results: Over the concentration range of 0.01 -1.0μM (n=3/conc.), AMN107 dose-dependently inhibited hERG peak tail current (over 2 sec. at clamped -50 mV) up to 90%, with an $IC_{25\%}$ and $IC_{50\%}$ of 0.04 and 0.13 μM, respectively, and temperature independence between room temp. and 35° C. The positive control (60 nM terfenadine) reduced hERG current by 79%,.

Conclusions:*Prima facie*, this is persuasive evidence of relatively potent inhibition of I_{kr} (the rapidly acting delayed rectifier cardiac potassium current).

2. Metabolite BJA783: **Study no. 0616144.** Study site not specified in report. This metabolite of AMN107 was tested at a single high concentration of 30 μM (n=4) vs. vehicle (0.1% DMSO) and positive control (100nM of E-4031). BJA783 non-significantly reduced hERG tail current by 5% (vehicle-corrected) vs. 0.3% for vehicle and 93% for E-4031. Sponsor concludes that this metabolite did not significantly inhibit residual hERG current. The absence of effect of DMSO vehicle supports sponsors contention in study 0380166 above of utility of this vehicle if used at ≤ 0.3%.

B. Electrophysiological studies in isolated rabbit heart.

Comments on selection of species/model/parameters to be monitored:
Rabbit heart was used because, like the human, the main repolarizing current in this species is I_{Kr} i.e., the rapid component of the delayed rectifier K^+, I_K, there being little I_{Ks} (Nattel, 1999). Moreover, agents which prolong clinical QT interval will lengthen APD in the rabbit heart; those which widen clinical QRS slow conduction in the rabbit heart (Hondeghem, *et al*, 2003). Hondeghem and Hoffman (2003) reported that APD changes and associated TRIaD status(see below) could, in the rabbit, be used as a biomarker for projecting risk of clinical TdP (also Valentin *et al*, 2004). Utility of TRIaD in projecting proarrhythmogenicity is advocated elsewhere as well (Antzelevitch, et al, 1998; Wisialowski et al, 2006 inter *alia*). Females were used as heart donors as they are reported to be more vulnerable to TdeP as is the case clinically (Ebert et, 1998 and Lu et al, 2001).]

1. Studies of AMN107
Two studies were performed with AMN107 at up to the limit of solubility - 0.5 μM in one study (no. 0618514), and 18- 30 μM in the other (no. 0350152). Both studies revealed, in the 0.3-0.5 μM range, decreases in perfusate flow (arteriolar vasoconstriction), but no electrophysiologic effects. However, at ≥ 3 μM, APD was prolonged, and shape of the action potential changed (triangulation) both of which justify a thorough clinical QTc study. The concentration-effect curve, especially the thresholds and slope, for the positive findings in study no 0350152 is suspect, however: study # 0618514 reports 0.5μM as the limit of solubility in sodium bicarbonate/ sodium phosphate-buffered physiological salt solution at even a slightly higher temperature (36-37° C).

a. Study no. 0618514: Electrophysiological Investigations in Isolated Rabbit Hearts
[redacted] Non-GLP i.e., neither periodic internal inspections nor final audit by QA was performed. However, a signed statement of quality standard asserts accuracy and integrity of the data.

Electrophysiological effects were studied in isolated hearts from female rabbits (n=6) using the Langendorff technique which is familiar to this reviewer. They were perfused with a physiological salt solution (PSS). The latter was buffered with sod. bicarbonate and sod. phosphate to a pH of 7.35, bubbled with 95% O_2 and 5% CO_2, and maintained at 36-37 °C. Limit of solubility under these conditions was in the range of 0.15-0.5μM. After equilibration, hearts were exposed for 30 min. to 0.005-0.5μM AMN107 (n=6) and parameters monitored at each conc. included coronary flow; action potential duration at 30, 60 ,and 90% recovery from depolarization (i.e., $APD_{30, 60, or 90}$); automaticity and escape cycle length; ectopic activity; threshold stim. current; conduction time; and beat-to-beat variability in septal vs. epicardial APD_{60} as a measure of dispersion of repolarization. Triangulation (APD90-APD30), reverse use dependence, and instability (beat-to-beat variability in APD) were monitored as this triad is associated with prolongation of APD and leads to dispersion. Number of early after depolarizations and TdeP were monitored with an automated computer algorithm.
Results: At up to 0.5μM (limit of solubility in the PSS)), the coronary perfusion rate was significantly decreased (evidence of vasoconstriction as the Langendorff technique involves low perfusion pressures and is sensitive to constriction of the coronary art. bed). It is asserted that no electrophysiological effects were detected in any of the monitored parameters including the triad defined above, ectopic activity, or on ventricular depolarization. However, one run of ventricular fibrillation developed. However, sponsor believes this does not reflect proarrythmic potential because neither APD change nor triangulation/ reverse use dependence/ instability were manifest; rather micro-emboli induced by drug precipitation at that concentration is offered as the reason.
Conclusions: *Over the concentration range tested (i.e., up to the 0.5μM limit of solubility)*, the absence of TRIaD (triangulation; reverse use dependance; or instability) is reassuring sinceTRIaD in the presence of a normal, or especially, decreased APD is reported to be highly pro-arrhythmic scenario (sponsor provides multiple references). However, in the presence of 0.5μM nilotinib, the coronary perfusion rate significantly decreased, and one run of ventricular fibrillation developed in 1/6 hearts.

b. Study no. 0350152: Electrophysiological Investigations in the Isolated Rabbit Heart [redacted] Non-GLP i.e., neither periodic internal inspections nor final audit by QA was performed. However, a signed statement of quality standard asserts accuracy and integrity of the data.

Electrophysiologic effects were studied in isolated hearts from female rabbits (n=3) using the Langendorff technique. As indicated in the variability of results noted below, this may be too few animals to afford an appreciation of extent and range of effect. They were perfused with a physiological salt solution (PSS). The latter was buffered with sod. bicarbonate and sod. phosphate to a pH of 7.35, bubbled with 95% O_2 and 5% CO_2, and maintained at 34 °C. Limit of solubility under these conditions was less than 18μM since "the preparation started to deteriorate from 18μM due to the precipitation of the compound into the system"
After equilibration, hearts were exposed to 0.3- 30μM AMN107, and parameters monitored included coronary flow; conduction velocity; action potential duration at 30, 60 ,and 90% recovery from depolarization (i.e., $APD_{30, 60, or 90}$); TRIaD (Triangulation

=APD_{90}-APD_{30}; reverse use dependence, and instability=beat-to-beat variability in APD); early after depolarizations;TdeP; and Poincare plots (APD plotted vs. immediately priorAPD.

Results: Perfusion was decreased at ≥ 0.9 μM (1/3) and at 18 μM (1/3). APD was prolonged at ≥ 3 μM (1/3 hearts) and 9 μM (2/3 hearts). Triangulation (APD_{90}-APD_{30} value significantly increased) also developed at ≥ 3 μM; and instability at 18 μM (1/3) or 30 μM (1/3).
The compound precipitated at a concentration of 18 μM in two preparations, and at 30 μM in one preparation. Sponsor does not address the marked difference in solubility of their compound between this study and study Study no. 0618514 despite comparable composition, pH, and temperature of the perfusate. Although their compound was more soluble in this study, the temperature was actually several degrees (34°C vs 36-37 °C in study no. 0618514.
Conclusions: No significant electrophysiological effects at up to 0.3 μM (0.16 μg/ml), which confirms results from study 0618514 above. The primary effect was to prolong APD at a threshold of 3-9 μM. Instability developed at 18 -30 μM. The ostensible threshold for the positive electrophysiological effects i.e., ≥ 3 is moot since that would have exceeded the threshold for solubility observed in the prior study – namely 0.5 μM.

2. Metabolite BJA783: Study 0618547 Electrophysiological Investigations in the isolated rabbit heart. [redacted] Non-GLP i.e., neither periodic internal inspections nor final audit by QA was performed. However, a signed statement of quality standard asserts accuracy and integrity of the data.

This metabolite was tested at up to 2 μM in six rabbit (female) hearts using the Langendorff technique, and monitoring as above which prominently included $APD_{60\%}$ and TRIad as defined above.
Results: This metabolite did not affect any of the electrophysiological parameters, - including APD, reverse use - dependency, instability, and triangulation - at up to the maximum concentration tested. results are consistent with absence of effect on hERG channel at 30 μM noted above (study no. 0616144)

C. *In vivo* cardiovascular assessment:

1. Study no. 0380165: A pharmacological assessment of the oral (gavage) administration of AMN107 on the cardiovascular system of the conscious telemetered male beagle dog.

Methods:
[The beagles, because they were used after a three week wash-out from a prior study, were already instrumented for telemetric CV monitoring and acclimated to the test environment. Only dogs with normal ECG, hematology, and clinical chemistries were "recruited."]
After acclimation to the oral gavage procedure, eachof 4 dogs, except the single control animal, was treated with a single dosage, at approx. 5-10 day intervals, of 30, 100, and 300 mg/kg. p.o., and with a 24-hr post-treatment observation period. Parameters monitored included clinical signs, and blood pressure (BP), heart rate (HR), and

electrocardiogram (ECG) intervals (PR, RR, QRS, QT and Van de Water –formula-corrected QT_C. Base line hematology and clin. chem. was monitored to assure participation of healthy specimens. Repeated measures ANOVA tested for statistically significant perturbations. Drug assays assured chemical stability of the test substance under the experimental conditions of the study.
Results: According to Sponsor, there were no test article related clinical, hemodynamic, or electrocardiographic alterations when considered as mean, or individual, changes. Lack of biologically important change in HR obviates the need for correction, although, as pre-specified, the van de Water algorithm for such correction was used. Reviewer confirmed lack of effect by examining mean changes only; other than BP and HR data, individual animal data for ECG parameters was not provided in the report.
PK: The report is silent on blood levels achieved in the course of this oral study, although stability in the vehicle (0.5% HPMC), as used, is documented.

2. Other in vivo CV studies (dog: GLP-compliant **study 0370147; monkey;** GLP-compliant study **0370147):**
According to the non-clinical overview provided to me (full study report not provided), no effects were seen in ECG measurements in the course of 4-week exposure to dogs at up to 45 mg/Kg [which afforded 2-4 μM max. blood concentrations i.e., 1200ng/ml in males; 2040 ng/ml in females), and 39 week exposure at up to 600mg/Kg in monkeys [maximum blood levels achieved were not provided].
The peak blood levels achieved in the canine study , namely 2-4 uM, exceeded by 20-40 fold the IC50% for inhibiting hERG channel current (0.13 uM.), and approached the threshold for prolonging APD in the isolated rabbit heart ((3 uM : study no 0350152).

D. Vasoactivity:
Study 0680132: safety study to assess test compound (s) activity in human subcutaneous resistance arteries and coronary arteries.

Methods: Changes in isometric tension in human sc and coronary arteries (surgically extracted) were monitored in vitro during exposure to AMN107 at up to 10 uM, and dissolved in DMSO (AMN107 is not soluble in PSS). Responses both to this and to reference agents (10 uM angiotensin II; 1 uM nor-epinephrine) were normalized as % contractile response to 65 mM K^+.
Results. No concentration-related vasoactivity of AMN107 on sc arterioles at up to 10 uM was manifest vs. expected strong contractile response to angiotensin II and norepinephrine. Lack of such activity was also revealed on the coronary arteries where mild spasm (29 % ref. response to high K+) on coronary arteries was observed at 3 uM but not at 1 or 10uM.
Conclusions. These essentially negative findings of concentration-related ability to promote isometric tension generated by arterial vascular smooth muscle *in vitro* do not project risk of vasoconstrictor activity *in vivo* in the corresponding intact arterial beds.

E. Coagulation and thrombosis:

Study 0680135: *In vitro* effects on platelet and coagulation biomarkers in human subjects

This testing of effects on platelet function, coagulation, and fibrinolysis biomarkers (PCFB) was performed because AMN107 is a tyrosine kinase inhibitor, and protein tyrosine kinase is recognized as playing a critical role in platelet function. Platelet activation is accompanied by a large increase in tyrosine phosphorylation of a variety of signaling proeins.

Blood was obtained from 20 healthy volunteers, and incubated for 1 hour with AMN107 present at 1, 3, or 10 ug/ml vs untreated control blood. Such concentrations are stated to be within the targeted therapeutic range. Effect on platelet aggregation provoked by ADP and collagen, and on flow cytometry were assessed.

Results:

Over the concentration tested, a mild but significant promotion of platelet activation by ADP and collagen was observed as was the expression of *six* PCFB markers including platelet surface receptors P-selectin, GP IIb/IIIa, PECAM-1, and PAR-1.However, Sponsor asserts that the changes in platelet function and morphology, albeit statistically significant, did not exceed the laboratory reference range either in healthy volunteers or in volunteers carrying multiple risk factors for coronary artery disease.

Comment:

The enhanced platelet aggregability and increased expression of platelet surface receptors, including CD31, CD41, CD62p, and WEDE-15 are established predictors of increased risk of coronary vascular events. Although the changes in platelet function and morphology may not be egregious, they clearly are in the "wrong direction".

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Christine Garnett
10/23/2007 10:47:37 AM
BIOPHARMACEUTICS
Roshni Ramchandani was the clinical pharmacology reviewer.

Norman Stockbridge
10/23/2007 01:25:00 PM
MEDICAL OFFICER

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION	Office of Clinical Pharmacology Genomics Group Office of Clinical Pharmacology White Oak Building 21 Tracking/Action Sheet for Formal/Informal Consults

From: **Michael Orr, PhD**			**To: DOCUMENT ROOM (LOG-IN and LOG-OUT)** **Please log-in this consult and review action for the specified IND/NDA submission**	
DATE: 7-9-2007	IND No.: Serial No.:	NDA No. **22068**	Document ID:	DATE OF DOCUMENT **September 11, 2006**
NAME OF DRUG **Nilotinib (AMN107)**	PRIORITY CONSIDERATION ☐ Yes ☐ No ☐ N/A			Date of informal/Formal Consult:

NAME OF THE SPONSOR: **Novartis**

TYPE OF SUBMISSION

CLINICAL PHARMACOLOGY/BIOPHARMACEUTICS/GENOMICS RELATED ISSUE

☐ PRE-IND	☐ DISSOLUTION/IN-VITRO RELEASE	☐ FINAL PRINTED LABELING
☐ ANIMAL to HUMAN SCALING	☐ BIOAVAILABILITY STUDIES	☐ LABELING REVISION
☐ IN-VITRO METABOLISM	☐ IN-VIVO WAIVER REQUEST	☐ CORRESPONDENCE
☐ PROTOCOL	☐ SUPAC RELATED	☐ DRUG ADVERTISING
☒ PHASE II PROTOCOL	☐ CMC RELATED	☐ ADVERSE REACTION REPORT
☐ PHASE III PROTOCOL	☐ PROGRESS REPORT	☐ ANNUAL REPORTS
☐ DOSING REGIMEN CONSULT	☐ SCIENTIFIC INVESTIGATIONS	☐ FAX SUBMISSION
☐ PK/PD- POPPK ISSUES	☐ MEETING PACKAGE (EOP2/Pre-NDA/CMC/Pharmacometrics/Others)	☐ OTHER *(SPECIFY BELOW):*
☐ PHASE IV RELATED		

REVIEW ACTION

☐ NAI (No action indicated) ☐ E-mail comments to: ☐ Medical ☐ Chemist ☐ Pharm-Tox ☐ Micro ☐ Pharmacometrics ☐ Others (Check as appropriate and attach e-mail)	☐ Oral communication with Name: [ ] ☐ Comments communicated in meeting/Telecon. see meeting minutes dated: [ ]	☐ Formal Review/Memo (attached) ☒ See comments below ☐ See submission cover letter ☐ OTHER *(SPECIFY BELOW)*: [ ]

REVIEW COMMENT(S)

☒ **NEED TO BE COMMUNICATED TO THE SPONSOR** ☐ **HAVE BEEN COMMUNICATED TO THE SPONSOR**

See attached comment.

SIGNATURE OF REVIEWER: _____ Date _____

SIGNATURE OF TEAM LEADER: _____ Date _____

CC.: DCP Genomics: M Orr **Associate Director, Genomics (ADG):** F Frueh
CC.: DCP-5: Reviewer – Q Liu; **Secondary Reviewer TL** - R Ramchandani; **TL/DDD** - BBooth **DD** - A Rahman

This review evaluates the pharmacogenomic/pharmacogenetic research study CAMN107A2101-03, which is a biomarker development study for Nilotinib.

BACKGROUND
Nilotinib (AMN107) a synthetic aminopyrimidine is a new selective inhibitor of the kinase activity of the Bcr-Abl oncoprotein. This protein is the product of the BCR-ABL fusion gene, which results from a reciprocal chromosome translocation in a bone marrow haematopoietic stem cell (HSC).

The clinical studies are a Phase I/II open-label study, Study 2101, included in this submission as 3 separate clinical study reports:
• The Phase IA dose-finding component (Study 2101) included 119 patients with imatinib resistant
CML in CP, AP, and BC and relapsed/refractory Ph+ acute lymphoblastic leukemia (ALL).
• The Phase II component intended for registration included
• 282 patients imatinib-resistant/intolerant Ph+ CML in CP (Study 2101E2) (Arm 4, Group A of Study 2101) and
• 89 imatinib-resistant/intolerant Ph+ CML in AP (Study 2101E1) (Arm 3, Group A of Study).

Study Number and Title:
CAMN107A2101, "Pharmacogenetic analysis of UGT1A1 polymorphism and hyperbilirubinemia in the phase II component of study CAMN107A2101"

Study design:

Details for the clinical trial are described in the study protocol (Study 2101 Appendix 16.1.1). A separate, voluntary consent was used for collection of blood for phamacogenetic analysis.

Sample Collection and Processing:
Baseline blood samples were obtained and DNA was extracted by [redacted] using [redacted] isolation kits in accordance with GLP standards. In Study 2101E1, 39 of 89 patients were genotyped and in Study 2101E2, 62 of 282 patients were genotyped.

STUDY SUMMARY PHARMACOGENOMICS/NOVEL BIOMARKERS:
The Phase I/II clinical trial explores the efficacy and safety of nilotinib in patients with imatinib-resistant CML in chronic phase (CP), accelerated phase (AP) or blastic crisis (BC), relapsed/refractory Ph+ALL and other hematological malignancies.

In the biomarker development section of the accelerated phase (Study 2101E1) and chronic phase (Study 2101E2) arms of the (Study 2101 clinical trial were genotyped at a promoter polymorphism in UGT1A1 gene in order to identify any association between genetic variation and hyperbilirubinemia during nilotinib therapy. The $(TA)_7/(TA)_7$ genotype was associated with

a statistically significant increase in risk of hyperbilirubinemia relative to the $(TA)_6/(TA)_6$ and $(TA)_6/(TA)_7$ genotypes.

Patient Demographics by Genotype-accelerated phase and chronic phase

Table 4-1 Patient demographics by genotype – accelerated phase

	(TA)5/(TA)6	(TA)6/(TA)6	(TA)6/(TA)7	(TA)6/(TA)8	(TA)7/(TA)7	(TA)7/(TA)8
Number	0	15	16	1	6	1
Baseline total bilirubin (µmol/L) Mean±SD	--	9.2±3.9	7.2±2.7	10.3	14.4±5.3	23.9
Baseline total bilirubin (µmol/L) Min, med, max	--	[redacted]				
Age Mean±SD (median)	--	60.7±15.3 (64.0)	62.6±8.9 (63.5)	54.0 (54.0)	60.5±9.7 (60.0)	28.0 (28.0)
Caucasian	0	11	15	0	5	0
Black	0	2	1	1	1	1
Asian	0	2	0	0	0	0

Source: [Study 2101E1 Table 14.2-11.1] and [Study 2101E1 Table 14.2-11.2]

Table 4-2 Patient demographics by genotype – chronic phase

	(TA)5/(TA)6	(TA)6/(TA)6	(TA)6/(TA)7	(TA)6/(TA)8	(TA)7/(TA)7	(TA)7/(TA)8
Number	1	26	28	1	6	0
Baseline total bilirubin (µmol/L) Mean±SD	5.8	6.4±2.0	8.4±3.0	10.3	17.7±10.3	--
Baseline total bilirubin (µmol/L) Min, med, max	[redacted]					--
Age Mean±SD (median)	39.0 (39.0)	60.7±13.5 (61.5)	55.9±12.0 (57.0)	45.0 (45.0)	56.3±16.11 (59.0)	--
Caucasian	0	24	25	0	6	0
Black	1	1	3	1	0	0
Asian	0	1	0	0	0	0

Source: [Study 2101E2 Table 14.2-11.1] and [Study 2101E2 Table 14.2-11.2]

UGT1A1 Genotype and Bilirubin levels

Table 4-3 **Post-treatment maximum within patient total bilirubin (micro mol/L) by genotype – accelerated phase**

Genotype	Number	Mean±SD	Median	Minimum	Maximum
(TA)5/(TA)5	--	--	--		
(TA)6/(TA)6	15	33.4±29.7	21.0		
(TA)6/(TA)7	14	22.4±9.1	20.5		
(TA)6/(TA)8	1	18.8	--		
(TA)7/(TA)7	6	66.9±41.6	60.5		
(TA)7/(TA)8	1	25.7	--		

Source: [Study 2101E1 Table 14.2-11.2]

Table 4-4 **Post-treatment maximum within patient total bilirubin (micro mol/L) by genotype – chronic phase**

Genotype	Number	Mean±SD	Median	Minimum	Maximum
(TA)5/(TA)5	1	13.7	--		
(TA)6/(TA)6	26	18.8±5.9	17.6		
(TA)6/(TA)7	28	29.3±21.7	22.8		
(TA)6/(TA)8	1	29.1	--		
(TA)7/(TA)7	6	59.6±23.0	57.4		
(TA)7/(TA)8	--	--	--		

Source: [Study 2101E2 Table 14.2-11.2]

For each patient, the maximal total bilirubin after the first dose of treatment is used.

