Published in final edited form as:
Clin Cancer Res. 2009 September 15; 15(18): 5910–5916. doi:10.1158/1078-0432.CCR-09-0542.

A Phase I Study of Single-Agent Nilotinib (AMN107) or in Combination with Imatinib in Patients with Imatinib-Resistant Gastrointestinal Stromal Tumors

George D Demetri[1], **Paolo G Casali**[2], **Jean-Yves Blay**[3], **Margaret von Mehren**[4], **Jeffrey A Morgan**[1], **Rossella Bertulli**[2], **Isabelle Ray-Coquard**[3], **Philippe Cassier**[3], **Monica Davey**[4], **Hossein Borghaei**[4], **Daniel Pink**[5], **Maria Debiec-Rychter**[6], **Wing Cheung**[7], **Stuart M Bailey**[7], **Maria Luisa Veronese**[7], **Annette Reichardt**[8], **Elena Fumagalli**[2], and **Peter Reichardt**[5]

[1]Ludwig Center at Dana Farber Cancer Institute and Harvard Medical School, Boston, MA, USA [2]Fondazione IRCCS –Istituto Nazionale dei Tumori, Milan, Italy [3]Centre Léon Bérard, Departement de Medecine, Lyon, France [4]Fox Chase Cancer Center, Philadelphia, PA, USA [5]Helios Klinikum Bad Saarow, Department of Hematology, Oncology and Palliative Medicine, Bad Saarow, Germany [6]Center for Human Genetics, Catholic University of Leuven, Leuven, Belgium [7]Novartis Pharmaceuticals AG, Basel, Switzerland [8]Helios Klinikum Berlin-Buch, Sarcoma Center, Berlin, Germany

Abstract

Purpose—To study the safety, tolerability and pharmacokinetics (PK) of the selective tyrosine kinase inhibitor nilotinib as a single-agent or in combination with imatinib in patients with advanced imatinib-resistant gastrointestinal stromal tumors (GIST).

Experimental Design—A Phase I intercohort dose-escalation trial was performed in patients who received either (1) single-agent nilotinib 400 mg bid or (2) escalating doses of nilotinib (200 mg qd, 400 mg qd, or 400 mg bid) plus imatinib 400 mg bid (10- and 14-hour interval daily), or (3) nilotinib 400 mg bid plus imatinib 400 mg qd. Safety, PK and tumor assessments were performed.

Results—Oral clearance (CL/F) of nilotinib was similar across the combination groups (mean CL/F=19.1-25.6 L/h), and lower than in the single-agent cohort (mean CL/F=35.6 L/h). A linear relationship between nilotinib daily dose and peak concentration (C_{max}) was observed in the combination cohorts. Observed adverse events (AEs) were mostly non-hematological. Frequently reported AEs were rash (40%), fatigue (38%), abdominal pain (36%) and nausea (36%). Severe AEs (grade 3 or 4) included abdominal pain (13%) and rash (9%), the latter mainly with the

Address for reprint requests: George D Demetri, MD, Ludwig Center, Dana-Farber Cancer Institute, Dana 1212, 44 Binney Street, Boston, MA 02115, USA, Tel: 617-632-3985, Fax: 617-632-3408, gdemetri@partners.org.

Note: This study has been presented in part at the annual meeting of American Society of Clinical Oncology (ASCO), Atlanta, June, 2006, at the European Society for Medical Oncology Congress, Istanbul, September, 2006, and at the annual meetings of ASCO, Chicago June, 2007 and May, 2008.

Statement of Translational Relevance: Although imatinib has been shown to be successful in the treatment of surgically incurable GIST, some patients develop resistance to imatinib. Sunitinib, another RTK inhibitor, has been used to treat imatinib-resistant patients, but the short benefit and strong adverse effects make it a less desirable drug. This Phase I trial in patients with imatinib-resistant GIST showed that nilotinib alone or in combination with imatinib could provide an alternative to imatinib-resistant patients, and possibly prevent or overcome imatinib resistance. This initial study explored the use of nilotinib as third-line treatment, and identified possible doses for further Phase II evaluations.

combination. Thirty-eight patients had stable disease and two patients achieved partial response with a median progression-free survival of 134 days for the entire group.

Conclusions—Nilotinib alone or in combination with imatinib was well tolerated overall and showed clinical activity in imatinib-resistant GIST patients. This Phase I trial identified single-agent nilotinib 400 mg bid or combined with imatinib 400 mg qd as possible Phase II doses for further evaluation.

Keywords

nilotinib; imatinib; gastrointestinal stromal tumors (GIST); Phase I; pharmacokinetics (PK)

Introduction

Gastrointestinal stromal tumors (GISTs) are the most frequent mesenchymal tumors of the gastrointestinal tract (1). The majority of GISTs (90%) harbor activating mutations in the *KIT* or platelet-derived growth factor receptor alpha (*PDGFRA)* receptor tyrosine kinases (RTK). The characteristic pathogenic feature of any GIST is the presence of aberrantly activated signaling through either KIT or PDGFRA RTKs (2,3).

Imatinib is a highly effective therapy for surgically incurable GIST, with >80% tumor control rates and median survival close to 5 years (4–6). However, a subset of patients (estimated between 5-14%) may exhibit primary resistance to imatinib within the first 6 months of therapy, especially patients with wild-type *KIT* tumors or *KIT* exon 9 mutations (5). Secondary resistance evolves in most patients after a median of 2 years of therapy (7,8). Sunitinib, the only second-line tyrosine kinase inhibitor (TKI) currently available for GIST, has shown significant clinical benefit in patients whose disease has progressed during, or who are intolerant to imatinib therapy (9). Unfortunately, the benefit of such second-line therapy is even shorter (approximately 9 months), and a relatively high incidence of serious adverse events (AEs) including hypothyroidism and cardiotoxicity has been reported (10,11).

Many studies have shown that treatment of GIST with TKIs, such as imatinib and sunitinib, eventually results in the development of tumor clones which are resistant to these agents (8,12–16). These observations suggest that no single kinase inhibitor will effectively inhibit all mutant clones, and provide the rationale for developing alternative agents so that effective broad-spectrum, non-cross-resistant combination therapies will eventually be feasible.

Nilotinib (Tasigna®, formerly known as AMN107; Novartis, Basel, Switzerland) is a rationally designed second-generation selective TKI, which potently inhibits BCR-ABL, KIT and PDGFRs and has demonstrated clinical activity in chronic myeloid leukemia (CML) (17,18). Nilotinib and imatinib exhibit similar *in vitro* potency against KIT and PDGFR kinases (19). However, they differ in their mechanism of cellular transport, resulting in 5-10 fold higher intracellular levels of nilotinib than imatinib (20,21). Furthermore, nilotinib has shown *in vitro* anti-proliferative activity at physiologically-relevant concentrations in imatinib-resistant human GIST cell lines, as well as in TKI-resistant GIST patients (22,23). Due to the polyclonal nature of GIST, evidence suggests that part of a tumor might still be under partial imatinib control at progression, suggesting that combining AMN107 with imatinib may have a synergistic effect in GIST.

This Phase I trial was designed to evaluate the safety, tolerability, and pharmacokinetics (PK) of nilotinib in patients with imatinib-resistant GIST, either as a single agent or in combination with imatinib. The rationale for testing the combination of nilotinib with

imatinib in GIST patients was based on two major justifications: first, *in vitro* data from CML cell lines suggesting that this combination might have synergistic cytotoxic activity (24,25), and second, there is a strong mechanistic structural biology argument in favor of simultaneously targeting multiple different mutationally-activated molecular variants of the KIT and PDGFRA kinases in GIST.

Patients and Methods

Patients

Patients ≥18 years with histologically-confirmed unresectable and/or metastatic GIST, who had demonstrated objective disease progression using Response Evaluation Criteria in Solid Tumors (RECIST) (26) during previous imatinib therapy at a dose of at least 800 mg daily were eligible. Up to 6 GIST patients intolerant to imatinib 800 mg daily were also allowed in the study. Previous therapy with other TKIs was permitted, provided patients had fully recovered from any toxicity attributed to these agents. Previous chemotherapy was also allowed, provided it had been discontinued ≥4 weeks earlier. A World Health Organization (WHO) performance status of 0 or 1 was required. Patients had normal electrolytes and adequate bone marrow, hepatic and kidney function (Absolute Neutrophil Count (ANC) ≥1500/μl; platelets ≥100,000/μl; potassium, calcium, magnesium or phosphorus ≥ LLN (lower limit of normal); ALT and AST, or alkaline phosphatase ≤2.5 × ULN; serum bilirubin, amylase and lipase, or creatinine ≤1.5 × ULN). Major exclusion criteria included abnormal cardiac function (LVEF <45%; right and left bundle branch block; pacemaker use; ST depression of >1mm; congenital long QT syndrome; ventricular or atrial tachyarrhythmias; bradycardia (<50 beats per minute); QTc >450 msec on screening ECG; myocardial infarction 12 months prior to starting AMN107; unstable angina during the past 12 months; significant heart disease) and prior or concomitant malignancies other than GIST. All patients provided written informed consent. The institutional review boards at all participating sites approved the study protocol.

Study design

In this open-label, Phase I, dose-escalation study, patients were assigned sequentially either to: (1) single-agent nilotinib 400 mg bid (the dose currently recommended for hematological malignancies) (18); or (2) inter-cohort escalating doses of nilotinib (200 mg qd, 400 mg qd or 400 mg bid) in combination with imatinib 400 mg bid (10- and 14-hour interval daily); or (3) nilotinib 400 mg bid plus imatinib 400 mg qd. Nilotinib and imatinib were each administered by continuous daily oral dosing. Morning doses were taken with a light breakfast; nightly doses of imatinib were taken with dinner while nilotinib was taken 2 hrs after imatinib. Study drug administration was discontinued with disease progression, unacceptable toxicity, or withdrawal of consent. Assignment to treatment was based on Bayesian inference of a logistic model describing the dose-toxicity relationship and was guided by the escalation with overdose control principle (see Statistical Methods Section) (27). Once the dose-escalation part of the study was completed and maximum tolerated dose determined, further safety, PK, and tumor response data were collected by expanding the cohort at the recommended Phase II dose level.