Table 4-5 **Post-treatment maximum total bilirubin (CTC grade) by genotype – accelerated phase (number of patients per maximum grade)**

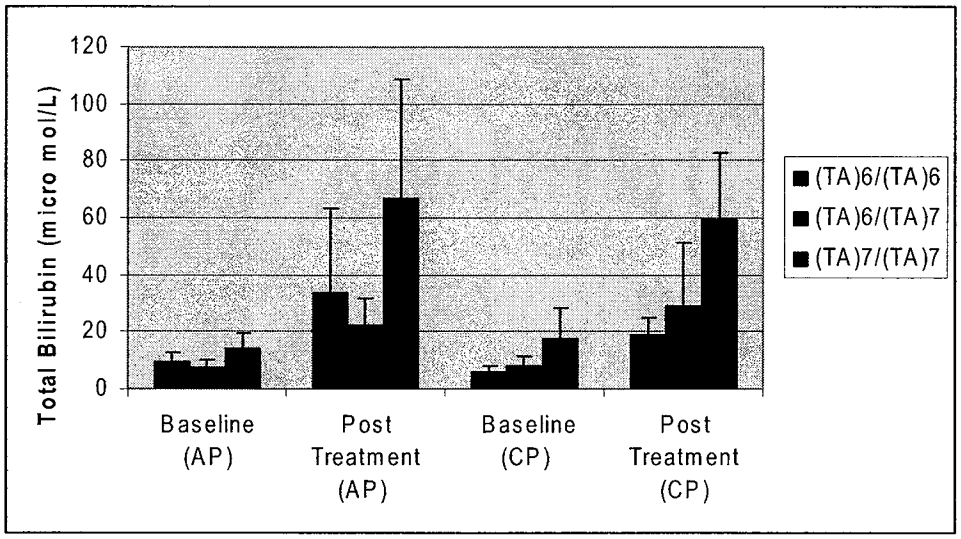
Genotype	Total	Grade 0	Grade 1	Grade 2	Grade 3	Missing
(TA)5/(TA)5	--	--	--	--	--	--
(TA)6/(TA)6	15	6	5	2	2	0
(TA)6/(TA)7	16	7	4	3	0	2
(TA)6/(TA)8	1	0	1	0	0	0
(TA)7/(TA)7	6	0	1	2	3	0
(TA)7/(TA)8	1	0	1	0	0	0

Source: [Study 2101E1 Table 14.2-11.2]

Summary Table from Study 2101E1 and 2101E2:

	Study 2101E1 (micro mol/L)		Study 2101E2 (micro mol/L)	
Genotype	Baseline (AP)	Nilotinib Post-treatment (AP)	Baseline (CP)	Nilotinib Post-treatment (CP)
(TA)5/(TA)5	N/A (n=0)	N/A (n=0)	5.8 (n=1)	13.7 (n=1)
(TA)6/(TA)6	9.2±3.9 (n=15)	33.4±29.7 (n=15)	6.4±2.0 (n=26)	18.8±5.9 (n=15)
(TA)6/(TA)7	7.2±2.7 (n=16)	22.4±9.1 (n=14)	8.4±3.0 (n=28)	29.3±21.7 (n=14)
(TA)7/(TA)7	14.4±5.3 (n=6)	66.9±41.6 (n=6)	17.7±10.3 (n=6)	59.6±23 (n=6)
(TA)6/(TA)8	10.3 (n=1)	18.8 (n=1)	10.3 (n=1)	29.1 (n=1)
(TA)7/(TA)8	23.9 (n=1)	25.7 (n=1)	N/A (n=0)	N/A (n=0)

Summary Graph 1: Baseline levels of Bilirubin versus Post Treatment with Nilotinib



Summary

The goal of this particular study, as indicated by the sponsor, was to confirm the association between UGT1A1 genotype and hyperbilirubinemia that was observed in patients treated with nilotinib in the Phase I part of the study BMD CAMN107A2101. In the analysis, 111 patients with CML or ALL were genotyped. Relative risk of hyperbilirubinemia was significantly greater than 1.0 in "7/7 patients" relative to "6/6 and 6/7 patients" and in "7/7 patients" relative to "6/7 patients, under both definitions of hyperbilirubinemia (grade $\geq$ 3, grade $\geq$ 2).

It should be noted that a significant increase in relative risk with a "6/7 genotype patients" relative to "6/6 genotype patients" was not seen in Phase I or Phase II but there was a trend observed. However, this study deals with a small number of patients.

With the caveat that only 111 patients were evaluated, there is reasonable evidence to support that notion that "7/7 genotype patients, n = 12" are at risk of developing hyperbilirubinemia when treated with nilotinib. There were 97 patients evaluated for UGT 6/6, 6/7, 7/7 genotype and the association of the genotypes with hyperbilirubinemia.

In both the (AP) and (CP) studies, the largest increases in total bilirubin levels were observed in the (TA)7/(TA)7 genotype individuals. It should be noted that increases in total bilirubin levels were observed in the all of the genotypes studied following administration of Nilotinib.

The question becomes: How do regulators use this type of information? Could the concomitant addition of agents that inhibit UDPGT enzyme activity such as NSAIDs like naproxen or agents that deplete UDPGA stores such as acetaminophen and chloramphenical potentially lead to adverse effects from drug-drug interactions with nilotinib. At this point in time, we just do not

know. Furthermore, a few patients with the "7/7 genotype are categorized as being grade 2 or less based on total bilirubin. Clearly, the genotype is only one factor that is contributing to the development of the hyperbilirubinemia, as there are potentially many other factors that could be contributing to the hyperbilirubinemia as well as one patients with the 7/7 genotype was grade 0.

Comments from OCP Genomics Review:

1. Does the hyperbilirubinemia reverse in the "7/7 genotype patients" after the administration of nilotinib is stopped?
2. **Pharmacogenomic Labeling Section 12.4**:
   a. A pharmacogenetic analysis of 97 patients evaluated the polymorphisms of UGT1A1 and its potential association with hyperbilirubinemia during nilotinib treatment. In this study, The (TA)7/(TA)7 genotype was associated with a statistically significant increase in the risk of hyperbilirubinemia relative to the (TA)6/(TA)6 and (TA)6/(TA)7 genotypes. [redacted] However, the largest increases in bilirubin were observed in the (TA)7/(TA)7 genotype patients.
3. Requested that the sponsor send a data set that links the genotype data with the AE information since it is currently not available. The data recently arrived for analysis, which will be conducted in the very near future.
4. Is there a link between 7/7 patients and liver toxicity that is observed in the clinical trial? This analysis will be conducted once the data is available for review.
5. Will patients with 1.5 x upper limit of normal range for bilirubin be restricted from using nilotinib similar to the clinical trial? If yes, will most of the patients with the UGT1A1 7/7 phenotype would be removed from the equation? If no, then patients with the UGT1A1 7/7 phenotype would be at risk of hyperbilirubinemia.
6. [redacted]
7. [redacted]

Signatures:

Reviewer: Michael Orr, PhD Division of Clinical Pharmacology, Genomics	Associate Director: Felix Frueh, PhD Division of Clinical Pharmacology, Genomics

Cc: **CC.: DCP Genomics:** M Orr **Associate Director, Genomics (ADG):** F Frueh
CC.: DCP-5: Reviewer – **Q Liu;** Secondary Reviewer - **R Ramchandani;** DDD - **B Booth**
DD - **A Rahman**

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Michael Orr
7/9/2007 12:09:45 PM
BIOPHARMACEUTICS

Felix Frueh
7/17/2007 11:55:35 PM
BIOPHARMACEUTICS

CLINICAL PHARMACOLOGY REVIEW

NDA:	22-068
Submission Type:	NDA-NME
Submission Dates:	29-Sept-2006; 8-Nov-2006; 16-Feb-2007; 3-May-2007; 10-May-2007
Brand Name:	Tasigna®
Generic Name:	Nilotinib
Indication:	Treatment of chronic phase and accelerated phase Ph+ CML in adult patients resistant to or intolerant to imatinib
Formulation:	200 mg capsules
Dosing Regimen:	400 mg orally twice daily
Sponsor:	Novartis
OCP Division:	Division of Clinical Pharmacology V
OND Division:	Division of Oncology Drug Products (HFD-150)
Primary/Pharmacometric Reviewer:	Qi Liu, Ph.D. Roshni Ramchandani, Ph.D.
Team Leader:	Brian Booth, Ph.D.
Pharmacometric Team Leader:	Yaning Wang, Ph.D.
QT Reviewer:	Roshni Ramchandani, Ph.D. Rajnikanth Madabushi, Ph.D.
Secondary QT Reviewer:	Christine Garnett, Pharm.D.
PG Reviewer:	Michael Orr, Ph.D.
Secondary PG Reviewer:	Felix Frueh, Ph.D.

TABLE OF CONTENTS:

1. EXECUTIVE SUMMARY

Tasigna (nilotinib) is an inhibitor of the tyrosine kinase activity of the Bcr-Abl oncoprotein. The current submission is the original NDA for Tasigna for the treatment of chronic phase (CP) and accelerated phase (AP) Philadelphia chromosome positive chronic myelogenous leukemia (CML) in adult patients resistant to or intolerant to imatinib. The proposed dosing regimen of Tasigna is 400 mg orally twice daily. Because food can increase the bioavailability of Tasigna, no food should be consumed for at least 2 hours before the dose and at least one hour after the dose.

The clinical efficacy and safety of nilotinib, at the proposed dosing regimen, has been evaluated in imatinib-resistant/intolerant Ph+ CML in CP or AP patients in the Phase II component of a Phase IA/II trial (study CAMN107A2101). The surrogate efficacy endpoints of cytogenetic response for CML-CP patients and hematologic response for CMP-AP patients were employed in this study. The safety, efficacy and pharmacokinetic data suggested that the proposed dosing regimen is reasonable.

In a healthy volunteer study designed to assess the effects of Tasigna on the QT interval, administration of Tasigna was associated with concentration-dependent QT prolongation. At exposures that were 26% lower than the therapeutic exposures observed in patients, the maximum mean placebo-adjusted QTcF change from baseline was 18 msec (1-sided 95% Upper CI: 26 msec).

The major metabolic pathway of nilotinib in humans involves hydroxylation of the methyl group in the methyl imidazole ring (P41.6) with further oxidation of the hydroxyl group to a carboxylic acid (P36.5). Cytochrome P450 3A4 (CYP3A4) is expected to be the main contributor to the oxidative metabolism of nilotinib in humans. Based on the results from the drug-drug interaction studies with ketoconazole and rifampin, labeling changes were made to reflect dose adjustment for concomitant administration of CYP3A4 inhibitors and inducers. Excretion of nilotinib occurred exclusively through the fecal route with no renal elimination of the drug or its metabolites.

In vitro data suggested that nilotinib is a potent inhibitor of CYP2C8, CYP2C9, CYP3A4/5 and UGT1A1, and a moderately potent inhibitor of CYP2C19 and CYP2D6. In vitro data also suggest that nilotinib may induce CYP2B6, CYP2C8, CYP2C9, CYP1A1, CYP1A2 and CYP3A4. In vitro data also suggested that nilotinib is a substrate and an inhibitor for P-gp mediated efflux. Coadminstration of a single oral 600 mg dose of nilotinib was found to increase the midazolam (CYP3A4 substrate) exposure by 30%. The impact of multiple doses of nilotinib on the pharmacokinetics of the substrates of these enzymes is unknown. Caution should be exercised when coadministering Tasigna with substrates of these enzymes having a narrow therapeutic index.

The sponsor has two clinical pharmacology studies currently ongoing, the hepatic

impairment study and the absolute bioavailability study. The completion of both studies are to be phase 4 commitments.

1.1 RECOMMENDATION

The Office of Clinical Pharmacology (OCP) finds the NDA to be acceptable from a clinical pharmacology perspective.

1.2 PHASE 4 COMMITMENTS

1) Submit the completed report and datasets for the hepatic impairment study.
2) Submit the completed report and datasets for the absolute bioavailability study.
3) Given the fact that nilotinib is both an inhibitor and a inducer of CYP2C8, CYP2C9 and CYP3A4, and that the currently completed DDI study with midazolam only used a single dose of nilotinib, we recommend a phase 4 commitment for the sponsor to conduct clinical studies to evaluate if multiple dose of nilotinib alter the metabolism of a sensitive CYP2C9 substrate (for example, S-warfarin). If significant interaction was demonstrated, additional clinical studies to evaluate if multiple doses of nilotinib alter the metabolism of a sensitive CYP2C8 substrate (for example, repaglinide) and/or a sensitive CYP3A4 substrate (for example, midazolam) may be needed.
4) Given the fact that nilotinib has pH dependent solubility, we recommend a phase 4 commitment for the sponsor to conduct clinical studies to evaluate if antacids and H2 blockers/proton pump inhibitors alter the pharmacokinetics of nilotinib.

COMMENTS:

1) Since the in vitro studies suggest that nilotinib is an inhibitor and a substrate for P-glycoprotein, you may wish to consider conducting an in vivo drug interaction study with a P-glycoprotein substrate (for example, digoxin) and an in vivo drug interaction study with a P-glycoprotein inhibitor.
2) Since the in vitro data demonstrated that nilotinib is an inducer of CYP2B6, you may wish to consider conducting clinical studies to evaluate if multiple doses of nilotinib alter the metabolism of a sensitive CYP2B6 substrate (for example, efavirenz).

1.3 SUMMARY OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FINDINGS

Tasigna (nilotinib) is an inhibitor of the Abl tyrosine kinase activity of the Bcr-Abl oncoprotein. The current submission is the original NDA for Tasigna for the treatment of chronic phase (CP) and accelerated phase (AP) Philadelphia chromosome positive chronic myelogenous leukemia (CML) in adult patients resistant to or intolerant to imatinib. The proposed dosing regimen of Tasigna is 400 mg orally twice daily.

Nilotinib is absorbed following oral administration. The bioavailability of nilotinib was increased when given with a meal. Compared to the fasted state, the systemic exposure (AUC) increased by 15% (drug administered 2 hours after a light meal), 29% (30 minutes after a light meal), or 82% (30 minutes after a high fat meal). The median time to reach C_{max} of nilotinib (t_{max}) was 3 hours. Drug elimination half-lives averaged about 17 hours. Steady-state conditions are achieved by day 8 after initiating nilotinib treatment. There is a 2-fold or 3.8-fold accumulation with once daily dosing or twice daily dosing in serum concentrations between the first-dose and steady-state. The increase in nilotinib AUC was generally dose-proportional over the dose range of 50 mg to 400 mg, but AUC appeared to plateau at dose levels starting at 400 mg. Daily steady-state nilotinib serum exposure with 400 mg b.i.d. was approximately 35% greater than with 800 mg given once daily.

The extent of nilotinib binding to human plasma protein is high (98% on average), and independent of concentration. The α_1-acid glycoprotein was found to be the primary binding protein as compared to serum albumin in human plasma.

The major metabolic pathway of nilotinib in humans involved hydroxylation of the methyl group in the methyl imidazole ring (P41.6) with further oxidation of the hydroxyl group to a carboxylic acid (P36.5). Metabolites P36.5 and P41.6 were present at the highest levels in human serum accounting for 6.1% and 4.7% of the total drug-related exposure, respectively. Unchanged nilotinib represented 88% of the total drug-related serum exposure. Metabolite P36.5 showed no activity on Bcr-Abl inhibition or in the hERG channel assay. CYP3A4 is expected to be the main contributor to the oxidative metabolism of nilotinib in humans with CYP2C8 making a minor contribution. In healthy subjects, co-administration of nilotinib with ketoconazole, a strong inhibitor of CYP3A4, increased nilotinib Cmax by 84% and AUC by 3-fold on average. In healthy subjects, co-administration of rifampin with nilotinib decreased C_{max} and AUC of nilotinib by 64% and by 80% on average, respectively. Concurrent treatment with strong CYP3A4 inhibitors and inducers should be avoided if possible; otherwise, dose adjustment of nilotinib will be needed. Excretion of nilotinib occurred exclusively through the fecal route with no renal elimination of the drug or its metabolites. Therefore, dose adjustment in patients with renal impairment is not necessary. Caution should be exercised when using nilotinib in patients with hepatic impairment.

There is a potential of nilotinib to inhibit the activity of cytochrome P450 and UGT enzymes. *K*i values of 0.24, 0.13, 3.8, 1.5, 0.45, and 0.19 µM were determined for CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5, and UGT1A1 respectively. The in vitro data suggested that nilotinib is a potent inhibitor of CYP2C8, CYP2C9, CYP3A4/5 and UGT1A1, and a moderately potent inhibitor of CYP2C19 and CYP2D6. When a single oral 600 mg dose of nilotinib was co-administered with a 4 mg dose of midazolam (CYP3A4 substrate) in healthy subjects, nilotinib was found to increase the midazolam exposure by 30%. In vitro studies suggest that nilotinib may induce CYP2B6, CYP2C8, CYP2C9, CYP1A1, CYP1A2 and CYP3A4. Nilotinib was found to be a substrate and an inhibitor for P-gp mediated efflux.

Age, body weight, or ethnic origin were not found to significantly affect the pharmacokinetics of nilotinib, whereas there is an effect of gender, with exposure to nilotinib in female patients being approximately 12% greater than in male patients.

The dose and dosing regimen were selected based upon safety, PK and efficacy response. Analysis of the exposure-response relationships suggested that higher nilotinib exposure were associated with increased efficacy response rates (hematologic response for CML-AP patients and cytogenetic response for CP patients). Based on the model predictions, exposures higher than that of the proposed 400 BID regimen may be associated with higher response rates, however, there was no further increase in exposure of the 600 mg BID dose as compared to the 400 mg BID dose. Therefore, the proposed 400 mg BID dosing regimen appears to be reasonable. Some lab abnormalities (total bilirubin, ALT, AST and lipase increase) were found to be nilotinib exposure-dependent. Nilotinib prolongs the QT interval. In a healthy volunteer study designed to assess the effects of Tasigna on the QT interval, administration of Tasigna was associated with concentration-dependent QT prolongation. At exposures that were 26% lower than the therapeutic exposures observed in patients, the maximum mean placebo-adjusted QTcF change from baseline was 18 msec (1-sided 95% Upper CI: 26 msec).

A pharmacogenetic analysis examining the polymorphism of UGT1A1 and its potential association with hyperbilirubinemia during nilotinib treatment was conducted [Study CAMN107A2101]. In this study, The $(TA)_7/(TA)_7$ genotype was associated with a statistically significant increase in risk of hyperbilirubinemia relative to the $(TA)_6/(TA)_6$ and $(TA)_6/(TA)_7$ genotypes.

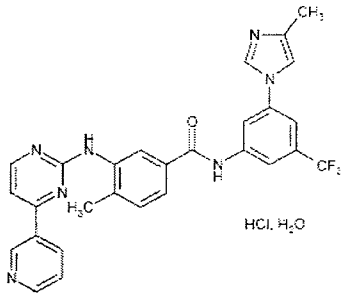
2 QUESTION BASED REVIEW

2.1 General Attributes of the Drug

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

The drug substance is the hydrochloride monohydrate salt of nilotinib. It is a white to slightly yellowish or slightly greenish yellowish powder.

Chemical structure of AMN107



4-Methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide, monohydrochloride, monohydrate

Molecular formula
Salt form as monohydrate: C28H22F3N7O.HCl.H2O
Salt form on anhydrous basis: C28H22F3N7O.HCl
Relative molecular mass
Salt form as monohydrate: 583.99
Salt form on anhydrous basis: 565.98

The solubility of Nilotinib hydrochloride monohydrate in aqueous solutions strongly decreases with increasing pH. Nilotinib hydrochloride monohydrate is slightly soluble in 0.1 N HCl, very slightly soluble in water, 0.01 N HCl and 0.001 N HCl and practically insoluble in buffer solutions of pH 4.5 and higher pH values.

The pH value of a 0.02% (m/V) solution of Nilotinib hydrochloride monohydrate (nilotinib) in water / ethanol 50:50 (v/v) is 4.3. The pH value of a 0.1% (m/V) suspension of Nilotinib hydrochloride monohydrate in water was determined to be 5.3. pKa1 for Nilotinib hydrochloride monohydrate (nilotinib) was found to be 2.1, and pKa2 was estimated to be around 5.4.

The distribution coefficient of nilotinib drug substance in n-octanol/0.1 N HCl at 37°C ± 0.5°C is 0.08 (Log D = -1.1).

Nilotinib can be classified as a moderately permeable compound based on the

permeability across confluent Caco-2 cell monolayers.

According to the Biopharmaceutics Classification System, nilotinib has been classified as a class 4 compound.

Nilotinib hard capsules are [redacted] dosage form for oral administration.

2.1.2 What are the proposed mechanism(s) of action and therapeutic indication(s)?

Tasigna (nilotinib) is an inhibitor of the Abl tyrosine kinase activity of the Bcr-Abl oncoprotein both in cell lines and in primary Philadelphia-chromosome positive leukemia cells. The drug binds tightly to the inactive conformation of the kinase domain in such a manner that it is an inhibitor of wild-type Bcr-Abl and maintains activity against 32/33 imatinib-resistant mutant forms of Bcr-Abl.

Nilotinib is indicated for the treatment of chronic phase and accelerated phase Philadelphia chromosome positive chronic myelogenous leukaemia (Ph+ CML) in adult patients resistant to, or intolerant to imatinib.

2.1.3 What are the proposed dosage(s) and route(s) of administration?

The proposed dose of Tasigna is 400 mg oral dose twice daily (approximately 12 hours apart). Treatment should continue as long as the patient continues to benefit. Tasigna should not be taken with food. No food should be consumed for at least 2 hours before the dose is taken and no food should be consumed for at least one our after the dose is taken.

2.2 General clinical pharmacology

2.2.1 What are the design features of the clinical studies used to support dosing or claims?

The clinical efficacy of nilotinib in imatinib-resistant/intolerant Ph+ CML in CP or AP, the indications claimed in this submission, has been demonstrated based on the Phase II component of a Phase IA/II trial (study CAMN107A2101, Table 1).

Study CAMN107A2101 is currently still ongoing. It was initially planned as a Phase IA, maximum tolerated dose (MTD)-determining trial employing a range of q.d. and b.i.d. nilotinib regimens. Later, Study CAMN107A2101 was modified by a protocol amendment to include a Phase II component consisting of six expansion arms for patients with various hematologic malignancies, who received the nilotinib treatment (400 mg b.i.d., with dose escalation to 600 mg if there was no response after 3 months). Patients in Phase II of [Study CAMN107A2101] were enrolled in the following disease categories:

- Arm 1: Relapsed/refractory Ph+ ALL
- Arm 2: Imatinib-resistant or -intolerant Ph+ CML in BC (Group A and Group B)
- Arm 3: Imatinib -resistant or -intolerant Ph+ CML in AP (Group A or Group B)
- Arm 4: Imatinib -resistant or -intolerant Ph+ CML in CP (Group A or Group B)
- Arm 5: Hypereosinophilic Syndrome and Chronic Eosinophilic Leukemia (HES/CEL)
- Arm 6: Systemic Mastocytosis (SM)

CML patients (Arms 2, 3 and 4) were divided into two separate groups. Group A had no prior treatment with other TKIs except imatinib; Group B included patients who had prior treatment with other investigational TKIs in addition to imatinib. Group A of Arms 3 and 4 represents the pivotal efficacy data for this submission.

Table 1. Pivotal Phase II studies in CP and AP CML patients and supportive Phase I study

Study No./ data cutoff date	Patient population	Purpose	n (total*)	Dose of nilotinib
Pivotal Phase II efficacy and safety trials				
ARM 4, Group A Study 2101E2 Pivotal Phase II trial 04 May 2006	Imatinib-resistant/intolerant Ph+ CML in CP, No prior TKI treatment except imatinib (Gr A)	safety, efficacy, PK, PD	132 efficacy and safety 150 safety only	400 mg b.i.d., (may be dose-escalated to 600 mg b.i.d)
ARM 3, Group A Study 2101E1 Pivotal Phase II trial 23 May 2006	Imatinib-resistant/intolerant Ph+ CML in AP, No prior TKI treatment except imatinib (Gr A)	safety, efficacy, PK, PD	64 efficacy and safety 25 safety only	400 mg b.i.d., (may be dose-escalated to 600 mg b.i.d)
Phase IA MTD-determining trial (supportive efficacy; blood level/response; safety)				
Study 2101 Phase IA trial 03 Mar 2006	Imatinib-resistant CML in CP, AP, and BC; and Ph+ ALL	safety, PK, preliminary efficacy, PD	119 total (17 CP) (46 AP)	dose-escalation; 50 mg q.d. - 1200 mg q.d; 400 mg b.i.d., 600 mg b.i.d.
Source: [Study 2101], [Study 2101E1], [Study 2101E2]				

Clinical Pharmacology studies with nilotinib in healthy subjects are summarized in Table 2.

Table 2. Summary of clinical pharmacology studies in healthy subjects

Study No.	Objectives	Nilotinib dose	Nilotinib treatment duration	No. of subjects
2104	Mass balance	400 mg	Single dose	4
2106	Food effect	400 mg	3 single doses	48
2108	DDI with midazolam	600 mg	2 single doses	18
2110	DDI with ketoconazole	200 mg	2 single doses	26
2119	QT/QTc	400-800 mg/day	Single dose, q.d. for up to 8 days, bi.d. for 3 days	102
2115	DDI with rifampin	400 mg	2 single doses	15

2.2.2 What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics (PD)) and how are they measured in clinical pharmacology and clinical studies?

Efficacy was assessed using endpoints of cytogenetic response for CML-CP patients and hematologic response for CMP-AP patients which have been used in the imatinib clinical development program, where they have been shown to predict clinical benefit in Ph+ CML patients. The achievement of both MCyR and HR with imatinib has been shown to be associated with prolonged survival.

The criteria used to evaluate efficacy in CML-CP and CML-AP patients in the Phase IA trial are provided in Table 3.

Table 3. Definitions of efficacy criteria for nilotinib Phase II studies in imatinibresistant or -intolerant CML-CP and CML-AP

Disease classification	Response criteria
CML-CP	Study 2101E2 and Study 2101E8
Complete hematologic response (CHR)	• WBC <10 x 10^9/L • PLT < 450 x 10^9/L • ≤ 5% myelocytes and metamyelocytes • Absence of blasts and promyelocytes in PB • No extramedullary involvement • Basophils ≤ 5% in PB and BM
CML-AP (responses confirmed after 4 weeks)	Study 2101E1 and Study 2101E7
Complete hematologic response (CHR)	• Myeloblast count <5% in BM • No myeloblasts in PB • ANC ≥1.5 x 109/L • PLT ≥100 x 109/L • No extramedullary involvement
Marrow response/no evidence of leukemia	• Myeloblasts <5% in BM • No myeloblasts in PB • ANC ≥1 x 10^9/L • PLT ≥20 x 10^9/L (no transfusions or bleeding) • No evidence of extramedullary involvement
Return to chronic phase (RTC)	• <15% myeloblasts in PB and BM • <30% myeloblasts + promyelocytes in PB and BM • <20% peripheral basophils • No other extramedullary involvement other than liver and spleen
Cytogenetic response (CyR)* CML-CP, CML-AP	• Complete: 0% Ph+ cells • Partial: 1% - 35% Ph+ cells • Minor: 36% - 65% Ph+ cells • Minimal: 66% - 95% Ph+ cells • None: > 95% Ph+ cells

*Major CyR includes complete response + partial response.

Source: [Study 2101E2-Section 9.7.1], [Study 2101E1-Section 9.7.1]

The overall CyR was calculated based on all bone marrow assessments. Bone

marrow assessments were used for cytogenetic response only if the number of metaphases examined was ≥ 20. If the number of metaphases examined was < 20, then FISH assessments were used for cytogenetic response. The overall CyR assessment was identical for both indications (CML-CP and CML-AP) and for both phases (Phase IA and Phase II) of Study CAMN107A2101.

2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

The results from the human mass balance study demonstrated that unchanged nilotinib (the major active moietie) was the predominant circulating component detected in the serum, accounting for 87.5 ± 9.2% of the total nilotinib-related radioactivity from 0-48 h. The plasma pharmacokinetics of nilotinib was assessed in several clinical studies. The nilotinib concentrations in human serum were determined by a validated LC-MS/MS method with the lower limit of quantification of ng/mL.

In the human mass balance study (CAMN107A2104), in addition to nilotinib pharmacokinetics, the pharmacokinetics of its minor pharmacologically active metabolite (BEJ866) was also measured using a validated bioanalytical LC/MS/MS method (lower limit of quantification: 1.00 ng/mL). The decision to quantitatively monitor BEJ866 was made based on preliminary data indicating that BEJ866 might possess some biological activity towards the same targets as nilotinib. The average serum exposure of BEJ866 was determined to be only about 1.0% of the exposure to nilotinib. This low level of exposure to BEJ866 suggests that BEJ866 may not need to be monitored in future clinical studies.

2.2.4 Exposure-response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy?

Efficacy of nilotinib is claimed for two indications: CML-CP and CML-AP adult patients who are resistant to or intolerant of imatinib. The efficacy claims are supported by the phase II trial (Phase II component of Study CAMN107A2101). In this Phase II trial, patients were administered with a starting oral dose of 400 mg BID with possible dose-escalation to 600 mg BID or dose-reduction to 400 or 200 mg QD. The treatment cycle was defined as 28 days of treatment. An exploratory population PK/PD analysis was performed for this Phase II study by the sponsor to evaluate the relationship between the Tasigna exposure and efficacy. This exposure-efficacy analyses were limited to Phase II, Arm 3 (CML-AP) and Arm 4 (CML-CP), Group A (patients who did not have prior tyrosine kinase inhibitors (TKI) other than imatinib) patients with PK data collected.

Binary logistic regression was used to model the efficacy response. Such models were fitted to data obtained during the first 3 months, and during the second 3 months conditional on nonresponse during the first 3 months. The primary predictor was the average of the patient's daily AUC values, calculated from the beginning of the treatment until the time of response or, for non-responders, until the end of the interval. The other covariates explored were Gleevec-resistant/intolerant status, baseline mutation, percentage of Ph+ metaphases at baseline, and white blood cell counts (WBC) at baseline. AUC and the other covariates were all included as predictors in a full model which was then pruned using a backwards stepwise procedure, where models were successively simplified by the elimination of terms if doing so reduced the Akaike Information Criterion (AIC).

(1) For CML-AP patients:

Hematological response (overall confirmed HR) was the primary efficacy variable for CML-AP. Overall hematologic response (HR) rate was reported at 3 months and at 6 months, which was confirmed by the same or better response at least 4 weeks later.

There were 32 CML-AP patients in this PK/efficacy analysis dataset. Of these 32 patients, 18 experienced confirmed hematologic response by 3 months. With this dataset for PK/efficacy analysis, none of the nonresponders from the first 3-month interval responded during the second 3-month interval, therefore, no regression was done for the second 3-month interval. For the first 3-month interval, logistic regression was performed. For CML-AP patients during the first 3-month interval, only average daily AUC (units: ng.hr/mL) was retained in the selected final logistic model:

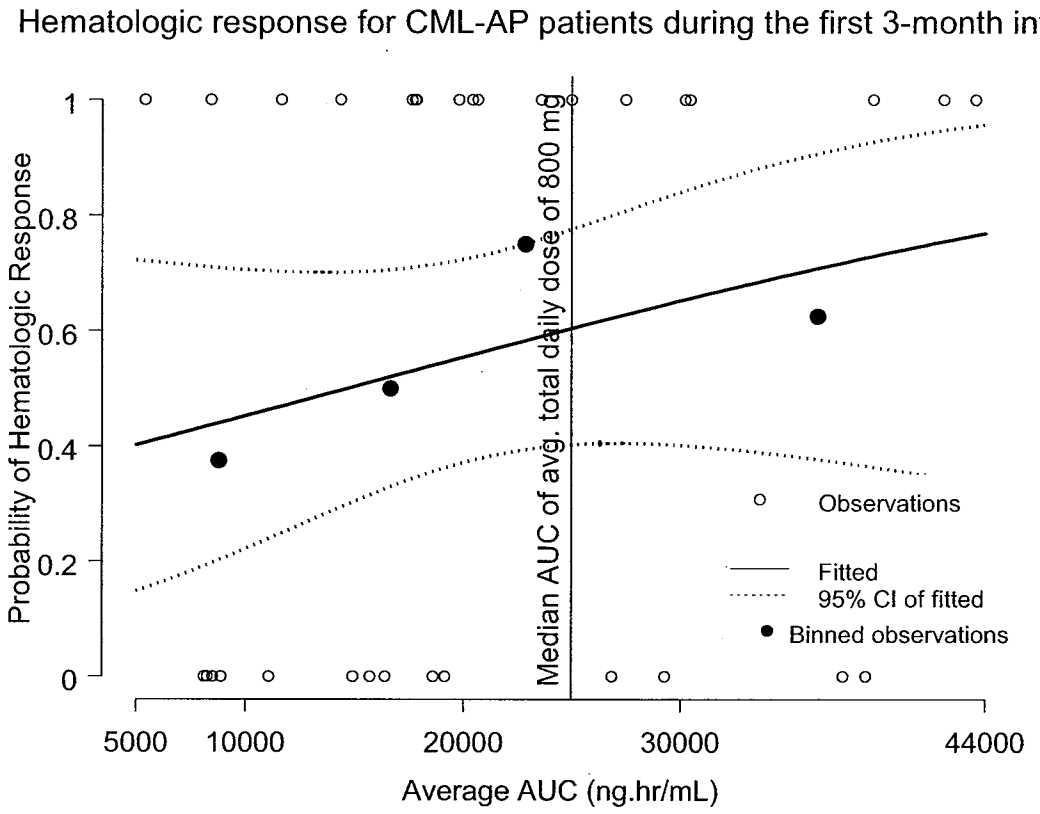
Logit(Hematologic Response) = -0.60+0.000041*AUC.

Parameter estimates and their standard errors are tabulated in Table 4. The observations and fitted probability are shown in Figure 1. For a 10,000 ng.hr/mL increase in average AUC, the odds that the patient will achieve hematologic response will be increased by a factor of 1.51. An increase of AUC from 20,000 ng.hr/mL to 30,000 ng.hr/mL increases the response rate from 0.55 to 0.65.

Table 4. Parameter estimates of logistic model for CML-AP in the first 3-month interval

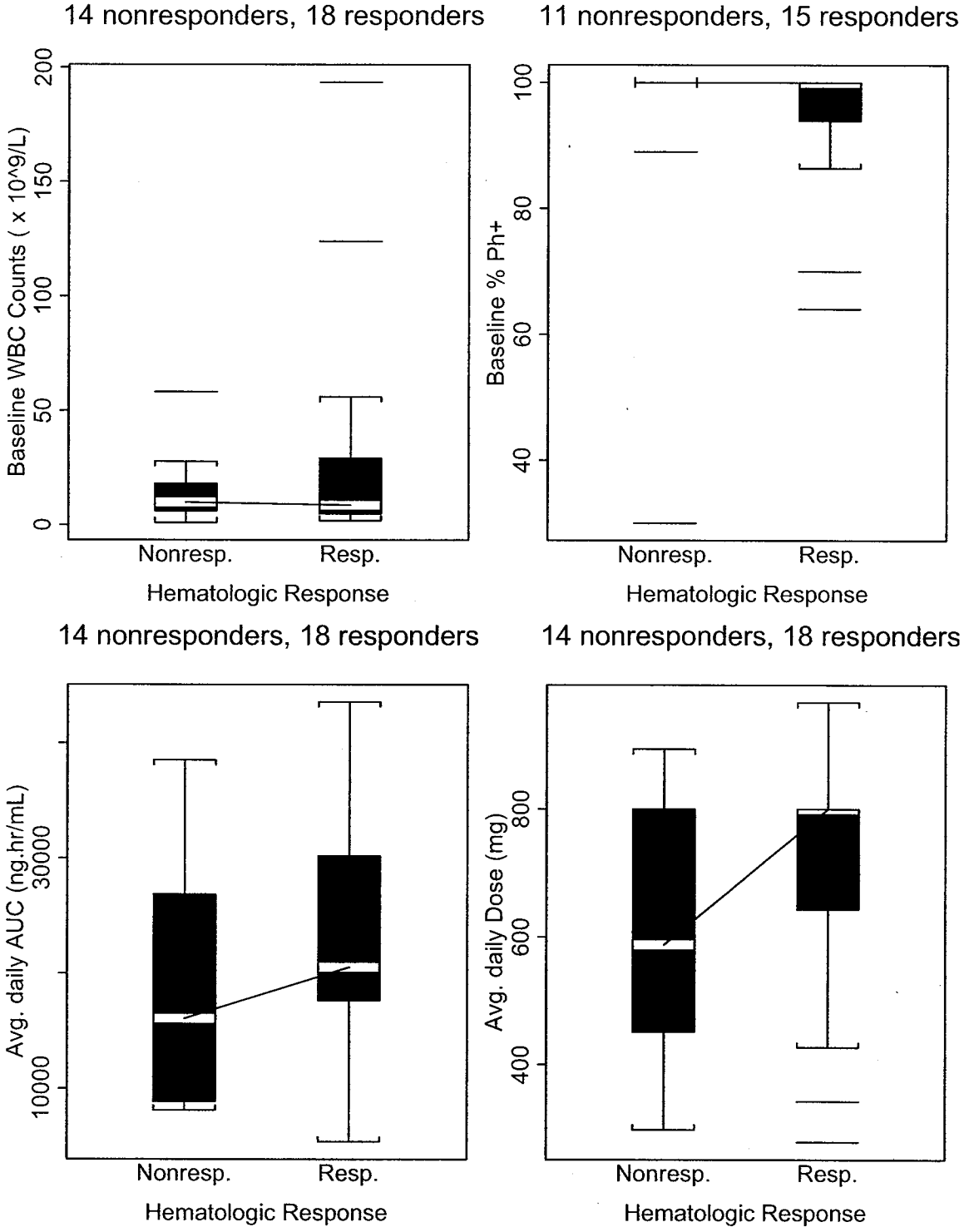
	Parameter Estimates	Standard Error
Intercept	-0.60	0.82
AUC (ng·hr/mL)	0.000041	0.000036

Figure 1. Probability of hematologic response for CML-AP patients during the first 3-month interval



The box plots in Figure 2 displays distributions of white blood cell counts at baseline, % Ph+ metaphases at baseline, average daily AUC, and average daily dose, stratified by hematologic response for the first 3 months. Patients who had confirmed hematologic response by 3 months tended to have higher average AUC and dose until the response or the end of 3-month interval. It should be noted that the median average daily dose of the nonresponders is only about 600 mg, much lower than the initial starting daily dose of 800 mg (400 mg BID). This may be explained, at least partially, by the fact that for patients who had to go through dose reductions due to toxicity, their nilotinib exposure is relatively low, and hence their chances of achieving the hematologic response by 3 months is relatively low.

Figure 2. Covariates for CML-AP patients versus hematologic response by first 3 months



(2) For CML-CP patients:
Major cytogenetic response was the primary efficacy variable for CML-CP. Bone marrow assessments for cytogenetic response were performed at the end of cycle 1, 3, 6, 9, and 12, and every 3 months thereafter.

There were 75 CML-CP patients from Phase II in the population PK analysis dataset who were assessed for cytogenetic response. Of these 75 patients, 65 were assessed for cytogenetic response in PK/efficacy analysis of the first 3-month interval. Of these 65 patients, 34 were responders, and 31 were non-responders. The latter were eligible for PK/efficacy analysis during the second 3-month interval conditional on nonresponse during the first 3-month interval. Two patients who had no cytogenetic assessments during the first 3-month interval did have cytogenetic assessments performed during the second 3-month interval, and they were included in the PK/efficacy analysis of the second 3 months. However, of the other 31, 3 dropped out before the end of the second 3-month interval and were excluded. Thus, there were 30 patients in the second 3-month interval, of whom six were responders. In addition, 2 and 6 patients of the first and second 3-month intervals, respectively, were missing baseline % Ph+ metaphases.

For CML-CP patients attaining response during the first 3-month interval, average AUC and WBC at baseline were retained in the final logistic regression model (after excluding data from one patient with extreme average AUC [> 80,000 ng.hr/mL]):

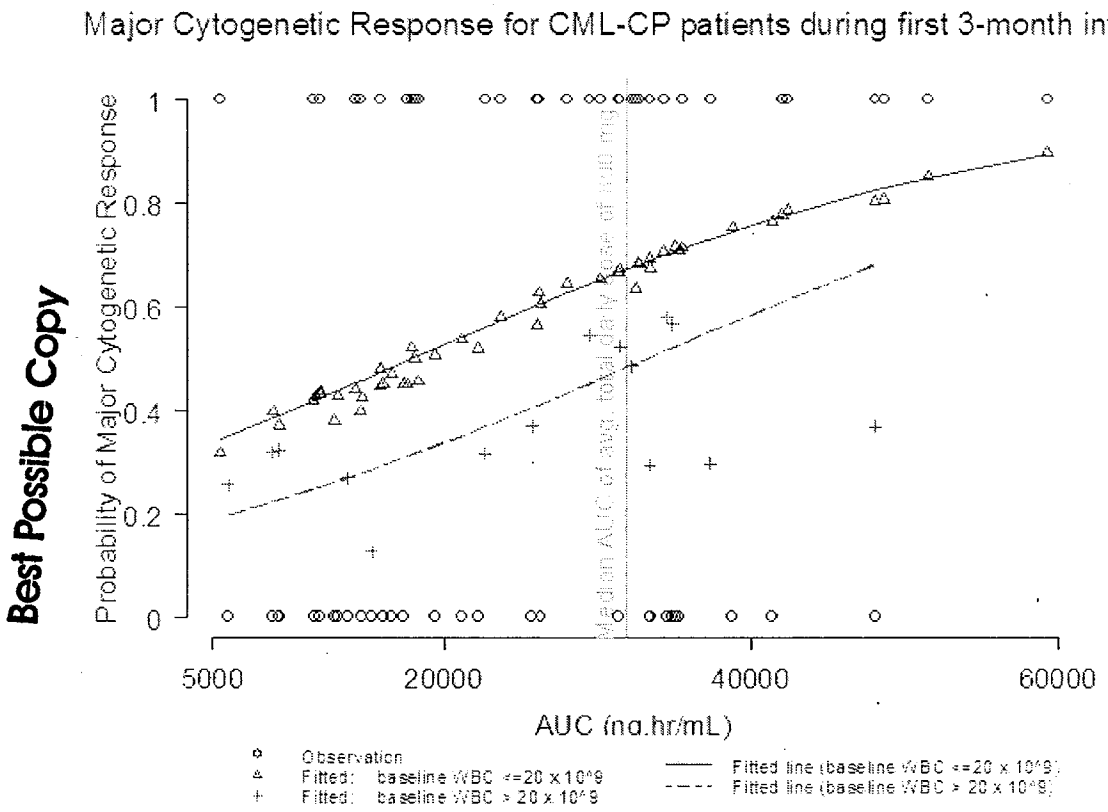
Logit(Major Cytogenetic Response) = -0.79 - 0.019*(Baseline WBC) +0.000051*AUC.