Dose escalation was stopped when patients experienced dose-limiting toxicity (DLT), defined as grade 4 neutropenia ANC <500/mm^3 lasting >7 days; grade 3 or 4 febrile neutropenia ANC <1000/mm^3 with fever greater than 101°F, grade 4 thrombocytopenia, platelet counts less than 25,000/mm^3 lasting >7 days; serum creatinine ≥2.0 to ≤3.0× ULN lasting >7 days; serum creatinine >3.0× ULN; total bilirubin ≥2 to ≤3.0× ULN lasting >7 days; total bilirubin >3.0× ULN (unless the hyperbilirubinemia was due to an indirect component only); grade 3 or grade 4 AST/ALT for ≥7 days; grade ≥2 pancreatitis; any

clinically significant grade ≥3 serious AEs (SAEs) related to study treatment causing ≥7 days of interruption of drug therapy; skin toxicity sufficiently severe to require dose reduction; nausea and vomiting if severe (> grade 1) and refractory to antiemetics; or diarrhea if severe (> grade 1) and refractory to anti-diarrheal therapies.

Study assessments

Safety was assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (28). Following baseline evaluations, physical examination, WHO performance status assessment, hematology (hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential) and serum biochemistry (sodium, calcium, potassium, chloride, magnesium, phosphorus, bicarbonate, creatinine, glucose, urea, uric acid, albumin, total protein, AST, ALT, total bilirubin, alkaline phosphatase, lipase, amylase, triglycerides and cholesterol) were performed every 2 weeks during the first 2 months of treatment and every month thereafter. Cardiac safety was monitored during the study by serial electrocardiograms on Day 1 and 8, then monthly thereafter or if clinically indicated. Tumor assessments included computed tomography scan or magnetic resonance imaging at baseline, after 1 and 2 months on study and then every 2 months thereafter. Tumor responses were analyzed according to RECIST (26). Available baseline, pre-treatment GIST biopsies were analyzed for *KIT* and *PDGFRA* genotype.

Pharmacokinetics

Blood sampling for determination of the serum concentrations of nilotinib at steady-state was performed on Day 8 or Day 15 as follows: 0 (pre-dose), 1, 2, 3, 5, 10 and 24 hours post-dose for the daily regimens and pre-dose, 1, 2, 3, 5, 10, 12 and 24 hours post-dose for the bid regimens. Blood sampling for determination of plasma concentrations of imatinib and des-methyl-imatinib metabolite were performed on Day -1 (imatinib alone) and Day 8 or Day 15 (imatinib in combination with nilotinib) as follows: 0 (pre-dose), 1, 2, 3, 5, 10, and 24 hours post-dose. Drug concentrations were determined using the validated assay methods of liquid chromatography-tandem mass spectrometry (29). The lower limit of quantification was 10 ng/mL for both compounds and metabolite.

Statistical methodology

A five-parameter logistic model was used to describe the safety profile for single-agent nilotinib or in combination with 3 doses of imatinib (400, 600 or 800 mg). Historical data for single-agent nilotinib and imatinib DLT rates were used to set up the model. The primary objective of this design was to find the dose maximizing the probability that the true DLT rate lie in the interval of 20% to 35%. Upon completion of 28 days, dosing and safety observation for each cohort estimates of the 5 parameters were updated and the dose-toxicity relationship for single-agent and combination therapy was derived. New cohorts ranged in size from 3 to 6 patients, whilst some combinations below the new level were expanded with additional patients. Any imatinib intolerant patients went into the nilotinib 400 mg bid and any additional imatinib-resistant patients went into the previously tested combination. Using data from all patients completing cycle 1 or experiencing DLT during it at the completion of a new cohort, the Bayesian inference (30,31) on the model parameters allowed quantification of the estimated risk of a dose or combination being unacceptably toxic, i.e. risk of the true rate of DLT for any dose or combination being >35%. A limit on this risk was set to 25% and any dose estimated to exceed this risk was excluded. This conservative escalation approach to find the MTD defined above allowed investigators to select the next dose or combination from a predicted set of acceptable doses. Other clinical data, *e.g.* drug-specific DLTs, PK information or emerging efficacy data, could be used to determine appropriate doses of nilotinib and imatinib (30,31).