Parameter estimates and their standard errors are provided in Table 5. At a given baseline WBC count, the effect of increasing average AUC by 10,000 ng.hr/mL will increase the odds of major cytogenetic response by a factor 1.67. Figure 3 illustrates the observed and fitted probability of achieving major cytogenetic response versus average AUC. At baseline WBC counts of 20 x 10^9, an increase of average AUC from 20,000 to 30,000 ng.hr/mL is predicted to increase the response rate from 0.46 to 0.59. The lower the baseline WBC count, the higher the response rate.

Table 5. Parameter estimates of logistic model for CML-CP in the first 3-month interval

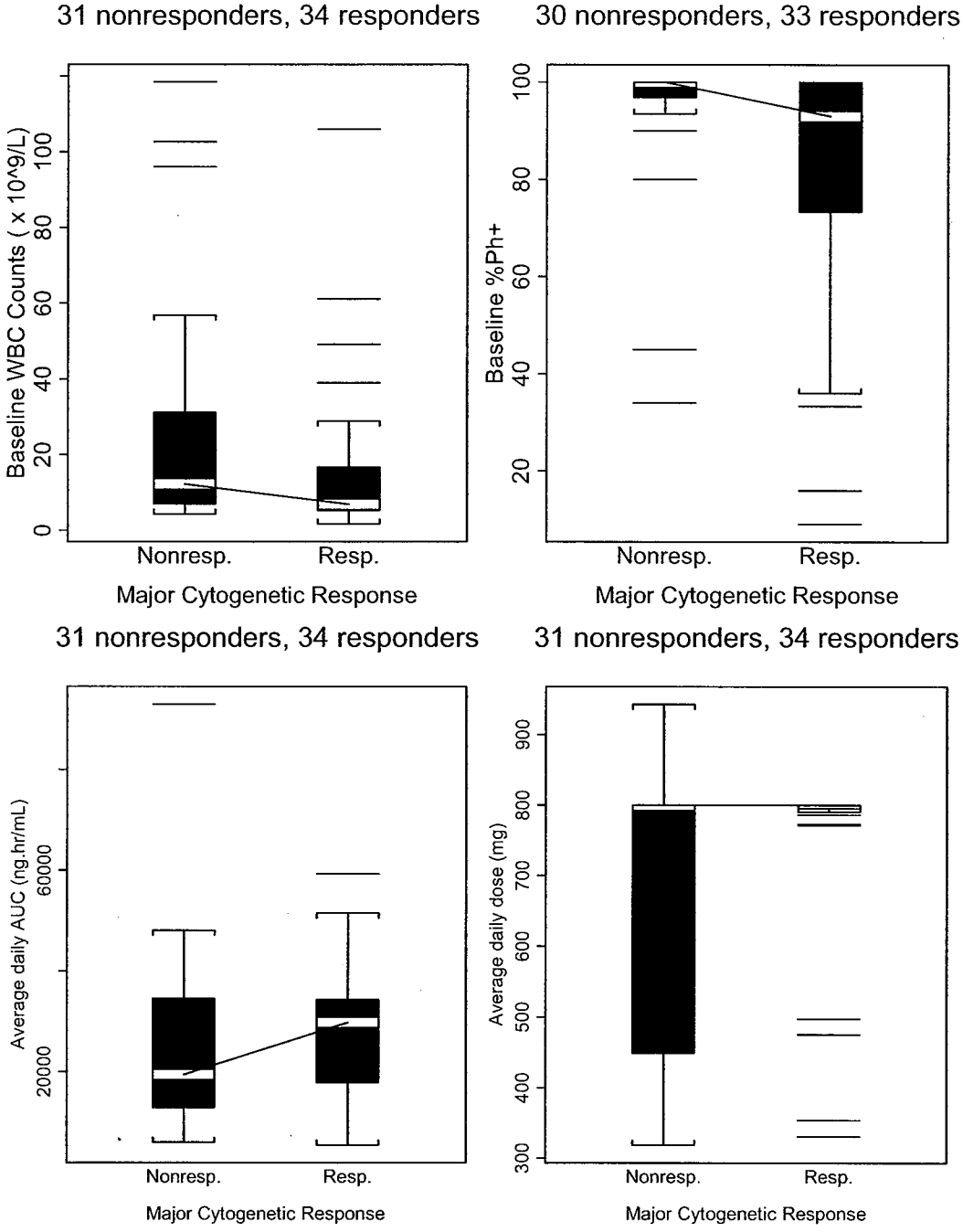
	Parameter Estimates	Standard Error
Intercept	-0.79	0.63
Baseline WBC	-0.019	0.011
AUC (ng·hr/mL)	0.000051	0.000024

Figure 3. Probability of achieving major cytogenetic response versus average AUC, stratified by WBC count at baseline. Every symbol represents either an observed or fitted response of a patient



The boxplots in Figure 4 show the covariate effects stratified by major cytogenetic response. Patients who had major cytogenetic response by 3 months tended to have lower white blood cell counts at baseline, lower % Ph+ metaphases at baseline, and higher average AUC and dose. For about 50% of the nonresponders, the average daily dose is below the initial starting daily dose of 800 mg (400 mg BID), while majority of the responders stayed at the daily dose of 800 mg. This may be explained, at least partially, by the fact that for patients who had to go through dose reductions due to toxicity, their nilotinib exposure is relatively low, and hence their chances of achieving the hematologic response by 3 months is relatively low.

Figure 4. Covariates for CML-CP patients versus major cytogenetic response by 3 months



In this limited subset of patients, no evidence was found to support the relationship between the new cytogentic response during the second 3-month interval and the nilotinib exposure.

In conclusion, there is an exposure-response relationship for the effectiveness of

nilotinib. Higher nilotinib dose and systemic exposure were associated with increased efficacy response rates (hematologic response for CML-AP patients and cytogenetic response for CP patients).

2.2.4.2 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety?

Various adverse events (AE) and lab abnormalities were found to be nilotinib exposure-dependent.

(1) QT prolongation

Nilotinib prolongs QT interval. In a healthy volunteer study designed to assess the effects of Tasigna on the QT interval, administration of Tasigna was associated with concentration-dependent QT prolongation. At exposures that were 26% lower than the therapeutic exposures observed in patients, the maximum mean placebo-adjusted QTcF change from baseline was 18 msec (1-sided 95% Upper CI: 26 msec).

For details, please refer to Section 2.2.4.3 and the QT Study Review.

(2) Lipase, total bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

In the phase IA/II study (CAMN107A2101), the sponsor examined increases in lipase and total bilirubin vs. predicted median AUC over seven days prior to a given visit, accounting for baseline lab values. Considering that patient samples may have been processed by different labs, they used the normalized values (NR), calculated from the lab result (R), the lab's reported upper limit of normal (U), and the lab's reported lower limit of normal (L), according to the following equation:

$$NR = (R - L)/(U - L)$$

Separately for Phase I and Phase II, the sponsor fit the linear mixed effects model:

$$Log(Lab_i(t) / Lab_i(baseline)) = \alpha_0 + \alpha_i + \beta AUC7_i(t) + \gamma Log(Lab_i(baseline)) + \varepsilon_{it}$$

where $Lab_i(t)$ is the normalized lab value of patient i at visit t, Lab_i (baseline) is the normalized lab value of patient i at the baseline visit, $AUC7_i(t)$ is the median daily AUC for patient i over the 7 days prior to visit t, and ε_{it} is residual error in the fit for patient i at visit t.

The sponsor conducted these analyses for Phase I and Phase II separately, and their analysis was performed on all patients with available PK information.

For the lipase, parameter estimates are displayed in Table 6 (Phase I) and Table 7 (Phase II).

Table 6. Linear mixed effects model parameters for change in log, normalized lipase vs. AUC and baseline log, normalized lipase in Phase I

Parameter	Value	Standard error	DF	t-value	p-value
α_0	-0.51	0.085	837	-6.01	< 0.0001
β (on AUC7)	3E-07	7E-07	837	0.41	0.683
γ (on log baseline)	-0.65	0.036	837	-17.8	< 0.0001
896 observations in 57 patients. Source: p1p2_safety.ssc -> Tab6_6to9.txt					

Table 7. Linear mixed effects model parameters for change in log, normalized lipase vs. AUC and baseline log, normalized lipase in Phase II

Parameter	Value	Standard error	DF	t-value	p-value
α_0	-0.53	0.06	1800	-8.82	< 0.0001
β (on AUC7)	4.1E-06	1.5E-06	1800	2.71	0.0067
γ (on log baseline)	-0.56	0.027	1800	-21.0	< 0.0001
2087 observations in 285 patients. Source: p1p2_safety.ssc -> Tab6_6to9.txt					

These linear mixed effects models for the entire time series do not show the coefficient on AUC7 to be statistically significant in Phase I. These results do show statistical significance (p= 0.0067) in Phase II. For a 10000 ng·h/mL increase in exposure, the model would predict a factor of exp(10000*4.1E-06), a 4% increase in lipase. The FDA reviewer repeated the analysis and found the sponsor's analysis agreeable. However, since the sponsor's data set for phase II data included patients other than the CML-AP and CML-CP population, the FDA reviewer has run another analysis for the phase II subset including the CML-AP and CML-CP patients only. The result of this subset analysis (summarized in Table 8) is generally consistent with the sponsor's analysis for the bigger phase II population. Based on the reviewer's analysis in the phase II CML-AP and CML-CP patients, for a 10000 ng·h/mL increase in exposure, the model would predict a factor of exp(10000*8.5E-06), a 9% increase in lipase.

Table 8. Linear mixed effects model parameters for change in log, normalized lipase vs. AUC and baseline log, normalized lipase in Phase II CML-AP and CML-CP patients

Parameter	Value	Standard error	DF	p-value
α_0	-0.57	0.07	1339	<.0001
ß (on AUC7)	8.5E-06	1.8E-06	1339	<.0001
γ (on log baseline)	-0.54	0.032	1339	<.0001
1524 observations in 183 patients				

For the total bilirubin, parameter estimates are displayed in Table 9 (Phase I) and Table 10 (Phase II).

Table 9. Linear mixed effects model parameters for change in log, normalized total bilirubin vs. AUC and baseline log, normalized total bilirubin in Phase I

Parameter	Value	Standard error	DF	t-value	p-value
α_0	-0.48	0.057	2371	-8.3	< 0.0001
β (on AUC7)	12E-06	5E-07	2371	23.5	< 0.0001
γ (on log baseline)	-0.67	0.037	2371	-17.9	< 0.0001
2489 Observations in 116 patients. Source: p1p2_safety.ssc -> Tab6_6to9.txt					

Table 10. Linear mixed effects model parameters for change in log, normalized total bilirubin vs. AUC and baseline log, normalized total bilirubin in Phase II

Parameter	Value	Standard error	DF	t-value	p-value
α_0	-0.37	0.045	3994	-8.09	< 0.0001
β (on AUC7)	20E-06	8.5E-07	3994	23.95	< 0.0001
γ (on log baseline)	-0.55	0.027	3994	-20.25	< 0.0001
4306 observations in 310 patients. Source: p1p2_safety.ssc -> Tab6_6to9.txt					

These linear mixed effect models for the entire time series show the coefficient on AUC7 to be statistically significant for both Phase I and Phase II ($p < 0.0001$ for each). The coefficient on AUC7 is 12E-06 in Phase I and 20E-06 in Phase II. For a 10000 ng-h/mL increase in exposure, the Phase II model would predict a factor of exp(10000*20E-06), a 22% increase in total bilirubin. The FDA reviewer repeated the analysis and found the sponsor's analysis agreeable. However, since the sponsor's data set for phase II data included patients other than the CML-AP and CML-CP population, the FDA reviewer has run another analysis for the phase II subset including the CML-AP and CML-CP patients only. The result of this subset analysis (summarized in Table 11) is generally consistent with the sponsor's analysis result for the bigger phase II population. Based on the reviewer's analysis in the phase II CML-

In conclusion, in vitro studies suggest that nilotinib may induce CYP2B6, CYP2C8, CYP2C9, CYP1A1, CYP1A2 and CYP3A4. Given the fact that nilotinib is also an inhibitor of multiple CYP enzymes (for example, CYP3A, CYP2C8 and CYP2C9), we recommend the following studies:

1) Given the fact that nilotinib is both an inhibitor and a inducer of CYP2C8 and CYP2C9, we recommend a phase 4 commitment for the sponsor to conduct clinical studies to evaluate if multiple dose of nilotinib alter the metabolism of a sensitive CYP2C9 substrate (for example, S-warfarin). If significant interaction was demonstrated, another clinical study with a sensitive CYP2C8 substrate (for example, repaglinide) may be needed.

2) Since the in vitro data demonstrated that nilotinib is an inducer of CYP2B6, the sponsor may wish to consider conducting clinical studies to evaluate if multiple dose of nilotinib alter the metabolism of a sensitive CYP2B6 substrate (for example, efavirenz) substrate.

2.4.2.4 Is the drug a substrate and/or an inhibitor of P-glycoprotein (P-gp) transport processes?

The *in vitro* permeability of nilotinib and its potential for transporter interactions were studied using bi-directional transport assay and functional flow cytometry assays. Nilotinib was found to be a substrate and an inhibitor for P-gp mediated efflux.

In the absence of inhibitors, the efflux ratio measured for nilotinib (determined by dividing the permeability in the basolateral to apical direction by the permeability in the apical to basolateral direction) is about 4 at a nilotinib concentration of 6 µM. In the presence of PSC833 (1.0 µM), a known P-gp inhibitor, the nilotinib (6 µM) efflux ratio was reduced to near unity (0.80-1.2). Permeability measurements performed in the presence of MK571 (200 µM), a known MRP inhibitor, had no effect on the nilotinib efflux ratio. These results indicate that nilotinib is a substrate for P-gp mediated efflux but not for MRP mediated efflux. When the nilotinib concentration was increased to 30 µM in the absence of inhibitors, the efflux ratio dropped to about 2, indicating that the P-gp mediated efflux of nilotinib can be saturated.

The intrinsic permeability of nilotinib (at 6 µM), estimated from the average permeability values for transport in either direction in the presence of sufficient levels of PSC833 to inhibit any P-gp efflux, was measured to be 24-30 x 10^{-5} cm/min. Under the same experimental conditions, the intrinsic permeabilities measured for mannitol (a low permeability compound) and propranolol (a high permeability compound) were 3.8-4.1 and 95-103 x 10^{-5} cm/min at concentrations of 4.1 and 5.6 µM, respectively. These results indicate that nilotinib can be classified as a moderately permeable compound.

The potential of nilotinib to inhibit the activity of P-gp mediated efflux was investigated using flow cytometry experiments with Rhodamine 123 (Rho123), a known P-gp

AP and CML-CP patients, for a 10000 ng-h/mL increase in exposure, the model would predict a factor of exp(10000*22E-06), a 25% increase in total bilirubin.

Table 11. Linear mixed effects model parameters for change in log, normalized total bilirubin vs. AUC and baseline log, normalized total bilirubin in Phase II CML-AP and CML-CP patients

Parameter	Value	Standard error	DF	p-value
α_0	-0.47	0.06	2797	<.0001
ß (on AUC7)	22E-06	1E-06	2797	<.0001
γ (on log baseline)	-0.59	0.034	2797	<.0001
2993 observations in 194 patients				

The FDA reviewer has also applied the above mentioned linear mixed effects models to examine the increase in ALT and AST vs predicted AUC over seven days prior to a given visit, accounting for the baseline values.

For ALT, parameter estimates are displayed in Table 12 (Phase I) and Table 13 (Phase II). These linear mixed effect models for the entire time series show the coefficient on AUC7 to be statistically significant for both Phase I and Phase II ($p < 0.0001$ for each). The coefficient on AUC7 is 7.6E-06 in Phase I and 7.3E-06 in Phase II. For a 10000 ng-h/mL increase in exposure, both the Phase I and II model would predict an 8% increase in ALT.

Table 12. Linear mixed effects model parameters for change in log, normalized ALT vs. AUC and baseline log, normalized ALT in Phase I

Parameter	Value	Standard error	DF	p-value
α_0	-0.25	0.07	2247	0.0003
ß (on AUC7)	7.6E-06	0.7E-06	2247	<.0001
γ (on log baseline)	-0.49	0.04	2247	<.0001
2363 observations in 114 patients				

Table 13. Linear mixed effects model parameters for change in log, normalized ALT vs. AUC and baseline log, normalized ALT in Phase II CML-AP and CML-CP patients

Parameter	Value	Standard error	DF	p-value
α_0	-0.13	0.06	2715	0.03
ß (on AUC7)	7.3E-06	1.2E-06	2715	<.0001
γ (on log baseline)	-0.50	0.03	2715	<.0001
2912 observations in 195 patients				

For AST, parameter estimates are displayed in Table 14 (Phase I) and Table 15 (Phase II). These linear mixed effects models for the entire time series show the coefficient on AUC7 to be statistically significant in Phase I (P<0.0001), but only marginally significant in Phase II (P=0.07). For a 10000 ng-h/mL increase in exposure, the Phase I and II model would predict 6% and 2% increase in AST, respectively.

Table 14. Linear mixed effects model parameters for change in log, normalized AST vs. AUC and baseline log, normalized AST in Phase I

Parameter	Value	Standard error	DF	p-value
α_0	-0.52	0.06	2093	<.0001
ß (on AUC7)	5.6E-06	0.6E-06	2093	<.0001
γ (on log baseline)	-0.59	0.04	2093	<.0001
2203 observations in 108 patients				

Table 15. Linear mixed effects model parameters for change in log, normalized AST vs. AUC and baseline log, normalized AST in Phase II CML-AP and CML-CP patients

Parameter	Value	Standard error	DF	p-value
α_0	-0.38	0.05	2724	<.0001
ß (on AUC7)	2.0E-06	1.1E-06	2724	0.07
γ (on log baseline)	-0.47	0.03	2724	<.0001
2921 observations in 195 patients				

To understand how the effect of nilotinib exposure on total bilirubin and lipase varied over time, the sponsor also fit a linear regression model for each scheduled visit separately for Phase I and Phase II, and the trend of the concentration-response relationship based on this modeling results is generally consistent with the linear mixed effects modeling results discussed above. Based on this visit-by-visit model, the sponsor predicted, for each visit in each phase, the percentage change in total bilirubin and lipase that would be due to increasing exposure by 10000 ng·h/mL. For total bilirubin, the relationship between the increase in total bilirubin and nilotinib exposure appeared to sustain and relatively consistent over time. Similar temporal patterns were observed in both phase I and phase II patients. For lipase, a statistically significant ($p<0.05$) exposure-response relationship was observed during the earlier visits (about the first two months) in phase I. However, this early trend was not sustained over time in Phase I, and is also not observed on a visit-by-visit basis in Phase II patients. However, this result needs to be interpreted with caution, because the power of this visit-by-visit analysis could be limited, due to the relatively small sample size at each of the visits, especially for the later ones.

In conclusion, there was evidence of increased total bilirubin, ALT, AST and lipase with nilotinib exposure. Caution is recommended in patients with hepatic impairment and patients with a history of pancreatitis.

2.2.4.3 Does this drug prolong the QT or QTc interval?

Nilotinib prolongs the QT interval. In a healthy volunteer study designed to assess the effects of Tasigna on the QT interval, administration of Tasigna was associated with concentration-dependent QT prolongation. At exposures that were 26% lower than the therapeutic exposures observed in patients, the maximum mean placebo-adjusted QTcF change from baseline was 18 msec (1-sided 95% Upper CI: 26 msec).

The risks associated with QT prolongation can be mitigated by correcting electrolyte abnormalities, conditions that would be expected to raise exposure to nilotinib (for example, food and CYP 3A4 inhibitors), and by prohibiting other QT-prolonging drugs. All of these seem like reasonable parts of a risk management strategy. Monitoring of QT is also a possibility, best performed around the time of C_{max}.

For details, please refer to the QT Study Review.

2.2.4.4 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The dose and dosing regimen were selected based upon safety, PK and efficacy response. Based on the exposure response for efficacy, a higher nilotinib

exposure may have better response rate; however, there was no further increase in exposure of the 600 mg BID dose as compared to the 400 mg BID dose.

The 400 mg BID dosing regimen appears reasonable.

2.2.5 What are the PK characteristics of the drug and its major metabolite?

2.2.5.1 What are the single dose and multiple dose PK parameters?

The bioavailability of nilotinib was increased when given with a meal. In a randomized, three period crossover study (CAMN107A2106), the pharmacokinetics of a single 400 mg oral dose of nilotinib was determined in healthy subjects under both fasting and fed conditions. The mean concentration-time profiles by treatment groups are depicted in Figure 5. Pharmacokinetic parameters under fasting and fed conditions are summarized in Table 16.