The following PK parameters were estimated using standard non-compartmental methods: peak concentration (C_{max}) time to reach C_{max} (t_{max}); area under the concentration-time curve from time zero to t_{last} in a dosing interval ($AUC_{0\text{-}last}$), where t_{last} is the time of the last available quantifiable drug concentration in a dosing interval; area under the concentration-time curve from time zero to the end of a dosing interval (AUC_{τ}); oral clearance at steady-state (CL/F), and average serum drug concentration in a dosing interval (C_{avg}) estimated by dividing $AUC_{0\text{-}last}$ by t_{last}. Descriptive statistics of PK parameters included mean, standard deviation, and range. Median values with ranges are presented for t_{max}.

Kaplan-Meier estimates were computed for progression-free survival (PFS).

Results

Patients

A total of 53 patients were enrolled in the study from August 2005 to July 2006, at 5 participating centers (2 in the United States and 1 each in France, Germany and Italy). Data are presented as of November 2006. Fourteen patients were still on study at the data cut-off date.

Twenty one (40%) patients had a primary cancer site in the small intestine and the majority of tumors (n=48; 91%) were imatinib-resistant (Table 1). Most patients (n=42, 79%) had previously received imatinib for ≥24 months. Approximately 60% of patients experienced disease progression after 1 year of imatinib therapy and 20% during the first 6 months. The majority of patients had experienced disease progression on additional TKIs therapies, most commonly sunitinib (n=33; 62%).

The median dose intensity of nilotinib and imatinib corresponded to 98-100% of the planned daily dose for the single-agent cohort and all combination cohorts, except for the nilotinib 800 mg plus imatinib 800 mg cohort where it was approximately 60%.

The median duration of treatment was 134 days across all cohorts (range 8-430 days). Thirty-nine patients (74%) discontinued the study. The most common primary reason for discontinuation was disease progression (n=32, 60%), followed by AEs (n=3, 6%) and death (n=2, 4%).

Tolerability and Dose-limiting Toxicities

Dose-limiting toxicities were rash and elevated bilirubin. Rash was the DLT in 5 patients receiving combination therapy, occurring in 2/5 (40%) patients receiving nilotinib 400 mg bid and imatinib 400 mg bid, and 3/16 (19%) patients receiving nilotinib 400 mg bid and imatinib 400 mg daily. The high frequency of severe skin rash in patients receiving nilotinib 400 mg bid and imatinib 400 mg bid resulted in dose reduction in all 5 patients, and no further dose escalation was undertaken. One of 18 (6%) patients in the single-agent nilotinib 400 mg bid cohort, with grade 2 elevated bilirubin at study entry, experienced grade 3 dose-limiting hyperbilirubinemia and was discontinued from the study.

The frequency of DLT in the nilotinib 400 mg bid plus imatinib 400 mg qd cohort (19%) was considered acceptable (i.e. the Bayesian model predicted only a 3% chance for this combination of being excessively toxic and a 47% chance of having an acceptable safety profile). Therefore, the combination dose of nilotinib 400 mg bid and imatinib 400 mg qd was deemed appropriate for future Phase II combination studies, as was the single-agent nilotinib 400 mg bid dose.

Safety

All patients experienced AEs during the study with the most frequently reported AEs being non-hematological AEs grade 1 or 2 (Table 2). The safety profiles of single-agent nilotinib or in combination with imatinib were generally similar. Overall, the most frequent AEs were rash (40%), fatigue (38%), abdominal pain (36%) and nausea (36%). Rash was more common in the combination cohorts and was generally manageable with temporary dose interruptions and/or topical corticosteroids. Fatigue and abdominal pain were more frequent in the single-agent nilotinib cohort. Peripheral edema was uncommon and reported only in the combination cohorts. Grade 3 or 4 toxicities occurred in 49% of patients overall (Table 2) and resulted in discontinuation from the study in 2 patients: hyperbilirubinemia in 1 patient treated with nilotinib 400 mg bid single-agent and rash in the other receiving nilotinib 400 mg bid plus imatinib 400 mg bid.

Hematological toxicities were uncommon with only anemia reported as AE. There were no episodes of thrombocytopenia. Neutropenia was reported as a laboratory abnormality in 1 patient treated with nilotinib 200 mg plus imatinib 400 mg bid, without neutropenic fever.

The most frequent grade 3 or 4 laboratory abnormalities were hypophosphatemia (12%), which was more common in the combination cohorts, and hyperbilirubinemia (8%), which was more frequent in the single-agent nilotinib cohort. Grade 3 elevations of AST or ALT were uncommon (<2% overall), did not result in drug discontinuation and were reversible upon dose interruption.

One patient with a history of episodes of junctional rhythm and supraventricular arrhythmias developed atrial fibrillation that was considered related to nilotinib; nilotinib was continued without dose reduction or interruption. Four patients (13%) exhibited clinically insignificant post-baseline QTcF interval >480 msec, 1 patient in the nilotinib 400 mg bid plus imatinib 400 mg bid cohort, and 3 patients in the nilotinib 400 mg bid plus imatinib 400 mg daily combination. None of these four patients experienced cardiac events during the study and no QTcF >500 msec was reported.