Figure 5. Mean nilotinib concentration-time profiles of a single 400 mg oral dose of nilotinib in healthy subjects under fasting and fed conditions (Study CAMN107A2106)

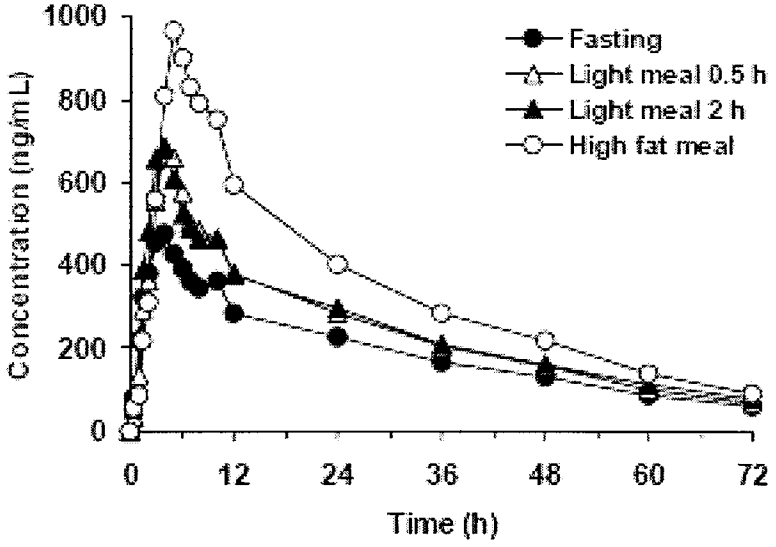


Table 16. Summary of nilotinib pharmacokinetic parameters of a single 400 mg oral dose of nilotinib in healthy subjects under fasting and fed conditions (Study CAMN107A2106)

PK parameter	Fasting N= 44	Light meal (0.5 h) N=20	Light meal (2 h) N=24	High fat meal N=44
t_{max} (h)	4.0 (2.0-10.0)	4.0 (4.0-8.0)	4.0 (3.0-5.0)	5.0 (0.5-12.0)
C_{max} (ng/mL)	508 (175)	743 (242)	716 (286)	1068 (319)
AUC_{0-t} (ng·h/mL)	13662 (4248)	17154 (8062)	17700 (6228)	24416 (6895)
$AUC_{0-\infty}$ (ng·h/mL)	14656 (5066)	17252 (8558)	19023 (7054)	25542 (7630)
CL/F (L/h)	32.8 (18.9)	30.2 (18.3)	23.8 (8.4)	17.0 (5.1)
Vz/F (L)	720 (267)	604 (302)	603 (204)	414 (115)
$t_{1/2}$ (h)	24.4 (21.2)	19.7 (10.3)	19.2 (6.4)	21.1 (8.7)

Values are median (range) for t_{max} and mean (SD) for all others.

Compared to the fasted state, the systemic exposure (AUC) increased by 15% (drug administered 2 hours after a light meal), 29% (30 minutes after a light meal), or 82% (30 minutes after a high fat meal), and the C_{max} increased by 33% (2 hours after a light meal), 55% (30 minutes after a light meal), or 112% (30 minutes after a high fat meal) (also see Section 2.2.5.3 and 2.4.1).

A dose escalation study of nilotinib was conducted in adult patients with Gleevec® (imatinib)-resistant/intolerant CML in chronic or accelerated phase or blast crisis, relapsed/refractory Ph+ ALL, and other hematologic malignancies (the Phase IA component of Study CAMN107A2101). Both once and twice daily dosing regimens were used in this study. The mean concentration-time profiles of nilotinib for all the once daily dose levels after the first dose are shown in Figure 6. Mean pharmacokinetic parameters after a single dose and multiple doses of nilotinib are summarized in Table 18 and Table 19. The median time to reach Cmax of nilotinib (t_{max}) was 3 hours. Drug elimination half-lives averaged 17 hours. For multiple dosing regimens, steady state conditions were achieved by day 8 after initiating nilotinib treatment. There was a 2-fold or 3.8-fold accumulation in serum concentrations with once daily dosing or twice daily dosing, respectively, between the first-dose and steady-state (Table 17). The accumulation ratios are slightly higher than what is expected based on an elimination half-live of 17 hours. Coefficients of variation for C_{max} and for AUC were generally in the range of about 30-70%.

The increase in nilotinib AUC was generally dose-proportional over the dose range of 50 mg to 400 mg, but AUC appeared to plateau at dose levels starting at 400 mg, remaining relatively constant over the dose range from 400 mg to 1200 mg (Figure 7). A similar pattern was also observed for C_{max} and C_{min}. Although the mechanism of the nonlinear pharmacokinetics is unknown, incomplete or limited absorption due to poor drug dissolution under gastrointestinal pH may have resulted in dose under-proportional bioavailability. Dividing the daily dose in a twice daily schedule partially overcame the dose-limiting exposure, which may be due to incomplete absorption of drug. Daily serum exposure of nilotinib at steady-state with 400 mg twice daily dose

was approximately 35% greater than with 800 mg given once daily (Figure 8), and 600 mg twice daily dose showed no further increase in exposure over 400 mg twice daily dose (Figure 9). The 400 mg twice daily dose was chosen for the Phase II component of Study CAMN107A2101.

Figure 6. Mean nilotinib concentration-time profiles after the first dose (Study CAMN107A2101 Phase I component)

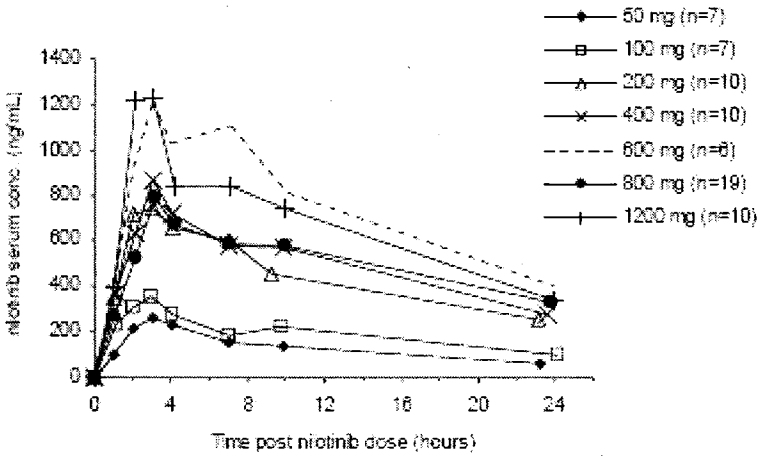


Table 17. Mean (±SD) nilotinib concentration-time profiles at representative doses

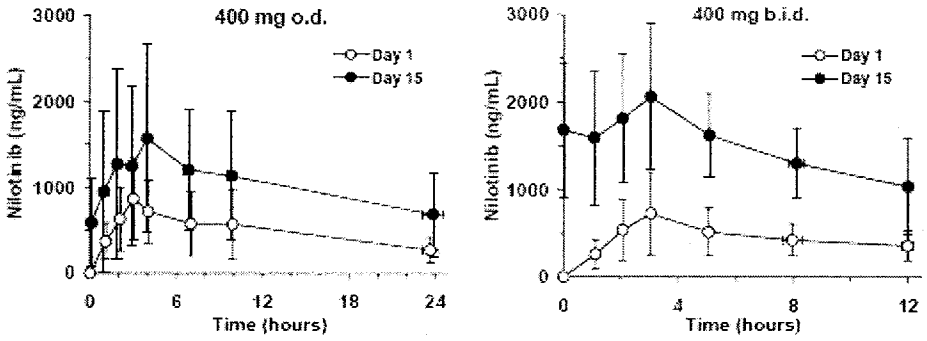


Table 18. Summary of nilotinib pharmacokinetic parameters for once-daily dosing

Day	Dose (mg)	N	t_{max} (h)	C_{max} (ng/mL)	C_{min} (ng/mL)	AUC_{0-t} (ng·h/mL)
1	50	7	3.0 (1.9-4.0)	278 (94)	65 (32)	2970 (960)
1	100	6	2.9 (1.1-3.0)	466 (285)	99 (66)	4530 (2300)
1	200	9	3.0 (1.9-7.0)	927 (562)	252 (126)	10300 (5000)
1	400	10	3.0 (1.1-10)	968 (469)	275 (149)	11300 (5800)
1	600	6	5.5 (3.0-7.3)	1450 (550)	406 (241)	17800 (8000)
1	800	19	3.3 (1.3-24)	956 (544)	331 (238)	11800 (6800)
1	1200	10	3.2 (2.1-9.8)	1540 (900)	337 (221)	15600 (8500)
15	50	2	3.0 (3.0)	403	99	4480
15	100	2	3.1 (3.0-3.1)	588	123	5400
15	200	1	4.0	1190	396	14600
15	400	9	4.0 (1.9-7.0)	1960 (1070)	686 (490)	24900 (15700)
15	600	4	3.1 (2.0-6.9)	1980 (1020)	476 (173)	22800 (9800)
15	800	17	3.1 (1.8-7.3)	2190 (1060)	607 (398)	26600 (14000)
15	1200	7	3.0 (2.0-7.6)	2490 (1420)	585 (326)	28000 (15000)
28	50	1	3.0	166	39	2230
28	100	0	ND	ND	ND	ND
28	200	2	3.0 (3.0-3.1)	1620	278	12100
28	400	6	3.0 (3.0-5.2)	1380 (970)	608 (431)	20400 (13100)
28	600	2	3.1 (3.0-3.2)	1840	544	23400
28	800	13	3.1 (3.0-9.1)	1580 (870)	446 (208)	19900 (9100)
28	1200	7	3.0 (0.8-5.2)	1930 (1400)	547 (338)	23200 (13700)

C_{min}: concentration at the last measurable time point (C_{last})
ND: not determined
Values are median (range) for t_{max}, and mean (SD) for others
t for AUC_{0-t} is 24±2 hours

Table 19. Summary of nilotinib pharmacokinetic parameters for twice-daily dosing

Day	Dose (mg)	N	t_{max} (h)	C_{max} (ng/mL)	C_{min} (ng/mL)	AUC_{0-t} (ng·h/mL)
1	400	30	3.0 (2.0-12)	808 (421)	359 (170)	5330 (2210)
1	600	16	2.6 (1.2-8.0)	1020 (600)	441 (263)	7050 (4060)
8	400	25	2.1 (0.0-5.3)	2040 (1160)	861 (471)	15700 (8300)
8	600	16	2.2 (0.0-8.0)	2610 (1190)	1130 (580)	20700 (8000)
15	400	17	3.0 (0.0-8.0)	2260 (800)	1030 (550)	18000 (5900)
15	600	12	2.2 (1.1-12)	2210 (780)	925 (581)	16400 (6900)

C_{min}: concentration at the last measurable time point (C_{last})
Values are median (range) for t_{max}, and mean (SD) for others
t for AUC_{0-t} is 12±2 hours

Figure 7. Steady state dose-exposure relationships with once daily dosing

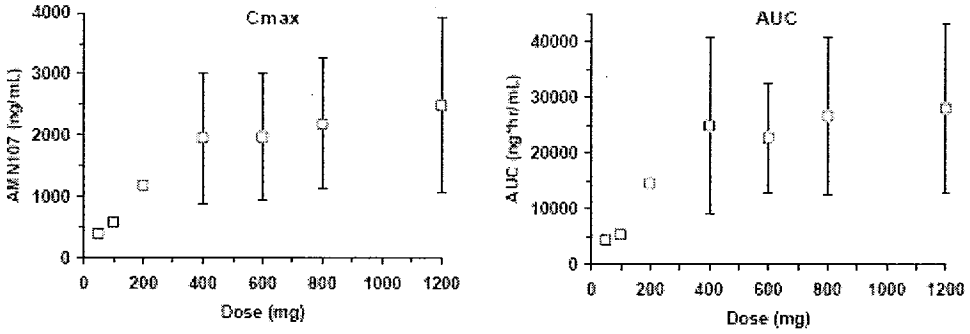


Figure 8. Steady state daily exposure with once or twice daily dosing

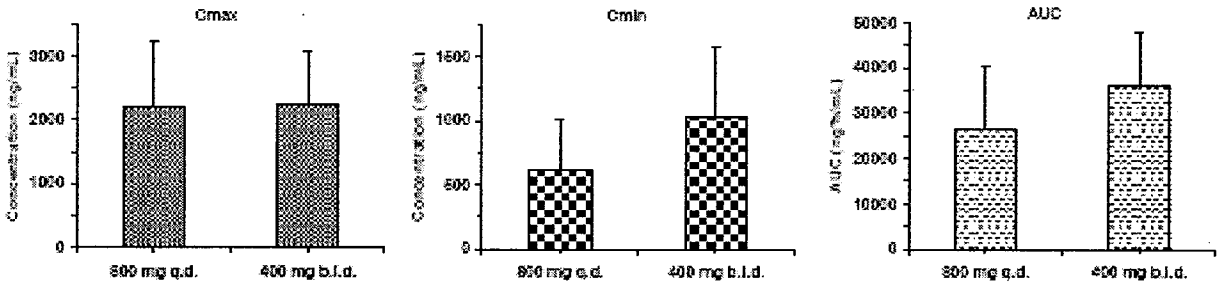
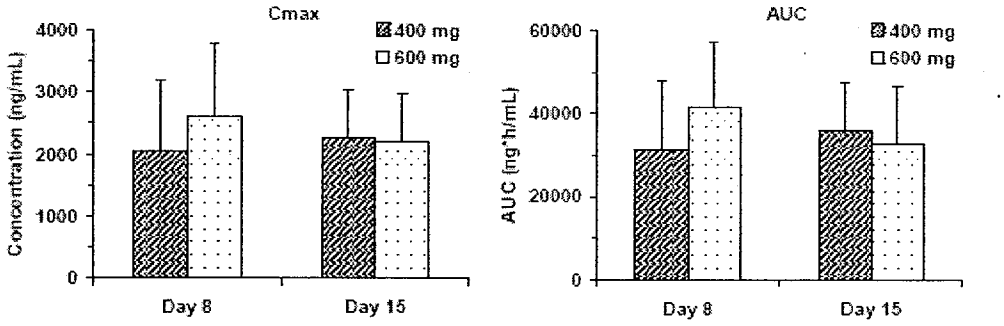


Figure 9. Steady state exposure with twice daily dosing



The extent of nilotinib binding to human plasma is high (98% on average), and independent of concentration. The α_1-acid glycoprotein was found to be the primary binding protein as compared to serum albumin in human plasma.

2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

To compare the pharmacokinetics between the healthy subjects and cancer patients, a pooled analysis of data with the 400 mg and 600 mg oral doses was conducted (Table 20). In general, the pharmacokinetics seen in healthy volunteers, especially the ones after light meals, were similar to those in cancer patients. However, it should be noted that the food intake was more restricted and carefully monitored in the healthy volunteer clinical pharmacology studies than the clinical studies in cancer patients. In the Phase IA component of Study CAMN107A2101, the once-daily dose of nilotinib was taken in the morning, 2 hours after a light breakfast. Patients then fasted for at least 2 hours after the administration of the morning nilotinib dose. For the twice-daily dose in the Phase IA component of Study CAMN107A2101, each morning/evening dose of nilotinib was taken 2 hours after a light breakfast/dinner ensuing fast of at least a 2-hours after the administration. It is known that food can increase the bioavailability of Tasigna; therefore, this comparison should be interpreted with caution.

Table 20. Pooled summary of pharmacokinetic parameters in cancer patients and healthy volunteers following single- and multiple-doses of Tasigna

Dosing regimen	Population	Study	N	Tmax (hr)	Cmax (ng/mL)		SS $AUC_{0-\tau}$ or SD AUC_{0-inf} (ng*h/mL)	
				median	mean	SD	mean	SD
400 mg QD day 1	Patients	2101 Phase I	10	3	968	469		
400 mg QD day 15	Patients	2101 Phase I	9	4			24900	15700
400 mg QD day 28	Patients	2101 Phase I	6	3			20400	13100
400 mg BID day 1	Patients	2101 Phase I	30	3	808	421		
400 mg BID day 8	Patients	2101 Phase I	25	2.1			15700	8300
400 mg BID day 15	Patients	2101 Phase I	17	3			18000	5900
400 mg SD fasted	Healthy	2106	44	4	508	175	14656	5066
400 mg SD light meal 0.5 h	Healthy	2106	20	4	743	242	17252	8558
400 mg SD light meal 2 h	Healthy	2106	24	4	716	286	19023	7054
400 mg SD high fat meal 0.5 h	Healthy	2106	44	5	1068	319	25542	7630
400 mg QD fasted day 8	Healthy	2119	15	5	542	265	13979	7776
400 mg SD fasted	Healthy	2115	15	3	426	150	11217	5155
600 mg SD day 1	Patients	2101 Phase I	6	5.5	1450	550		
600 mg BID day 1	Patients	2101 Phase I	16	2.6	1020	600		
600 mg BID day 8	Patients	2101 Phase I	16	2.2	2610	1190	20700	8000
600 mg BID day 15	Patients	2101 Phase I	12	2.2	2210	780	16400	6900
600 mg SD fasted	Healthy	2108	18	4	453	204	14576	5109

SS: Steady state
SD: Single dose
QD: Once-daily
BID: Twice-daily
$AUC_{0-\tau}$: AUC during a dosing interval

2.2.5.3 What are the characteristics of drug absorption?

The absolute oral bioavailability of nilotinib could not be determined, as a reference intravenous dosage form is not available. The sponsor attempted to estimate the oral bioavailability from the human mass balance study (CAMN107A2104), in which a single oral dose of 400 mg 100μCi [^{14}C] nilotinib was given to four healthy male volunteers under fasted condition. The results from this study suggest that nilotinib

and its related metabolites were exclusively cleared in the feces. The sponsor estimated the oral absorption from the amount of unchanged drug found in the feces (mean: 68.5%). Based on this value and the assumptions that nilotinib is not undergoing biliary secretion (the FDA reviewer thinks that the sponsor needs to add to their assumptions that nilotinib is also not undergoing GI secretion) and that nilotinib and its metabolites are stable in feces, the level of oral absorption was estimated by the sponsor to be approximately 30%. However, in the rat ADME study (ADME(US) R0300234-1), it was shown that approximately 25.4% of the dose were eliminated as unchanged drug in feces during the 0-48 h period following the intravenous dose, which could be attributed to, at least in part, the reduction of nilotinib N-oxide by gut flora and/or the GI secretion. Therefore, the FDA reviewer remains skeptical about the sponsor's estimation of the oral bioavailability of nilotinib, since the assumptions that the estimation is based upon have not been validated.

After oral administration, the median time to reach peak plasma concentration of nilotinib (t_{max}) was 3 hours. The increase in nilotinib AUC was generally dose-proportional over the dose range of 50 mg to 400 mg, but AUC appeared to plateau at dose levels starting at 400 mg, remaining relatively constant over the dose range from 400 mg to 1200 mg. Although the mechanism of this non-proportionality in nilotinib exposure is not known, incomplete *in vivo* drug dissolution resulting in incomplete absorption of this highly water-insoluble drug, and whose dissolution is pH-dependent, can be speculated. Daily steady-state nilotinib serum exposure with 400 mg b.i.d. was approximately 35% greater than with 800 mg given once daily, but 600 mg b.i.d. showed no further increase in AUC over 400 mg b.i.d. (CAMN107A2101).

The bioavailability of nilotinib is increased when the drug is given with a meal. In a randomized, three period crossover study (Study CAMN107A2106), healthy subjects received nilotinib during 3 treatment periods under conditions of (1) fasting (Treatment A), (2) 0.5 hour after a high fat meal (Treatment D) and (3) 0.5 hour (Treatment B) or 2 hours (Treatment C) after a light meal. It was demonstrated that the magnitude of the food effect depends on the type of food (light-fat meal vs. high-fat meal), as well as time between the dose and meal (see section 2.2.5.1 and 2.4.1).

Table 21. The effect of food on nilotinib pharmacokinetics (Applicant's Table 11-4 in CAMN107A2106 report, with sligt modification by the FDA reviewer)

	Ratio of geometric mean (90% CI)		
PK parameter	Light meal 0.5 h prior to dosing vs. fasting	Light meal 2 h prior to dosing vs. fasting	High fat meal 0.5 h prior to dosing vs. fasting
C_{max}	1.55 (1.36, 1.77)	1.33 (1.18, 1.50)	2.12 (1.93, 2.33)
AUC_{0-t}	1.32 (1.19, 1.45)	1.19 (1.09, 1.31)	1.82 (1.69, 1.95)
$AUC_{0-\infty}$	1.29 (1.14, 1.45)	1.15 (1.03, 1.28)	1.82 (1.67, 1.99)

2.2.5.4 What are the characteristics of drug distribution? (Include

protein binding)

Protein Binding
The extent of nilotinib binding to human plasma is high (98% on average), and independent of concentration. The α1-acid glycoprotein (AAG) was found to be the primary binding protein as compared to serum albumin in human plasma.

Blood/Plasma Ratio
Studies in mouse, rat, dog, monkey and human indicate that nilotinib uptake into blood cells was not significant. The blood to plasma ratios of nilotinib ranged from 0.68 to 0.84.