Anti-Tumor Activity and Clinical Outcomes

Overall, 2 RECIST-defined partial responses were observed, 1 in a patient who had progressed during adjuvant imatinib and was intolerant to imatinib 800 mg, the other in a patient who had failed multiple regimens including imatinib and sunitinib (Table 3). The median duration of response was 197 days. The majority of patients (78%) had stable disease (SD). Thirteen patients (72%) in the single-agent group had SD lasting for >4 months in 9 (50%) patients and >6 months in 5 (28%) patients. Of the 16 patients treated with the combination cohort selected for further Phase II studies (nilotinib 400 mg bid and imatinib 400 mg qd), 9 had SD. The median PFS for the patients in the nilotinib single-agent group was 168 days (range 1-393) and among all patients was 134 days (range 1-393). The median PFS was not reached for the patients in both the nilotinib 400 mg bid plus imatinib 400 mg bid and the selected Phase II dose cohorts (Table 3). The Kaplan-Meier estimate of PFS at 6 months was 56% for the Phase II combination cohort compared with 47% for the single-agent group (Table 3).

KIT mutations were found in 19/23 (83%) patients; none of the analyzed tumors had *PDGFR* mutations (data not shown).

Pharmacokinetics

Nilotinib dose proportionality was observed during combination treatments. There was a linear relationship between C_{max} or C_{avg} and total nilotinib daily dose for the combination

cohorts (slope=0.881 and 0.946, respectively, based on power model). Oral clearance (CL/F) of nilotinib was similar across the 4 combination groups, but lower than that in the single-agent cohort. In comparison with the single-agent cohort, the AUC_{0-t} values of niltotinib were 40% and 18% higher in the nilotinib 400 mg bid combination cohorts (Table 4).

As compared to monotherapy (Day -1), the AUC_{0-t} values of imatinib were found to be increased by 18-39% during the combination therapy with nilotinib 200 mg QD, 400 mg QD, or 400 mg BID doses (Table 5).

Discussion

The current study represents the first trial to examine single-agent nilotinib and in combination with imatinib in patients with imatinib-resistant GIST. In this resistant patient population, nilotinib alone or in combination with imatinib was generally well tolerated. We administered nilotinib and imatinib at doses similar to the single-agent standard doses with an acceptable safety profile: nilotinib 400 mg bid with or without the combination of imatinib 400 mg qd were the recommended doses for further clinical development. The Bayesian model provided a flexible dose escalation scheme where clinical experience could be combined with information on DLT probabilities leading to a more informed decision making on study.

Overall, toxicities noted in association with nilotinib-based treatments were mild to moderate, mostly non-hematological and consistent with the previous experience in CML (18). Most grade 3 or 4 toxicities were considered unrelated to study medication, and included abdominal pain, nausea, vomiting and increased bilirubin, AEs expected in patients with advanced GIST.

Skin toxicity is a common side-effect of treatment with imatinib or nilotinib in advanced GIST and CML, and skin rash in this study was particularly severe in the combination groups (4,18). This might be due to a potential biological effect of nilotinib and imatinib, as only a slight pharmacokinetic interaction was observed between these two drugs in this study.

The lower CL/F in all four combination cohorts compared with the single-agent group suggests that imatinib might have inhibitory effects on the clearance of nilotinib. This effect appeared to be dose-independent as the CL/F values of nilotinib were similar in the total daily dose range of 200 to 800 mg, when administered concurrently with either imatinib 400 mg qd or 400 mg bid. In single-agent nilotinib 400 mg bid cohort, the mean C_{max} and AUC values of nilotinib were comparable to values reported previously in the CML patients following nilotinib doses of 400 mg bid for 8 days (18). These results indicate that the pharmacokinetic profiles of nilotinib in this group of patients with GIST appeared to be similar to those in CML patients.

Nilotinib appeared to demonstrate clinical activity in refractory GIST, both as single-agent and in combination with imatinib. The majority of patients achieved SD for some period of time. Interpretation of the tumor responses and relationship to *KIT* mutational status is limited by the small sample size in each cohort. However, further investigation of the effect of nilotinib single-agent or in combination with imatinib in patients with advanced GIST with different *KIT* or *PDGFRA* mutations is warranted.

Our data demonstrate that nilotinib, either as a single agent or in combination with imatinib, was overall reasonably well tolerated and demonstrates clinical activity in some patients with imatinib-resistant GIST. Nilotinib alone or in combination with imatinib could provide an alternative strategy for preventing or overcoming imatinib resistance in patients with

GIST. A Phase III study of nilotinib versus best supportive care with or without TKIs in patients with GIST who have progressed during prior therapy with imatinib and sunitinib has been completely accrued and the results are pending.

Acknowledgments

We would like to acknowledge the patients who participated in this study, as well as the collaboration and commitment of all investigators and their staff, without whom the study would not have been possible.

Grant support: This study was sponsored in part by Novartis Pharmaceuticals Corporation, Oncology Business Unit, Florham Park, NJ.

Authors' Financial Support: G. Demetri received additional philanthropic support for this work from the following sources: The Virginia and Daniel K. Ludwig Trust for Cancer Research and the Stutman GIST Cancer Research Fund. M. von Mehren was supported by grant number P30 CA006927 and CA106588 from the National Cancer Institute. The following authors received funding from Novartis Pharmaceuticals: G. Demetri, E. Fumagalli, P.G. Casali, P. Reichardt, M. von Mehren, J.Y. Blay. The following authors received funding from Pfizer Inc. for other projects: G. Demetri, E. Fumagalli, P.G. Casali,

References

1. Miettinen M, Sarlomo-Rikala M, Lasota J. Gastrointestinal stromal tumors: recent advances in understanding of their biology. Hum Pathol 1999;30:1213–20. [PubMed: 10534170]
2. Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. J Clin Oncol 2004;22:3813–25. [PubMed: 15365079]
3. Corless CL, Heinrich MC. Molecular pathobiology of gastrointestinal stromal sarcomas. Annu Rev Pathol 2008;3:557–86. [PubMed: 18039140]
4. Blanke CD, Rankin C, Demetri GD, et al. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. J Clin Oncol 2008;26:626–32. [PubMed: 18235122]
5. Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002;347:472–80. [PubMed: 12181401]
6. Verweij J, Casali PG, Zalcberg J, et al. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. Lancet 2004;364:1127–34. [PubMed: 15451219]
7. Heinrich MC, Corless CL, Blanke CD, et al. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol 2006;24:4764–74. [PubMed: 16954519]
8. Wardelmann E, Thomas N, Merkelbach-Bruse S, et al. Acquired resistance to imatinib in gastrointestinal stromal tumours caused by multiple KIT mutations. Lancet Oncol 2005;6:249–51. [PubMed: 15811621]
9. Demetri GD, van Oosterom AT, Garrett CR, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 2006;368:1329–38. [PubMed: 17046465]
10. Chu TF, Rupnick MA, Kerkela R, et al. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. Lancet 2007;370:2011–9. [PubMed: 18083403]
11. Desai J, Yassa L, Marqusee E, et al. Hypothyroidism after sunitinib treatment for patients with gastrointestinal stromal tumors. Ann Intern Med 2006;145:660–4. [PubMed: 17088579]
12. Antonescu CR, Besmer P, Guo T, et al. Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. Clin Cancer Res 2005;11:4182–90. [PubMed: 15930355]
13. Debiec-Rychter M, Cools J, Dumez H, et al. Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. Gastroenterology 2005;128:270–9. [PubMed: 15685537]
14. Heinrich MC, Corless CL, Liegl B, et al. Mechanisms of sunitinib malate (SU) resistance in gastrointestinal stromal tumors (GISTs). J Clin Oncol 2007;25:10006. Meeting Abstracts.

15. Loughrey MB, Waring PM, Dobrovic A, et al. Polyclonal resistance in gastrointestinal stromal tumor treated with sequential kinase inhibitors. Clin Cancer Res 2006;12:6205–6. [PubMed: 17062700]

16. Wardelmann E, Merkelbach-Bruse S, Pauls K, et al. Polyclonal evolution of multiple secondary KIT mutations in gastrointestinal stromal tumors under treatment with imatinib mesylate. Clin Cancer Res 2006;12:1743–9. [PubMed: 16551858]

17. Hazarika M, Jiang X, Liu Q, et al. Tasigna for chronic and accelerated phase Philadelphia chromosome--positive chronic myelogenous leukemia resistant to or intolerant of imatinib. Clin Cancer Res 2008;14:5325–31. [PubMed: 18765523]

18. Kantarjian H, Giles F, Wunderle L, et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med 2006;354:2542–51. [PubMed: 16775235]

19. Manley PW, Cowan-Jacob SW, Mestan J. Advances in the structural biology, design and clinical development of Bcr-Abl kinase inhibitors for the treatment of chronic myeloid leukaemia. Biochim Biophys Acta 2005;1754:3–13. [PubMed: 16172030]

20. Prenen H, Guetens G, De Boeck G, et al. Cellular uptake of the tyrosine kinase inhibitors imatinib and AMN107 in gastrointestinal stromal tumor cell lines. Pharmacology 2006;77:11–6. [PubMed: 16534250]

21. White DL, Saunders VA, Dang P, et al. OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): reduced OCT-1 activity is the cause of low in vitro sensitivity to imatinib. Blood 2006;108:697–704. [PubMed: 16597591]

22. Dileo P, Bauer S, Van den Abbeele A, et al. Results with AMN107, a novel kinase inhibitor, in gastrointestinal stromal tumor (GIST): Preclinical rationale and early results in a patient (Pt) with imatinib (IM)-resistant GIST. Proc Am Soc Clin Oncol General Poster Session A. 2006

23. Montemurro M, Schoffski P, Reichardt P, et al. Nilotinib in the treatment of advanced gastrointestinal stromal tumours resistant to both imatinib and sunitinib. Eur J Cancer. 2009 Epub ahead of print.