Tissue and Organ Distribution
Tissue and organ distribution studies were conducted in the pigmented (Long Evans Hooded) and non-pigmented (HanWister) rat and pregnant rat using whole body autoradiography. Nilotinib-related radioactivity was widely and rapidly distributed to most rat tissues following a single oral dose of [14C] nilotinib, consistent with the large steady-state volume of distribution of nilotinib in the rat. Between 2 and 6 h post dose, blood and most tissue radioactivity concentrations reached the plateau and the majority of tissues had a higher concentration than blood. The highest tissue-to-blood concentration ratios (>8) were observed in the adrenal cortex, liver, uveal tract, and small intestine. The following tissues: bone mineral, brain, epididymis, eye, white fat, muscle, seminal vesicles, spinal cord, testis, and thymus had concentrations similar or lower than blood. Nilotinib-related radioactivity in bone marrow was slightly higher than the blood (1-1.7 fold). By 48 h post dose, the radioactivity in most tissues was below the limit of quantification (LOQ). At 168 h post dose only the artery, liver, lung, skin, and uveal tract were above the LOQ ([redacted] ngEq/g). Significant amounts of radioactivity were found in bile, consistent with the biliary/fecal excretion being a major elimination route. The nilotinib-related radioactivity showed a low level in brain and testis indicating that nilotinib has poor brain and testis penetration. The nilotinib-related radioactivity had a high affinity to melanin but it was apparently reversible. Following intravenous dose of [^{3}H] nilotinib, the distribution pattern, except for GI tract, was similar to those of the oral dose. Furthermore, the distribution of nilotinib to liver and muscle of female mice was also similar to the rats in terms of tissue-to-plasma ratio.

Following a single 20 mg/kg oral dose of [^{14}C] nilotinib to pregnant rats, the highest tissue-to maternal blood concentration ratios (2 to 10) at the tissue peak concentrations were observed in the maternal liver, kidney, uterus, heart, and amnion. The drug-related radioactivity was moderately distributed to the fetus (day 10 gestation) with a fetus-to maternal blood ratio of 1.5-2.3 over the 24 h time period post dose. However, the fetal tissue (day 17 gestation) concentrations were all below those observed in maternal blood except for the 24 h liver (1.6-fold higher).

In pregnant rabbits after oral daily nilotinib dosing, the nilotinib concentration in all

fetuses for the dose groups of 30 and 100 mg/kg was below the lower limit of quantification at 24 h after the last dose. The mean nilotinib concentration in fetuses in the 300 mg/kg group was 29.8 ng/g tissue, while the mean maternal serum concentration was 372 ng/mL. The relative exposure (fetus-to-maternal serum ratio) was estimated to be approximately 8%, indicating that the absorption of nilotinib to the fetus could be low.

Following an oral dose (20 mg/kg) of [^{14}C] nilotinib to lactating rats, the transfer to milk of parent drug and metabolites was observed. The overall milk : plasma (M/P) concentration ratio of total radioactivity was ~2 based on $AUC_{0-\infty}$ values. Unchanged nilotinib was present at higher concentrations in rat milk compared to that observed in plasma. There were no metabolites detected that were unique to rat milk. Projecting the rat data to humans, it is estimated that the maximum amount of nilotinib and/or its metabolites that a breast-fed infant could be exposed to by ingesting 1 L of milk daily is 0.26% of a 400 mg adult dose.

2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

The human mass balance study (CAMN107A2104) suggests that nilotinib and its related metabolites were exclusively cleared in the feces.

Following a single oral dose administration of 400 mg 100μCi [^{14}C] nilotinib to four healthy male volunteers under fasted condition, the majority of the dose (mean of all 4 subjects: 93.5%) was excreted in the feces within 168 hours post dose. The amount of unchanged nilotinib recovered in the feces averaged 68.5% of the dose. Urine excretion accounted for 4.5% (mean) of the radioactivity dose; however, all of the observed radioactive peaks in the urine were related to an unexpected contaminant of the drug product, 3-fluoro-4-trifluoromethyl-benzoic acid (IMP5) and its metabolites IMP1, IMP2, IMP3 and IMP4. There was no nilotinib or related metabolites detected in the urine.

Also see section 2.2.5.6 and 2.2.5.7.

2.2.5.6 What are the characteristics of drug metabolism?

There appeared to be three primary metabolic reactions involved in the in vivo biotransformation of nilotinib in man, namely:

1. Oxidation of the methyl-imidazole ring (aliphatic hydroxylation, carboxylic acid formation, and oxidative cleavage of the methyl-imidazole ring). This pathway led to the formation of the metabolites P25.6, P34.7, P37, P36.5, P38, P41.6, P43, P46.5, P47, P49B, and P50 which together accounted for elimination of approximately 15 % of the dose.
2. Oxidation at the pyridinyl-pyrimidinyl-amino-methyl-benzamide moiety (aliphatic and aromatic hydroxylation, and N-oxide formation). This pathway led to the

formation of the metabolites P25.6, P31, P33.1, P34.7, BEJ866 (P36, which might possess some biological activity towards the same targets as nilotinib), P37, P38, P40, P42.1, P45, P49A, which together accounted for elimination of approximately 9% of the dose.

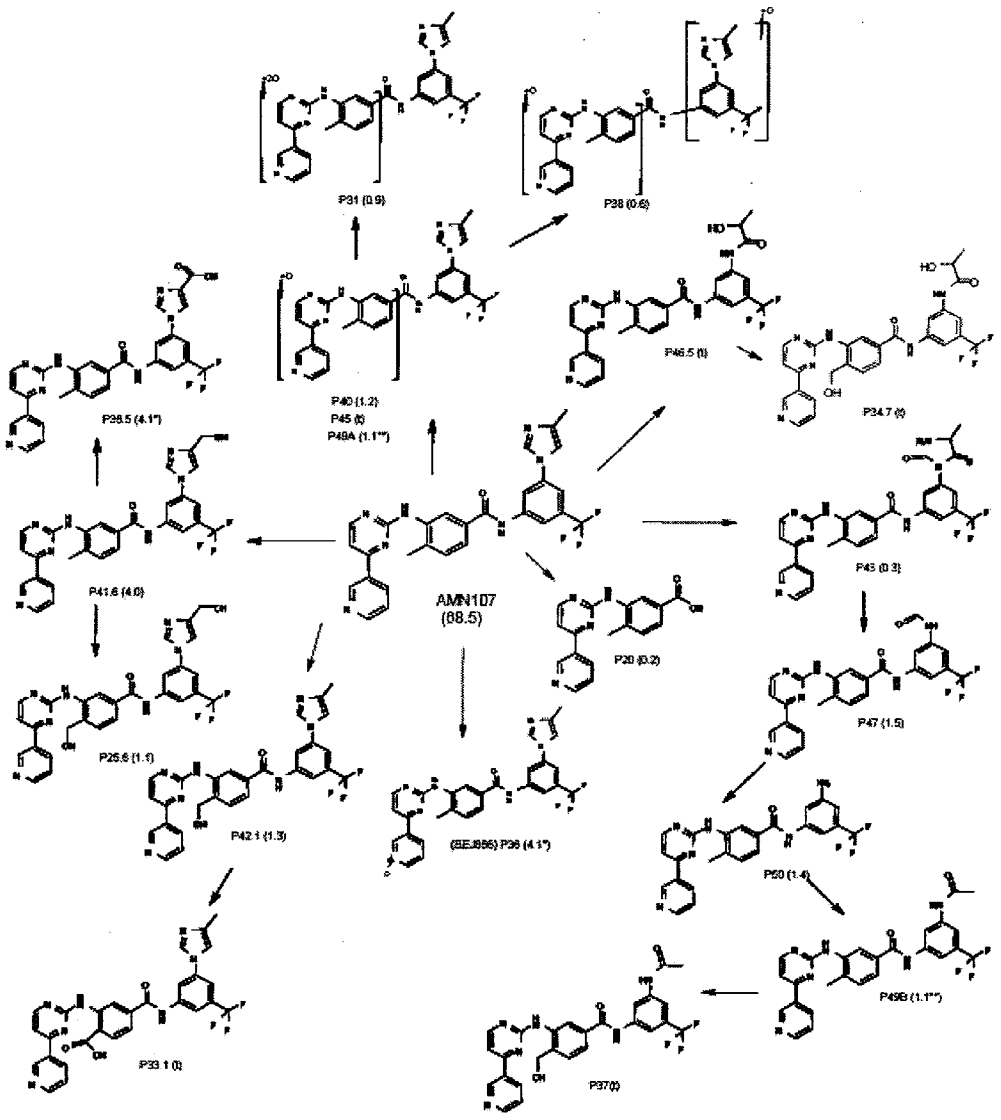
3. Amide hydrolysis which led to the formation of the metabolite P20, accounting for elimination of 0.2% of the dose.

Combinations of these primary pathways and further reactions led to the formation of at least 20 human metabolites. Based on the metabolites characterized in human excreta and in serum in the human ADME study (CAMN107A2104), a general biotransformation scheme is proposed in Figure 10.

The majority of hepatic oxidative clearance can be attributed to CYP3A4. Other hepatic P450s were able to catalyze the oxidation of nilotinib (e.g. CYP2C8, CYP2J2, and CYP1A2) however their contribution to overall clearance is expected to be minor compared to that of CYP3A4. The major metabolic pathway of [^{3}H] nilotinib metabolism in human liver microsomes was the formation of metabolite **P41.6** resulting from the hydroxylation at the methyl group of the imidazole moiety. This initial hydroxylation is subsequently combined with secondary hydroxylation events producing the additional metabolites (i.e. carboxylic acid metabolite, **P36.5**). Metabolite P36.5 showed no activity on Bcr-Abl inhibition or in the hERG channel assay. The kinetic parameters (K_m, V_{max}) associated with this pathway as catalyzed by pooled human liver microsomes were 0.55 µM and 35.8 nmol. h^{-1}. mg $protein^{-1}$, respectively. The intrinsic clearance (V_{max}/Km, CL_{int}) was estimated to be 65 ml·h^{-1}·mg $protein^{-1}$.

As predicted from the estimated CYP3A4 contribution to the hepatic clearance of nilotinib, the inhibition of CYP3A4 by ketoconazole and troleandomycin reduced nilotinib oxidative metabolism by > 95% in human liver microsomes, with apparent IC_{50} values of 0.012 and 1.2 µM, respectively. In healthy subjects, co-administration of nilotinib with ketoconazole increased nilotinib C_{max} by 84% and AUC by 3-fold on average (Study CAMN107A2110).

Figure 10. Biotransformation scheme of nilotinib in man (Applicant's Figure 11-8 in CAMN107A2104 report)



** BEJ866 (P36) and P36.5 were not chromatographically resolved, the % of dose reported was roughly estimated for each components*

*** P49A and P49B coeluted, the % of dose reported was for both components*

**** The value in parentheses next to the metabolite name corresponds to the % of dose that this metabolite contributes in the excreta. "t" corresponds to "trace".*

2.2.5.7 What are the characteristics of drug excretion?

The human mass balance study (CAMN107A2104) suggests that excretion of nilotinib and its metabolites occurred exclusively through the fecal route with no renal elimination of the drug or its metabolites observed.

Following a single oral dose administration of 400 mg 100µCi [^{14}C] nilotinib to four healthy male volunteers under fasted condition, the majority of the dose (mean of all 4 subjects: 93.5%) was excreted in the feces within 168 hours post dose. The amount of unchanged nilotinib recovered in the feces averaged 68.5% of the dose. Urine excretion accounted for 4.5% (mean) of the radioactivity dose; however, all of the observed radioactive peaks in the urine were related to an unexpected contaminant of the drug product, 3-fluoro-4-trifluoromethyl-benzoic acid (IMP5) and its metabolites IMP1, IMP2, IMP3 and IMP4. There was no nilotinib or related metabolites detected in the urine. The relative amount of moieties identified in excreta is shown in Table 22.

Table 22. [a] Amounts of nilotinib and its metabolites (expressed as % of radioactive dose) in urine (0-144 h) and feces (0-144 h or 0-168 h) of four healthy human volunteers following a single oral dose of 400 mg [^{14}C]nilotinib (Applicant's Table 11-6 in CAMN107A2104 report)

Metabolites[b]	Subject 2	Subject 3	Subject 4	Subject 7	Mean ± SD
P20					0.18 ± 0.19
P25.6					1.12 ± 0.88
P31					0.87 ± 0.42
(BEJ866) P36/P36.5[c]					8.08 ± 4.42
P38					0.62 ± 0.42
P40					1.20 ± 0.74
P41.6					3.97 ± 1.31
P42.1					1.32± 0.56
P43					0.27± 0.09
P47					1.49 ± 0.72
P49A/P49B[c]					1.05 ± 0.28
P50					1.36 ± 0.75
IMP1					0.50 ± 0.31
IMP2					1.12 ± 0.81
IMP3					1.18 ± 0.26
IMP4					1.29 ± 0.09
IMP5					0.28 ± 0.06
AMN107					68.5 ± 7.26
Sum in excreta					94.3 ± 4.9

[a] Values are calculated using the methods described in Section 9.5.4.5 and data from PTT 14.2-1.4. PTT 14.2-1.5 and integrated radiochromatogram peak areas

[b] IMP1, IMP2, IMP3 and IMP4 are acyl glucuronides of the contaminant 3-fluoro-4-trifluoromethyl-benzoic acid (IMP5) found in the AMN107 capsules. IMP1-IMP5 were only detected in urine and not in feces. IMP1-IMP5 were the only compounds detected in the urine. All of the other compounds in the table are metabolites of AMN107 and were detected only in the feces.

[c] Components not chromatographically resolved.

2.2.5.8 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

The increase in nilotinib AUC was generally dose-proportional over the dose range of 50 mg to 400 mg, but AUC appeared to plateau at dose levels starting at 400 mg, remaining relatively constant over the dose range from 400 mg to 1200 mg. A similar pattern was also observed for C_{max} and C_{min}. Although the mechanism of this nonlinear pharmacokinetics is unknown, incomplete or limited absorption due to poor drug dissolution under gastrointestinal pH may have resulted in dose under-proportional bioavailability (also see Section 2.2.5.1).

2.2.5.9 How do the PK parameters change with time following chronic dosing?

For multiple dosing regimens, steady state conditions were achieved by day 8 after initiating nilotinib treatment. There was a 2-fold or 3.8-fold accumulation in serum concentrations with once daily dosing or twice daily dosing, respectively, between the first-dose and steady-state (also see Section 2.2.5.1).

2.2.5.10 What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

The inter-subject variability of the drug exposure in the patients is moderate to high, with the coefficients of variation for C_{max} and AUC generally in the range of about 30-70%. The inter-subject variability in the healthy volunteers appears to be slightly lower (generally in the range of 30-50%). The major causes of variability may include the variability in the intrinsic factors of the patients (such as gender, hepatic function, disease stage) and extrinsic factors such as food and concomitant medications.

2.3 Intrinsic Factors

2.3.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

Based on the sponsor's population PK analysis, age, body weight, or ethnic origin were not found to significantly affect the pharmacokinetics of nilotinib, whereas there is an effect of gender, with exposure to nilotinib in female patients being approximately 12% greater than in male patients. It also appears that female patients had a slightly higher response rate than male patients (55 vs 45% for cytogenetic response in CML-CP patients; 60 vs 50% for the confirmed HR response in the CML-AP patients), which seems to be consistent with the finding that the exposure to nilotinib in female patients is slightly greater than in male patients. The population PK

analysis also suggested that the increase of AST or total bilirubin may predict the decrease in CL/F, but the magnitude of the effect is small (doubling of AST or total bilirubin is predicted to yield less than 10% increase in AUC).

Based on the population exposure-response analysis for efficacy in the CML-AP and CML-CP Phase II patients, responding patients tended to have lower white blood cell counts and lower percent Ph+ metaphase at baseline.

2.3.2 Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations (examples shown below), what dosage regimen adjustments, if any, are recommended for each of these groups?

No dose adjustment is recommended.

2.3.2.1 Elderly

Approximately 30% of subjects in clinical studies were 65 or over. No major differences were observed for safety and efficacy in patients ≥ 65 years of age as compared to adults 18 to 65 years.

2.3.2.2 Pediatric patients

Safety and effectiveness have not been established in pediatric patients.

2.3.2.3 Gender

Based on the sponsor's population PK analysis, the exposure to nilotinib in female patients is approximately 12% greater than in male patients. It also appears that female patients had a slightly higher response rate than male patients (55 vs 45% for cytogenetic response in CML-CP patients; 60 vs 50% for the confirmed HR response in the CML-AP patients).

2.3.2.4 Race

Based on the sponsor's population PK analysis, ethnic origin was not found to affect the pharmacokinetics of nilotinib.

2.3.2.5 Renal impairment

Clinical studies have not been performed in patients with impaired renal function. Clinical studies have excluded patients with serum creatinine concentration >1.5 times the upper limit of the normal range.

Since nilotinib and its metabolites are not renally excreted, altered renal function is not likely to affect nilotinib pharmacokinetics.

2.3.2.6 Hepatic impairment

Tasigna has not been investigated in patients with hepatic impairment. Clinical studies have excluded patients with ALT and/ or AST >2.5 (or >5, if related to disease) times the upper limit of the normal range and/or total bilirubin >1.5 times the upper limit of the normal range.

Metabolism of nilotinib is mainly hepatic. A hepatic impairment study is underway. Caution is recommended in patients with hepatic impairment.

2.3.2.7 What pharmacogenetics information is there in the application and is it important or not?

A pharmacogenetic analysis examining the polymorphism of UGT1A1 and its potential association with hyperbilirubinemia during nilotinib treatment was conducted [Study CAMN107A2101]. In this study, The $(TA)_7/(TA)_7$ genotype was associated with a statistically significant increase in risk of hyperbilirubinemia relative to the $(TA)_6/(TA)_6$ and $(TA)_6/(TA)_7$ genotypes. See the Pharmacogenetic Review by Michael Orr.

2.3.2.8 What pregnancy and lactation use information is there in the application?

Women of childbearing potential must be advised to use effective contraception during treatment with Tasigna (nilotinib). No effects on sperm count/motility, and fertility were noted in male and female rats up to the highest tested dose approximately 5 times the recommended dosage for human. Sexually active male or female patients taking Tasigna should use adequate contraception.

It is not known whether nilotinib is excreted in human milk. Studies in animals demonstrate that it is excreted into milk. Women should therefore not breast-feed while taking Tasigna.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or -response and what is the impact of any differences in exposure on response?

Administration of nilotinib under fed conditions increased the PK exposure of nilotinib. In study CAMN107A2106, it was shown that compared to the fasted state, the systemic exposure (AUC) increased by 15% (drug administered 2 hours after a light

meal), 29% (30 minutes after a light meal), or 82% (30 minutes after high fat meal), and the C_{max} increased by 33% (2 hours after a light meal), 55% (30 minutes after a light meal), or 112% (30 minutes after high fat meal). (Also see section 2.2.5.3). As administered in Phase II and to minimize the effect of food on nilotinib bioavailability, the sponsor proposed that nilotinib should be taken at least 2 hours after food intake, and food intake should be avoided for 1 hour after drug administration. However, the food effect for doses given 2 hours after high fat meal is not unknown.

With the exception of food and concomitant drugs, no studies were conducted to assess correlations between extrinsic factors and the pharmacokinetics of the nilotinib.

2.4.1.1 Based upon what is known about exposure-response relationships and their variability, what dosage regimen adjustments, if any, do you recommend for each of these factors? If dosage regimen adjustments across factors are not based on the exposure-response relationships, describe the basis for the recommendation.

With the exception of food and drugs, which appear in other sections of this review, no dosage regimen changes based on the extrinsic factors are recommended.

2.4.2 Drug-drug interactions

2.4.2.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?

Yes. See Section 2.4.2.2. to 2.4.2.5.

2.4.2.2 Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

Studies with pooled human liver microsomes and recombinant human CYP enzymes indicate that although multiple CYP enzymes are capable of metabolizing nilotinib, CYP3A4 is expected to be the main contributor to the oxidative metabolism of nilotinib in humans with CYP2C8 also making a contribution ($CL_{int,CYP2C8} \approx 24\%$ of $CL_{int,CYP3A4}$).

The conclusion that CYP3A4 is the main contributor to the oxidative metabolism of nilotinib was further supported by human microsomal incubation experiments performed in the presence of the known CYP3A4 inhibitors, ketoconazole and troleandomycin, where nilotinib oxidative metabolism was reduced by > 95%, with apparent IC_{50} values of 0.012 and 1.2 µM, respectively.

Furthermore, in a clinical study in which 26 healthy subjects [Study CAMN107A2110]

were administered a single 200 mg oral dose of nilotinib on day 4 while also being administered ketoconazole on days 1-6, a three-fold increase in the nilotinib serum AUC was observed (see Section 2.4.2.9).

2.4.2.3 Is the drug an inhibitor and/or an inducer of CYP enzymes?

Inhibition of CYP enzymes

The potential of nilotinib to inhibit the activity of CYP enzymes was investigated using pooled human liver microsomes and enzyme-specific probe substrates. *K*i values were determined for CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5, respectively (Table 23). *K*i values were not determined for CYP1A2 and CYP2E1 as IC_{50} values were higher than 100 µM.

Table 23. K_i values and C_{max}/K_i ratios of nilotinib for CYP isofroms.