24. Weisberg E, Catley L, Wright RD, et al. Beneficial effects of combining nilotinib and imatinib in preclinical models of BCR-ABL+ leukemias. Blood 2007;109:2112–20. [PubMed: 17068153]

25. White DL, Saunders VA, Quinn SR, Manley PW, Hughes TP. Imatinib increases the intracellular concentration of nilotinib, which may explain the observed synergy between these drugs. Blood 2007;109:3609–10. [PubMed: 17409347]

26. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–16. [PubMed: 10655437]

27. Babb J, Rogatko A, Zacks S. Cancer phase I clinical trials: efficient dose escalation with overdose control. Stat Med 1998;17:1103–20. [PubMed: 9618772]

28. National Cancer Institute: Cancer Therapy Evaluation Program. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). 2006. http://ctep cancer gov/reporting/ctc_v30 html

29. Yin OQP, Gallagher N, Li A, et al. Effect of grapefruit juice on the pharmacokinetics of nilotinib in healthy subjects. J Clin Pharmacol. 2009

30. Bailey S, Neuenschwander B, Laird G, Branson M. A Bayesian case study in oncology Phase I combination dose-finding using logistic regression with covariates. J Biopharm Stat 2009;19:469–84. [PubMed: 19384689]

31. Neuenschwander B, Branson M, Gsponer T. Critical aspects of the Bayesian approach to phase I cancer trials. Stat Med 2008;27:2420–39. [PubMed: 18344187]

Table 1
Patient demographics and baseline disease characteristics

Characteristics	N=53 (%)
Age, years	
Mean±SD	55±12
Range	24-83
Male gender, n (%)	31 (59)
WHO performance status, n (%)	
Grade 0	29 (55)
Grade 1	23 (43)
Grade 2	1 (2)
Primary site of cancer, n (%)	
Small intestine	21 (40)
Stomach	12 (23)
Colon	3 (6)
Other*	17 (32)
Time on imatinib, n (%)	
<6 months	2 (4)
≥6-<12 months	3 (6)
≥12-<24 months	6 (11)
≥24 months	42 (79)
Other prior tyrosine kinase inhibitor treatment, n (%)	
Sunitinib	33 (62)
AMG706, RAD001, dasatinib, or VEG10003	12 (23)

*Includes colon, peritoneum, rectum and other abdominal sites

SD=Standard deviation; WHO=World Health Organization

Table 2

Most frequent adverse events

Adverse events, n (%)	Nilotinib 400 mg bid n=18		Nilotinib 200 mg qd/imatinib 400 mg bid n=7		Nilotinib 400 mg qd/imatinib 400 mg bid n=7		Nilotinib 400 mg bid/imatinib 400 mg bid n=5		Nilotinib 400 mg bid/imatinib 400 mg qd n=16	
	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4
All events	18 (100)	9 (50)	7 (100)	3 (43)	7 (100)	3 (43)	5 (100)	4 (80)	16 (100)	7 (44)
Rash	3 (17)	0	2 (29)	0	3 (43)	1 (14)	3 (60)	2 (40)	10 (63)	2 (13)
Fatigue	12 (67)	0	2 (29)	0	2 (29)	0	1 (20)	0	3 (19)	0
Abdominal pain	11 (61)	4 (22)	2 (29)	1 (14)	2 (29)	0	0	0	4 (25)	2 (13)
Nausea	7 (39)	2 (11)	1 (14)	0	3 (43)	0	2 (40)	0	6 (38)	0
Diarrhea	6 (33)	0	2 (29)	0	2 (29)	0	1 (20)	0	6 (38)	0
Pruritus	4 (22)	0	1 (14)	0	2 (29)	0	0	0	9 (56)	0
Vomiting	7 (39)	2 (11)	2 (29)	0	1 (14)	0	1 (20)	0	5 (31)	0
Constipation	8 (44)	0	1 (14)	0	1 (14)	0	0	0	5 (31)	0
Pyrexia	6 (33)	0	2 (29)	0	0	0	0	0	5 (31)	0
Anemia	2 (11)	1 (6)	3 (43)	2 (29)	2 (29)	0	1 (20)	0	4 (25)	0
Headache	3 (17)	0	3 (43)	0	2 (29)	0	1 (20)	0	3 (19)	0
Anorexia	7 (39)	2 (11)	1 (14)	0	2 (29)	0	1 (20)	0	0	0
Edema peripheral	0	0	3 (43)	0	3 (43)	0	0	0	5 (31)	2 (13)
Asthenia	3 (17)	0	1 (14)	0	3 (43)	0	0	0	3 (19)	0
Depression	3 (17)	0	1 (14)	0	2 (29)	0	2 (40)	0	1 (6)	0
Myalgia	6 (33)	0	0	0	0	0	2 (40)	0	1 (6)	0
Eczema	3 (17)	0	1 (14)	0	3 (43)	0	1 (20)	0	0	0
Flatulence	5 (28)	0	2 (29)	0	1 (14.3)	0	0	0	0	0
Muscle spasms	2 (11)	0	2 (29)	0	2 (29)	0	0	0	2 (13)	0
Abdominal pain, upper	3 (17)	0	2 (29)	0	0	0	0	0	2 (13)	0
Back pain	3 (17)	0	0	0	1 (14)	0	2 (40)	0	1 (6)	0
Pain in extremity	4 (22)	0	1 (14)	0	1 (14)	0	1 (20)	0	0	0
Alopecia	2 (11)	0	1 (14)	0	0	0	0	0	3 (19)	0
Erythema	0	0	1 (14)	0	3 (43)	0	0	0	2 (13)	0