Isoform tested	nilotinib K_i (uM)	nilotinib C_{max}/K_i ratio
CYP2C8	0.236	18.2
CYP2C9	0.132	32.6
CYP2C19	3.82	1.1
CYP2D6	1.46	2.9
CYP3A4/5	0.448	9.6

Based on the steady state nilotinib serum Cmax value of ≈ 4.3 µM observed in patients receiving oral doses of 400 mg bid [Study CAMN107A2101], the C_{max}/K_i ratios of nilotinib for CYP isofroms were calculated (Table 23). Based on the fact that the estimated C_{max}/K_i ratios were all higher than 1, it is likely that nilotinib could act as an inhibitor of CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 activity in the clinic. In a clinical study with eighteen healthy subjects in which a single oral 600 mg dose of nilotinib was co-administered with a 4 mg dose of midazolam (CYP3A4 substrate) [CAMN107A2108], nilotinib was found to increase the midazolam exposure by 30% (see Section 2.4.2.8).

Based on the above data, caution should be exercised when coadministering Tasigna with substrates of these enzymes having a narrow therapeutic index. Since warfarin is metabolized by CYP2C9 and CYP3A4 it should be avoided if possible. Other medications for anticoagulation should be considered. Because the nilotinib C_{max}/K_i ratio was much higher for CYP2C9 and CYP2C8 than for CYP3A4, significant in vivo drug-drug interactions are likely when Tasigna is coadministered with sensitive CYP2C9 substrates or sensitive CYP2C8 substrates. Therefore, the FDA reviewer recommends a phase 4 commitment to perform clinical studies examining the ability of Tasigna to alter the metabolism of a sensitive CYP2C9 substrate (for example, S-warfarin) and a sensitive CYP2C8 substrate (for example, repaglinide).

Preincubation experiments using human liver microsomes and nilotinib showed no potential for timedependent inhibition (i.e., no mechanism-based inactivation) of CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5.

Induction of CYP enzymes

The potential of nilotinib (up to 10 µM) to act as an inducer of CYP enzymes and drug transporters was evaluated in primary human hepatocytes of three donors using both mRNA and CYP enzyme activity quantitation.

Nilotinib was found to induce CYP1A1 mRNA, CYP1A2 mRNA and activity in the primary human hepatocytes of the three donors examined. The inductions were more than 2-fold with nilotinib concentrations ≥ 1 µM, but did not exceed 40% of the positive ß-napthoflavone (BNF) control.

Nilotinib was found to induce UGT1A1 mRNA in the primary human hepatocytes of the three donors examined. Induction of UGT1A1 mRNA levels did not exceed 40% of the positive controls; the levels were low, only exceeding 2-fold (2.66-fold) in one liver (Liver 1) at 10 µM nilotinib.

Nilotinib was found to induce CYP2B6 (mRNA and activity), with induction levels close to 40% of the rifampicin (RIF) positive controls, but much lower than 40% of the Phenobarbital positive control. The level of CYP2B6 activity exceeds 2-fold (2.47- to 2.85-fold) in one out of three livers examined with nilotinib treatments ≥ 1 µM.

Nilotinib was found to induce CYP2C8 and CYP2C9 activities, with induction levels not exceeding 40% of the RIF positive controls. CYP2C8 and CYP2C9 activities were induced above 2-fold by nilotinib in one out of three liver examined. Although it is likely that nilotinib inhibited the activities of CYP2C8 and CYP2C9 after the induction period, the induction of CYP2C8 and CYP2C9 activity was still observed. Therefore, nilotinib would be considered to be an *in vitro* inducer of CYP2C8 and CYP2C9 activities.

Nilotinib was found to induce CYP3A4 and CYP3A5 mRNA dose-dependently in all three livers examined. CYP3A4 mRNA was induced up to 71.8-fold. The induction levels of CYP3A4 mRNA were, however, below 40% of the RIF positive controls. Measurement of CYP3A activity after the induction treatment found no induction of CYP3A activity above 2-fold in the three livers examined. Due to the potent inhibition of CYP3A activity by nilotinib, it is likely any induction of CYP3A activity was masked by nilotinib's inhibition of the CYP3A activity.

Nilotinib (up to 10 µM) was not found to be an inducer of CYP2C19 mRNA (< 1.1-fold) or activity (≤ 1.75-fold) in the three livers examined.

Nilotinib (up to 10 µM) was not found to be an inducer of ABCB1 or ABCC2 mRNA.

substrate. The P-gp mediated efflux of Rho123 was inhibited by nilotinib with an IC_{50} of 1.7 µM. This IC_{50} value was similar to the value obtained for cyclosporine (CsA), a strong P-gp inhibitor used as a positive control in these experiments. However, the maximum P-gp inhibition obtained for nilotinib was only about one-third of the amount obtained with the CsA. Based on the IC_{50} value of 1.7 µM determined for P-gp inhibition by nilotinib and the steady state nilotinib serum C_{max} value of ≈4.3 µM obtained in a 400 mg twice-daily oral dosing study with patients, it is possible that nilotinib could act as a P-gp inhibitor in the clinic. Based on the C_{max}/IC_{50} value of ~2.5, the sponsor may wish to consider conducting an in vivo drug interaction study with a P-gp substrate (for example, digoxin).

An *in vitro* study that monitored the effect that prazosin (a potent inhibitor of the OCT-1 cellular transporter) had on the uptake of nilotinib into blood cells suggested that the cellular uptake of nilotinib should not be impacted by OCT-1 mediated influx.

2.4.2.5 Are there other metabolic/transporter pathways that may be important?

The potential of nilotinib to act as an inhibitor of human UGT1A1 was investigated by examining the effect of increasing concentrations of nilotinib on bilirubin and estradiol glucuronidation activity in a number of *in vitro* assay systems. Inhibition of UGT1A1 activity by nilotinib was apparent using either probe reaction with an estimated IC_{50} below 1 µM. A follow-up kinetic study estimated a *K*i value of 0.19 µM for inhibition of estradiol glucuronidation that appeared to fit a competitive (full) inhibition model. This *K*i value is well below the steady state nilotinib serum Cmax value of ≈4.3 µM observed in patients receiving oral doses of 400 mg bid [Study CAMN107A2101], suggesting that nilotinib could inhibit the activity of UGT1A1 in clinical settings.

In the proposed label, the sponsor stated that Nilotinib is a competitive inhibitor of UGT1A1 in vitro, potentially increasing the concentrations of drug eliminated by these enzymes. Caution should be exercised when coadministering Tasigna with substrates of these enzymes having a narrow therapeutic index.

A pharmacogenetic analysis examining the polymorphism of UGT1A1_and its potential association with hyperbilirubinemia during nilotinib treatment was conducted [Study CAMN107A2101]. In this study, a positive correlation was seen between the (TA)7/(TA)7, genotype at the (A(TA)nTAA) element of UGT1A1 and hyperbilirubinemia, suggesting that genetic susceptibility may contribute to the development of hyperbilirubinemia with nilotinib treatment.

2.4.2.6 Does the label specify co-administration of another drug (e.g., combination therapy in oncology) and, if so, has the interaction potential between these drugs been evaluated?

The proposed label stated that Tasigna may be given in combination with hematopoietic growth factors such as erythropoietin or Filgrastim (G-CSF) if clinically

indicated, and that Tasigna may be given with hydroxyurea or anagrelide if clinically indicated. The interaction potential between these drugs has not been evaluated.

Erythropoietin (EPO) is a glycoprotein that regulates the production of red blood cells by stimulating the division and differentiation of committed erythroid progenitor cells in the bone marrow. EPO is administered intravenously or subcutaneously. Metabolism and elimination of EPO are not fully understood. About 10% of the dose appears to be excreted in the urine.

Filgrastim is a human granulocyte colony-stimulating factor (rhuG-CSF) produced by recombinant DNA technology. Filgrastim is administered intravenously or subcutaneously; however, the subcutaneous (SC) route is preferred clinically. The specific metabolic pathways of filgrastim have not been identified. In animal studies, 90% of radiolabeled filgrastim is excreted in the urine within 24 hours.

Hydroxyurea is rapidly absorbed following oral administration. Roughly half of an administered dose is metabolized by the liver, and these metabolites are excreted by the lungs as carbon dioxide and by the kidneys as urea. The remaining 50% is excreted as unchanged drug by the kidneys. The elimination half-life is 3.5—4.5 hours. Eighty percent of a dose is found in the urine as parent drug or metabolites in 12 hours.

Anagrelide is administered orally. Anagrelide is extensively metabolized with < 1% excreted unchanged in the urine. Greater than 70% of the dose is recovered in urine and 10% in the feces. Anagrelide is partially metabolized by CYP1A2. Furthermore, anagrelide demonstrates some inhibitory activity of CYP1A2, and theoretically may affect the clearance of drugs metabolized by CYP1A2.

2.4.2.7 What other co-medications are likely to be administered to the target patient population?

In the phase II study, 91.2% of CML-CP patients and 92.5% of CML-AP patients took concomitant medications and significant non-drug therapies after the start of study drug.

There are many kinds of concomitant medications used in the CML-CP and CML-AP patients in this study, for example, other antineoplastic agents, diuretic agents, antiemetic agents, anti-infective agents, anti-histamine agents and agents to treat gout.

In the CML-CP population in this study, 49 (15.4%) patients were given red blood cells, 28 (8.8%) patients received platelets or human blood, and three (0.9%) patients received blood or related products (WBC). In the CML-AP population in this study, 55 (45.8%) patients were given red blood cells, 37 (30.8%) patients received platelets or human blood, and one (0.8%) patient received blood or related products (WBC).

2.4.2.8 Are there any in vivo drug-drug interaction (DDI) studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

DDI between Nilotinib and Ketoconazole (a potent CYP3A4 inhibitor)
The effect of multiple doses of ketoconazole, a potent CYP3A4 inhibitor, on the single–dose pharmacokinetics of nilotinib was examined in healthy adult subjects (Study CAMN107A2110).

This was a single center, two period, open-label, single sequence crossover study. Twenty-five out of 26 enrolled subjects completed the study. Subjects were given the following treatment:

- Treatment period 1 (day 1): subjects received a single 200 mg oral dose of nilotinib (under fasted condition);
- Treatment period 2 (days 9 through 16): subjects received a single 200 mg oral dose of nilotinib (under fasted condition) on day 12, and ketoconazole (400 mg oral dose once daily) on days 9 through 14.

Nilotinib blood PK samples were collected up to 96 hours post-dose on Day 1 and 12. Ketoconazole blood PK samples was collected at pre-dose, 2 hours and 24 hours post-dose on Days 9, 10, 11, 12, 13 and 14.

According to the FDA drug-drug interaction guidance, the study design and choice of CYP3A4 inhibitor are appropriate.

Nilotinib (200 mg) with and without ketoconazole is generally safe and well tolerated in healthy subjects. However, the frequency of adverse events and/or laboratory abnormalities related to nilotinib and/or ketoconazole appears to be increased under circumstances when coadministration is required.

The mean concentration-time profiles of nilotinib are depicted in Figure 11. Pharmacokinetic parameters of nilotinib administered alone and in combination with ketoconazole are summarized in **Table 24**. The 90% confidence interval for the ratios of geometric mean for Cmax and AUC was not in the range of 0.80 and 1.25, and a statistically significant drug-drug interaction was concluded (**Table 25**). Coadministration of ketoconazole with nilotinib increased nilotinib Cmax by 84% and AUC by 3-fold on average. The half-life was prolonged by 115% on average.

Based on these results, the sponsor proposed in the label that concurrent treatment with strong CYP3A4 inhibitors (including but not limited to, ketoconazole, itraconazole, erythromycin,clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin) should be avoided if at all possible. Should treatment with any of these agents be required, the sponsor recommends that therapy with Tasigna be interrupted if possible. If transient interruption of treatment with Tasigna is not possible, the sponsor recommends that close monitoring of the

individual for QT prolongation of the QT interval is indicated for patients who can not avoid strong CYP3A4 inhibitors. In addition to the sponsor's recommendations, the FDA reviewer recommends that if a strong CYP3A4 inhibitor is unavoidable, the dose of nilotinib should be decreased at least by 1/2 (for example, if the patient was given 400 mg twice daily, then the dose should be reduced to 400 mg once daily).

Figure 11. Mean nilotinib concentration-time profiles (n=25) after administration alone and in combination with ketoconazole (Applicant's Figure 11-1 in CAMN107A2110 report)

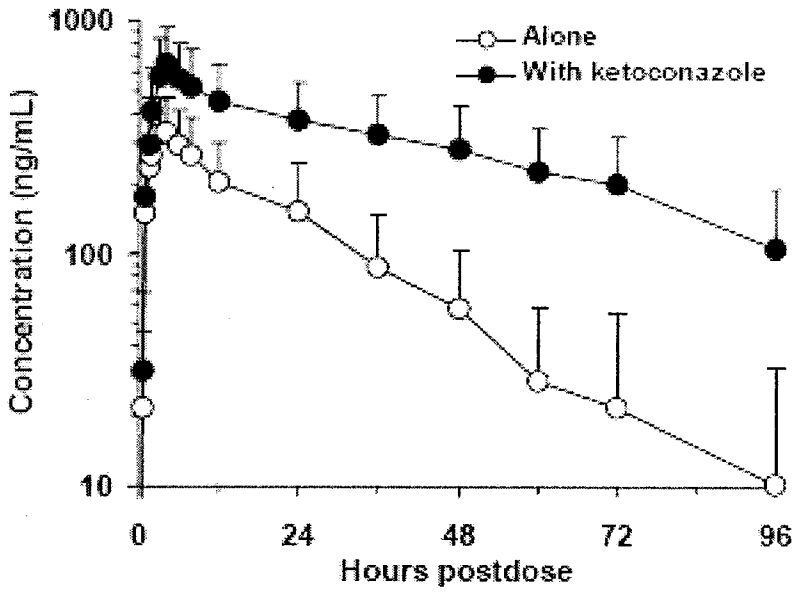


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Table 24. Summary of nilotinib pharmacokinetic parameters (Applicant's Table 11-3 in CAMN107A2110 report)

Parameter	AMN107 (N=25)	AMN107 + ketoconazole (N=25)
t_{max} (h)	4.0 (2.0-8.0)	4.0 (2.0-8.0)
C_{max} (ng/mL)	356 (142)	673 (279)
AUC_{0-t} (ng·h/mL)	8793 (4769)	27757 (13183)
AUC_{0-inf} (ng·h/mL)	8590 (4753)	26682 (13703)
CL/F (L/h)	31.0 (17.0)	12.3 (13.9)
Vz/F (L)	587 (385)	333 (219)
$t_{1/2}$ (h)	15.2 (9.3)	32.7 (17.9)

Values are median (range) for t_{max}, and mean (SD) for all others.

Table 25. Statistical Results (Applicant's Table 11-4 in CAMN107A2110 report)

Parameters	Point estimate (90% CI) AMN107 + ketoconazole/ AMN107 only
t_{max} ^	0.0 (0.0-1.0)
C_{max} *	1.84 (1.53, 2.20)
AUC_{0-t} *	3.13 (2.55, 3.84)
AUC_{0-inf} *	3.01 (2.31, 3.93)

^ point estimate refers to the estimated median difference with (25^{th}-75^{th} percentile)

*point estimate refers to the ratio of adjusted geometric means with associated 90% CI

Ketoconazole plasma PK was also evaluated in this study. It was shown that ketoconazole concentrations were fairly consistent at steady state when given either alone or in the presence of nilotinib.

DDI between Nilotinib and Rifampin (CYP3A4 Inducer)

The effect of 600 mg daily oral dose of rifampin (CYP3A4 inducer) on the pharmacokinetics of a single 400 mg oral dose of nilotinib was examined in healthy subjects (Study CAMN107A2115).

This is a phase I open-label, two period, single center study. 15 healthy male or sterile/postmenopausal female subjects (18 to 55 years of age) completed the study. Each subject received nilotinib on Days 1 and 16 and daily rifampin on Days 8 through 19. During the first treatment period, baseline oral nilotinib PK were assessed on Day 1. The second treatment period consisted of one oral 400 mg dose of nilotinib (Day 16) and 12 daily dosing days of 600 mg rifampin treatment (Days 8-19) to maintain CYP3A4 induction by rifampin. Serial blood samples for serum nilotinib concentration determination were collected up to 72 hours post-dose after nilotinib administration on Days 1 and 16. The ratio of 6β-hydroxycortisol to cortisol

was measured on Days -1, 11, 15, and 19, to monitor induction of CYP3A4.

The mean concentration-time profiles of nilotinib are depicted in Figure 12. PK parameters of nilotinib administered alone and in combination with rifampin are summarized in **Figure 12**. The results of the statistical analysis on Cmax and AUC are summarized in **Table 27**.

Figure 12. Mean nilotinib concentration-time profiles after 400 mg single oral dose of Tasigna administration alone and in combination with rifampin

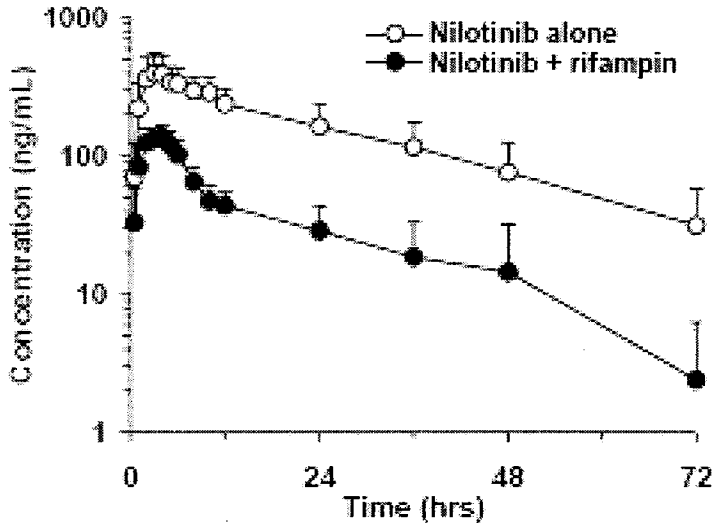


Table 26. Summary of nilotinib PK parameters after 400 mg single oral dose of Tasigna administration alone and in combination with rifampin

Parameter	AMN107 (N=15)	AMN107 + rifampin (N=15)
t_{max} (h)	3.0 (2.0-8.0)	4.0 (2.0-6.0)
C_{max} (ng/mL)	426 (150)	149 (30)
AUC_{0-t} (ng·h/mL)	10114 (3996)	2088 (801)
AUC_{0-inf} (ng·h/mL)	11217 (5155)	2010 (579)
CL/F (L/h)	45.2 (24.6)	216 (69)
Vz/F (L)	888 (305)	4157 (1950)
$t_{1/2}$ (h)	18.8 (10.0)	14.6 (6.5)

Values are median (range) for t_{max}, and mean (SD) for all others.

Table 27. Statistical Results of nilotinib PK parameters after 400 mg single oral dose of Tasigna administration alone and in combination with rifampin

Parameter	Adjusted geometric mean		Geometric mean ratio (AMN107 + rifampin) / AMN107 alone		
	AMN107 alone	AMN107 + rifampin	Point estimate	Lower 90% CI	Upper 90% CI
C_{max}	401	146	0.36	0.31	0.43
AUC_{0-t}	9308	1964	0.21	0.18	0.25
AUC_{0-inf}	10074	2029	0.20	0.16	0.25
$t_{1/2}$	16.5	13.5	0.82	0.61	1.09

Co-administration of rifampin with nilotinib decreased C_{max} and AUC of nilotinib by 64% and by 80% on average, respectively. The 90% confidence interval for the ratios of geometric mean for Cmax and AUC was not in the range of 0.80 and 1.25 and a statistically significant drug-drug interaction was concluded.

Based on these results, concurrent treatment with potent CYP3A4 inducers (including but not limited to, phenytoin, rifampicin, carbamazepine, phenobarbital, and St. John's Wort) should be avoided if at all possible. If a strong CYP3A4 inhibitor is unavoidable, the dose of nilotinib may need to be increased, depending on patient tolerability. However, it should be noted that the nilotinib exposure (AUC) appears to plateau at dose levels starting at 400 mg, therefore, to increase the exposure, dosing regimen more frequent than twice daily may be necessary. If the [redacted] dose is adjusted upward, the dose will need to be reduced to the indicated starting dose upon discontinuation of rifampicin or other inducers.

DDI between Midazolam (a sensitive CYP3A4 substrate) and Nilotinib

The effect of a single oral 600 mg dose of nilotinib on the pharmacokinetics of a single oral 4 mg dose of midazolam was studied in healthy subjects (Study CAMN107A2108).

This was a single-center, open label, randomized, three-period crossover study. In each treatment period, healthy male and female subjects (18 - 55 years of age) received one of the following:

(A) nilotinib 600 mg alone (3 x 200 mg hard gelatin capsules),
(B) Midazolam 4 mg alone (2 mL x 2 mg/mL oral syrup),
(C) Midazolam 4 mg dosed 2 hours after administration of nilotinib 600 mg.

There were 6 unique treatment sequences: ABC, ACB, BAC, BCA, CAB, and CBA. All study drugs were given under fasting conditions (at least 10 hours prior to dosing and 4 hours post administration of nilotinib, or 2 hours post administration of midazolam when midazolam was administered alone).

During the treatment period when midazolam was administered alone, PK samples were taken up to 24 hours post dosing. For the other treatments, PK samples were taken up to 72 hours post dosing. Systemic concentrations of nilotinib, midazolam, and 1-hydroxymidazolam were determined by validated liquid chromatography-tandem mass spectrometry assays with lower limit of quantifications of [redacted] ng/mL (nilotinib), and [redacted] ng/mL (midazolam and 1-hydroxymidazolam).

No adverse events were reported. No clinically relevant changes were seen in laboratory parameters, vital signs or ECGs.

Pharmacokinetic parameters of nilotinib, midazolam and 1-OH midazolam when administered either alone or in combination, are summarized in Table 28. The results of the statistical analysis on AUC_{0-t}, AUC_{0-inf}, C_{max} and t_{max} for midazolam are summarized in Table 29.

Table 28. Summary of pharmacokinetic parameters for nilotinib, midazolam and 1-OH midazolam

PK Parameter	AMN107		Midazolam		1-OH midazolam	
	AMN107 only	AMN107+ midazolam	Midazolam only	AMN107+ midazolam	Midazolam only	AMN107+ midazolam
AUC_{0-t} (ng·hr/mL)	13368 (4269)	13578 (5059)	111 (58)	143 (66)	28.7 (10.8)	35.6 (10.9)
AUC_{0-inf} (ng·hr/mL)	14576 (5109)	14512 (6302)	121 (73)	157 (71)	30.8 (10.4)	37.5 (11.6)
C_{max} (ng/mL)	453 (204)	511 (203)	38.5 (16.1)	45.0 (17.5)	12.7 (8.9)	13.4 (4.9)
t_{max} (hr)	4.0 (2.0-5.0)	4.0 (1.9-5.0)	1.0 (0.5-1.5)	0.5 (0.5-1.5)	1.0 (0.5-1.5)	1.0 (0.5-2.0)
λz (1/hr)	0.034 (0.014)	0.035 (0.017)	0.14 (0.07)	0.13 (0.06)	0.12 (0.06)	0.15 (0.10)
$t_{1/2}$ (hr)	26.4 (20.8)	24.6 (12.5)	5.8 (2.5)	6.8 (4.2)	6.6 (2.9)	6.2 (3.4)
Vz/F (L)	1490 (710)	1476 (763)	303 (99)	281 (184)	ND	ND
CL/F (L/hr)	45.3 (18.3)	47.5 (29.0)	40.9 (15.7)	29.8 (10.5)	ND	ND

t_{max} values are median (range). All others are mean (SD).
ND: not determined.

Table 29. Statistical results: Effect of nilotinib on midazolam pharmacokinetic parameters

Parameters	Ratio of geometric means (90% CI) AMN107+midazolam/ midazolam
AUC_{0-t}* (N=17)	1.30 (1.18,1.43)
AUC_{0-inf}* (N = 16)	1.31 (1.16,1.47)
C_{max}* (N = 17)	1.20 (1.03,1.38)
t_{max}^ (N =17)	0.0 (-0.5-0.0)
* = refers to adjusted geometric means with associated 90% CI ^ = refers to estimated median difference with (25th - 75th percentile)	

A statistically significant drug-drug interaction on the pharmacokinetics of midazolam was concluded based on the co-administration of nilotinib and midazolam. In the presence of single 600 mg of nilotinib, AUC of midazolam was increased by 30% and Cmax by 20%, which is only of a moderate magnitude despite the in vitro C_{max}/K_i of 9 for nilotinib against CYP3A4. However, the C_{max} associate with this 600 mg single dose of nilotinib was only about 1μM, much lower than the steady state C_{max} (~4μM) for the recommended 400 mg twice daily dosing regimen. On the other hand, since in vitro data also suggested that nilotinib may also induce CYP3A4 enzyme, it is challenging to predict the effect of long term use of nilotinib on the pharmacokinetics of Midazolam and other CYP3A4 substrates. The AUC of 1-OH Midazolam, the active metabolite of midazolam, increased by 23% in the combination treatment. Co-administration of both nilotinib and midazolam does not affect the pharmacokinetics of nilotinib, which is anticipated since midazolam is neither an inhibitor nor inducer of CYP3A4.

It is recommended as prudent clinical practice, to exercise caution when co-administering nilotinib with other medications that are CYP3A4 substrates. Other treatment alternatives should be carefully considered before using CYP3A4 substrates with narrow therapeutic windows. Additional DDI study to evaluate if multiple doses of nilotinib alter the metabolism of midazolam or other sensitive CYP3A4 substrate may be needed (see 2.4.2.10).

2.4.2.9 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

No pharmacodynamic drug-drug interactions have been predicted based on mechanisms.

2.4.2.10 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

Yes.

a. Given the fact that nilotinib is both an inhibitor and a inducer of CYP2C8, CYP2C9 and CYP3A4, and that the currently completed DDI

study with midazolam only used a single dose of nilotinib, we recommend a phase 4 commitment for the sponsor to conduct clinical studies to evaluate if multiple dose of nilotinib alter the metabolism of a sensitive CYP2C9 substrate (for example, S-warfarin). If significant interaction was demonstrated, additional clinical studies to evaluate if multiple doses of nilotinib alter the metabolism of a sensitive CYP2C8 substrate (for example, repaglinide) and/or a sensitive CYP3A4 substrate (for example, midazolam) may be needed.

b. Given the fact that nilotinib has pH dependent solubility, we recommend a phase 4 commitment for the sponsor to conduct clinical studies to evaluate if antacids and H2 blockers/proton pump inhibitors alter the pharmacokinetics of nilotinib.
c. Since the in vitro data demonstrated that nilotinib is an inducer of CYP2B6, the sponsor may wish to consider conducting clinical studies to evaluate if multiple dose of nilotinib alter the metabolism of a sensitive CYP2B6 substrate (for example, efavirenz) substrate.
d. Nilotinib was found to be an inhibitor and a substrate for P-gp mediated efflux. Therefore the sponsor may wish to consider conducting an in vivo drug interaction study with a P-glycoprotein substrate (for example, digoxin) and an in vivo drug interaction study with a P-glycoprotein inhibitor.

2.4.3 What issues related to dose, dosing regimens, or administration are unresolved and represent significant omissions?

The food effect for doses given 2 hours after high fat meal is not known.

2.5 General Biopharmaceutics

2.5.1 Based on the biopharmaceutics classification system (BCS) principles, in what class is this drug and formulation? What solubility, permeability, and dissolution data support this classification?

According to the Biopharmaceutics Classification System, nilotinib has been classified as a class 4 compound.

Nilotinib is very slightly soluble in water (0.29 mg/mL). Solubility in aqueous solutions is 2.8 mg/mL at gastric pH, and greatly decreases with increasing pH (<0.1 mg/mL at intestinal pH). Nilotinib is practically insoluble (<0.1 mg/mL) in buffer solutions of pH 4.5 and higher.

Dissolution of nilotinib at 0.1N hydrochloric acid is both rapid and complete (within 20 minutes) whereas dissolution under conditions of near neutral pH was very slow and incomplete due to the limited solubility of the drug substance.

Nilotinib can be classified as a moderately permeable compound. The permeability of nilotinib across confluent Caco-2 cell monolayers was investigated both with and without the addition of transport protein-specific inhibitors in order to assess the *in vitro* permeability of nilotinib and its potential for transporter interactions. The results indicate that when compared to mannitol (a low permeability compound) and propranolol (a high permeability compound), nilotinib can be classified as a moderately permeable compound.

2.5.2 What is the relative bioavailability of the proposed to-be-marketed (or final market image, FMI) formulation to the pivotal clinical trial?

Both the clinical service form (CSF) formulation and the proposed to-be-marketed (or final market image, FMI) formulation were used in the pivotal clinical trial (Phase II components of Study CAMN107A2101) in the target patient population. In the phase IA component of Study CAMN107A2101, the CSF formulation was used. The formulation used in Clinical Pharmacology studies, [Study CAMN107A2106] [Study CAMN107A2108] [Study CAMN107A2110] [Study CAMN107A2119], was the FMI. For the human ADME study [Study CAMN107A2104], the CSF formulation containing the radioactive dose was used, where the excipients and their mixture ratio were identical with those in the CSF.

The differences between the CSF and the FMI are the [redacted] Since only minor quantitative, and no qualitative modifications were made between the CSF and the FMI, no definitive bioequivalence study in humans was conducted.

The dissolution profiles of the CSF and the FMI were found to be similar.

In the 29-Sep-07 submission, pharmacokinetic data obtained by sparse sampling approach in the Phase II component of Study CAMN107A2101 were compared between the CSF in 44 patients and the FMI in 10 patients (Based on the cut-off date of 26-Jun-2005). Pharmacokinetic parameters, Cmax, Cmin, and the ratio of Cmax/Cmin, were obtained, and descriptive statistics of these pharmacokinetic parameters are summarized in Table 30.

Table 30. Descriptive statistics of pharmacokinetic parameters for CSF and FMI

Parameter	C_{max} (ng/mL)		C_{min} (ng/mL)		C_{max}/C_{min} ratio	
Formulation	CSF	FMI	CSF	FMI	CSF	FMI
Mean	1530	1454	1079	1187	1.60	1.25
SD	932	699	728	569	0.65	0.18
CV (%)	61	48	67	48	41	14
Minimum	[redacted]					
Maximum						
Geometric mean	1303	1303	864	1051	1.53	1.24

The two formulations showed comparable systemic exposure to nilotinib, assessed by C_{max} and C_{min}. The differences in mean C_{max} and C_{min} between the CSF and the FMI were 5% and 9%, respectively, and were not statistically significant.

In the updated population PK/PD modeling report submitted in 16-Feb-07, the sponsor explored whether there was a significant effect on bioavailability due to formulation (CSF, FMI, or missing) using the population PK modeling approach. This modeling analysis included more data than the previous mentioned analysis (comparison of the C_{max} and C_{min} between the FMI and CSF formulations) since they had different cut-off dates. After accounting for a difference in bioavailability parameterization with phase, the sponsor did not find a statistically significant effect from including formulation on bioavailability (p= 0.11).

The CSF and the FMI are considered similar based on similar *in vitro* dissolution profiles and the human PK data obtained from Study CAMN107A2101.

2.5.2.1.1 What data support or do not support a waiver of in vivo BE data?

Both the clinical service form (CSF) formulation and the proposed to-be-marketed (or final market image, FMI) formulation was used in the pivotal clinical trial (Phase II components of Study CAMN107A2101) in the target patient population. The differences between the CSF and the FMI are the [redacted]. Since only minor quantitative, and no qualitative modifications were made between the CSF and the FMI, no definitive bioequivalence study in humans was conducted.

The dissolution profiles of the CSF and the FMI were found to be similar. Based on the sparse PK data in study CAMN107A2101, the PK from the CSF and FMI formulations were considered to be similar.

Therefore, an in vivo BE study appears to be unnecessary based on current information.

2.5.2.2 What are the safety or efficacy issues, if any, for BE studies that fail to meet the 90% CI using equivalence limits of 80-125%?

An in vivo BE study appears to be unnecessary based on current information. See section 2.5.2.1.

2.5.2.3 If the formulations do not meet the standard criteria for bioequivalence, what clinical pharmacology and/or clinical safety and efficacy data support the approval of the to-be-marketed product?

An in vivo BE study appears to be unnecessary based on current information. See section 2.5.2.1.

2.5.3 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

Administration of nilotinib under fed conditions increased the PK exposure of nilotinib. Compared to the fasted state, the systemic exposure (AUC) increased by 15% (drug administered 2 hours after a light meal), 29% (30 minutes after a light meal), or 82% (30 minutes after high fat meal), and the C_{max} increased by 33% (2 hours after a light meal), 55% (30 minutes after a light meal), or 112% (30 minutes after high fat meal). (Also see section 2.2.5.3). To minimize the effect of food on nilotinib bioavailability, the sponsor proposed that nilotinib should be taken at least 2 hours after food intake, and food intake should be avoided for 1 hour after drug administration. However, the food effect for doses given 2 hours after high fat meal is still unknown.

2.5.4 When would a fed BE study be appropriate and was one conducted?

An in vivo BE study appears to be unnecessary based on current information. See section 2.5.2.1.

2.5.5 How do the dissolution conditions and specifications ensure in vivo performance and quality of the product?

In vitro dissolution of nilotinib (200 mg capsules) was tested using the basket method (apparatus 1) according to United States Pharmacopoeia (USP), <711> Dissolution at 100 rpm in 1000 mL 0.1N HCl as dissolution medium. The amount of nilotinib released from 200 mg capsules was determined by a UV detection method. The method was validated for selectivity, accuracy, precision and linearity. The dissolution profiles obtained in water, pH 4.5 and above are consistently low due to the limited solubility of the drug substance for both formulations. The use of a surfactant did not improve the dissolution of nilotinib.

2.5.6 If different strength formulations are not bioequivalent based on standard criteria, what clinical safety and efficacy data support the approval of the various strengths of the to-be-marketed product?

There is currently only one strength formulation of Tasigna.

2.5.7 If the NDA is for a modified release formulation of an approved immediate product without supportive safety and efficacy studies, what dosing regimen changes are necessary, if any, in the presence or absence of PK-PD relationship?

This NDA is not for a modified release formulation of an approved immediate product.

2.5.8 If unapproved products or altered approved products were used as active controls, how is BE to the approved product demonstrated? What is the basis for using either in vitro or in vivo data to evaluate BE?

Unapproved products or altered approved products were not used as active controls.

2.5.9 What other significant, unresolved issues related to in vitro dissolution or in vivo BA and BE need to be addressed?

None.

2.6 Analytical Section

2.6.1 How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

In the clinical pharmacology and biopharmaceutics studies, nilotinib was determined in serum by validated LC-MS/MS methods with the lower limit of quantification of 2.50 ng/mL.

In the human mass balance study (study CAMN107A2104), in addition to nilotinib, its minor pharmacologically active metabolite (BEJ866) was also determined in serum. It was analyzed by a validated LC-MS/MS methods with the lower limit of quantification of 1.00 ng/mL.

2.6.2 Which metabolites have been selected for analysis and why?

In the human mass balance study (study CAMN107A2104), in addition to nilotinib, its minor pharmacologically active metabolite (BEJ866) was also determined in serum.

2.6.3 For all moieties measured, is free, bound, or total measured? What

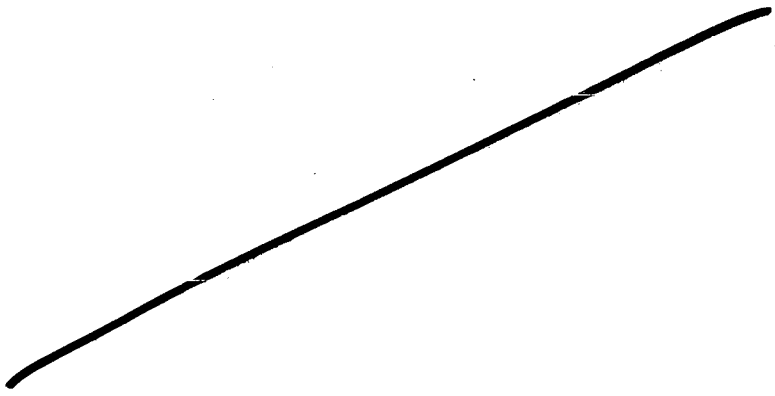
is the basis for that decision, if any, and is it appropriate?

Total drug was measured for all moieties. The basis for choosing to measure total drug was not presented in the submission.

The extent of nilotinib binding to human plasma is high (98% on average). The extent of nilotinib binding to human plasma is independent of concentration. This suggests that measurement of total drug is appropriate and may have been the basis of the Applicant's decision to measure total drug.

2.6.4 What bioanalytical methods are used to assess concentrations?

Nilotinib and BEJ866 were determined in serum by validated LC-MS/MS methods.



. The lower limit of quantitation (LLOQ) for nilotinib and BEJ866, respectively, was 2.50 and 1.00 ng/ml using 0.1 mL of human serum. Concentrations below the LLOQ were reported as 0 ng/mL.

2.6.4.1 What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?

For both nilotinib and BEJ866, the calibration model is a quadratic regression model:

$$y = ax^2 + bx + c$$

Where y is the peak area ratio of nilotinib or BEJ866 to the internal standard x is the concentration of nilotinib or BEJ866. The weighting factor is $1/x^2$.

The range of the standard curve for nilotinib is ng/mL.

The range of the standard curve for BEJ866 is ng/mL.

2.6.4.2 What are the lower and upper limits of quantification (LOQ)?

The lower and upper limits of quantification (LOQ) for nilotinib are ng/mL, respectively.

The lower and upper limits of quantification (LOQ) for BEJ866 are ng/mL, respectively.

2.6.4.3 What are the accuracy, precision, and selectivity at these limits?

The acceptance criteria for the standard curve in each run is that the bias should be within the range of ±20% at the LLOQ, and within the range of ±15% at the other concentration levels. There should be at least 6 validated standard levels including both extremes with no more than 25% of the values excluded.

The acceptance criteria for QC samples in each run is that the bias should be within the range of ±15% for at least 2/3 of the individual values. There should be at least one value at each QC level fulfilling the acceptance criteria.

3. OCPB Labeling Recommendations

The entirety of the Applicant's Proposed Package Insert appears as Appendix 1 of this review. The clinical pharmacology information submitted by the applicant in the current submission is acceptable, from the Office of Clinical Pharmacology perspective. We recommend some modifications to the sponsor's proposed labeling (changes highlighted in blue text as below).

In HIGHLIGHTS OF PRESCRIBING INFORMATION, DOSAGE AND ADMINISTRATION Section:

21 Page(s) Withheld

_____ Trade Secret / Confidential

✓ Draft Labeling

_____ Deliberative Process

Withheld Track Number: Clin Pharm/Bio- 5

4. ***Cover Sheet and OCPB Filing/Review Form***

XIX. Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	**22-068**	**Brand Name**	**Tasigna®**
DCP Division (I, II, III, IV, V)	**V**	**Generic Name**	**nilotinib (AMN107)**
Medical Division	**Oncology**	**Drug Class**	**second generation tyrosine kinase inhibitor**
OCP Reviewer	**Julie M. Bullock, Pharm.D.**	**Indication(s)**	**treatment of chronic phase and accelerated phase Ph+CML in adults patients resistant to or intolerant to [redacted] prior therapy**
OCP Team Leader	**Brian Booth, Ph.D.**	**Dosage Form**	**200 mg capsules**
		Dosing Regimen	**400 mg BID**
Date of Submission	**29 Sept 2006**	**Route of Administration**	**oral**
Estimated Due Date of OCP Review		**Sponsor**	**Novartis Oncology**
PDUFA Due Date		**Priority Classification**	**Priority review**
Division Due Date			

Clinical Pharmacology Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			Data submitted for studies: 1101 (Japanese study) 2101 (Phase 1/2 efficacy) 2101 (safety data set) 2101 (pop PK) 2101e1 (accelerated phase) 2101e2 (Chronic phase) 2103 (GIST study) 2109 (expanded access trial) 2119 (QT data)
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X	1		DMPK-R0300652B
I. Clinical Pharmacology				
Mass balance:	X	1		CAMN107A2104 (HV)
Isozyme characterization:				
Blood/plasma ratio:	X	2		ADME R0300252, DMPK R0500654
Plasma protein binding:	X	3		ADME R0300252, DMPK R0500654, DMPK R0400674
Pharmacokinetics (e.g., Phase I) -				

Healthy Volunteers-				
single dose:	X	4		ADME study CAMN107A2104, MDZ study CAMN107A2108, keto study CAMN107A2110, food effect study CAMN107A2106
multiple dose:				
XX. Patients-				
single dose:	X	1		Phase 1 component of CAMN107A2101
multiple dose:	X	1		
Dose proportionality -				
fasting / non-fasting single dose:	X	1		Phase 1 component of CAMN107A2101
fasting / non-fasting multiple dose:	X	1		
Drug-drug interaction studies -				
In-vivo effects on primary drug:	X	1		Keto study CAMN107A2110 (HV)
In-vivo effects of primary drug:	X	1		MDZ study CAMN107A2108 (HV)
In-vitro:	X	5		DMPK R0300237, R0400672, R0301332, R0500591; ADME R0300236
Subpopulation studies -				
ethnicity:	X			pop PK analysis of CAMN107A2101
gender:	X			
geriatrics:	X			
renal impairment:				
hepatic impairment:				
pediatrics:				
PD:				
Phase 2:	X	1		phase 2 component CAMN107A2101
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:	X	1		CAMN107A2101
Phase 3 clinical trial:				
Population Analyses -				
Data rich:	X	1		CAMN107A2101
Data sparse:	X	1		CAMN107A2101
II. Biopharmaceutics				
Absolute bioavailability:	X	1		Clinical trial form vs FMI CAMN107A2101
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:	X	1		CAMN107A2106 (HV)
QTC studies:	X	1		CAMN107A2119 (HV)
In-Vitro Release BE				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:	X	1		5 reports from study CAMN107A2101
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		**6 clinical trials 9 in-vitro reports**		
Filability and QBR comments				

	"X" if yes	*Comments*
Application filable?		Study 2101 is ongoing. A 120-day update submission is planned for Jan 2007. In addition the following will be submitted after the original submission: • Interim report for QT prolongation (Dec 2006) • Final report for a DDI study with CYP3A4/5 inducer (March 2007) • Hepatic impairment study is planned and final report will be available in 3Q07 • Population PK/PD analysis is in progress and a report will be submitted in Dec 2006.
Comments sent to firm?		Please submit the data for studies CAMN107A2104, CAMN107A2108, CAMN107A2110, and CAMN107A2106
QBR questions (key issues to be considered)		
Other comments or information not included above		
Primary reviewer Signature and Date	**Julie M. Bullock, Pharm.D.**	
Secondary reviewer Signature and Date	**Brian Booth, Ph.D.**	

CC:

HFD-150 (CSO - **C Cottrell;** MTL - **A Farrell;** MO - **M Hazarika**)

HFD-860 (Reviewer - **J Bullock;** TL - **B Booth;** DD - **A Rahman**)

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/s/

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7/9/2007 04:42:46 PM
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