Adverse events, n (%)	Nilotinib 400 mg bid n=18		Nilotinib 200 mg qd/imatinib 400 mg bid n=7		Nilotinib 400 mg qd/imatinib 400 mg bid n=7		Nilotinib 400 mg bid/imatinib 400 mg bid n=5		Nilotinib 400 mg bid/imatinib 400 mg qd n=16	
	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4
Sleep disorder	6 (33)	0	0	0	0	0	0	0	0	0

Table 3

Best overall response

Response, n (%)	Nilotinib 400 mg bid n=18	Nilotinib 200 mg qd/imatinib 400 mg bid n=7	Nilotinib 400 mg qd/imatinib 400 mg bid n=7	Nilotinib 400 mg bid/imatinib 400 mg bid n=5	Nilotinib 400 mg bid/imatinib 400 mg qd n=16
Complete response	0	0	0	0	0
Partial response	1 (6)	0	0	0	1 (6)
Stable disease	13 (72)	7 (100)	5 (71)	4 (80)	9 (56)
Progressive disease	2 (11)	0	1 (14)	0	6 (38)
Unknown*	1 (6)	0	0	0	0
N/A†	1 (6)	0	1 (14)	1 (20)	0
Progression-free survival – Kaplan-Meier estimates					
Rate at 4 months, %	59	83	43	50	56
Rate at 6 months, %	47	17	29	50	56
Median, days	168	143	112	Ongoing	Ongoing
Range	1-393	56-223	22-336	1-322	27-180
Median follow-up, days	186	153	113	167	126

*Best response could not be classified as partial or complete response, progressive or stable disease;

†Not assessed: patients had discontinued study prior to the first post-baseline tumor assessment.

Table 4

Summary statistics* of Nilotinib pharmacokinetic parameter values at steady-state (Day 8 or 15)

	Cohort 1 n=15	Cohort 2 n=6	Cohort 3 n=6	Cohort 4 n=5	Cohort 5 n=12
Nilotinib dose	400 mg bid	200 mg qd	400 mg qd	400 mg bid	400 mg bid
Imatinib dose	0 mg	400 mg bid	400 mg bid	400 mg bid	400 mg qd
t_{max}, h	2.8 (0-9.8)	4.0 2.9-6.3)	6.4 (1.9-23.8)	2.1 (0-12.5)	0 (0-5.0)
C_{max}, ng/mL	1644±828	655±277	1236±705	2160±756	2509±1266
C_{avg}, ng/mL	1172±587	438±150	863±472	1617±610	1642±701
AUC_{0-t}, ng*h/mL	13636±6617	10474±3491	20498±11135	18717±8516	16760±9450
CL/F, L/h	35.6±16.7†	21.5±9.3†	25.6±13.8†	24.8±10.0†	19.1±6.1‡

*Median (range) for t_{max} and mean±SD for other PK parameters.

†CL/F was estimated as dose/AUC_{0-t}.

‡n=3; CL/F was estimated as dose/AUC_{τ}.

Table 5

Summary statistics* of Imatinib pharmacokinetic parameter values of imatinib administered as 400 mg bid alone (Day -1) and during combination therapy with nilotinib (Day 8 or 15)

Cohort	Nilotinib dose	Study day	t_{max} h	C_{max} ng/mL	C_{avg} ng/mL	AUC_{0-t} ng.h/mL
Cohort 2 (n=6)	200 mg qd	Day -1	2.5 (1.8-9.8)	2768 ± 1142	2381 ± 1181	18070 ± 5686
		Day 8	2.9 (1.9-5.5)	3370 ± 1117	2768 ± 1039	24463 ± 4884
Cohort 3 (n=7)	400 mg qd	Day -1	4.8 (2.0-5.0)	4020 ± 2173	3594 ± 2195	33445 ± 23883
		Day 8	2.4 (0.9-5.0)	4203 ± 1121	3735 ± 1212	34593 ± 13319
Cohort 4 (n=5)	400 mg bid	Day -1	3.0 (0-9.8)	4170 ± 1757	3556 ± 1523	31703 ± 14614
		Day 8	2.5 (2.0-3.1)	5074 ± 2758	4285 ± 2325	42440 ± 24349

*Median (range) for t_{max} and mean ± SD for other PK parameters