

Phase II Study of Ponatinib in Advanced Gastrointestinal Stromal Tumors: Efficacy, Safety, and Impact of Liquid Biopsy and Other Biomarkers



Suzanne George¹, Margaret von Mehren², Jonathan A. Fletcher³, Jichao Sun⁴, Sen Zhang⁴, Justin R. Pritchard⁴, John Graeme Hodgson⁴, David Kerstein⁵, Victor M. Rivera⁴, Frank G. Haluska⁴, and Michael C. Heinrich⁶

ABSTRACT

Purpose: The purpose of this study is to evaluate ponatinib for advanced gastrointestinal stromal tumors (GIST).

Patients and Methods: This single-arm phase II trial enrolled patients with metastatic and/or unresectable GIST with failure of prior tyrosine kinase inhibitor (TKI) treatment into two cohorts based on presence or absence of *KIT* exon 11 (ex11) primary mutations. Patients initially received ponatinib 45 mg once daily. Following a temporary clinical hold in October 2013, dose reductions were implemented to reduce risk of arterial occlusive events (AOE). Primary endpoint was 16-week clinical benefit rate (CBR) in *KIT* ex11-positive cohort. *KIT* mutations in circulating tumor DNA (ctDNA) were assessed.

Results: Forty-five patients enrolled (30 *KIT* ex11-positive and 15 *KIT* ex11-negative); median follow-up was 14.7 and 13.6 months, respectively, as of August 1, 2016. Sixteen-week CBR was 36% (*KIT*

ex11-positive; primary endpoint) and 20% (*KIT* ex11-negative). ctDNA analyses ($n = 37$) demonstrated strong concordance of primary *KIT* mutations between plasma and tumor. At least two secondary mutations were detected in 35% of patients overall and 54% of *KIT* ex11-positive patients. Changes from baseline in mutated ctDNA levels were consistent with clinical activity. Ponatinib was ineffective in patients with *KIT* exon 9 primary mutations. Resistance was associated with emergence of V654A. AOE and venous thromboembolic events occurred in three and two patients, respectively. Six patients died; two deaths (pneumonia and pulmonary embolism) were considered possibly ponatinib-related.

Conclusions: Ponatinib demonstrated activity in advanced GIST, particularly in *KIT* ex11-positive disease. ctDNA analysis confirmed heterogeneous resistance mutations in TKI-pretreated advanced GIST. Safety was consistent with previous studies.

Introduction

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal malignancy of the gastrointestinal tract, with an estimated annual incidence of 5,000 to 6,000 new cases in the United States (1, 2). Oncogenic, gain of function mutations in *KIT* and *PDGFRA* are found in approximately 85% and 5% of GIST, respectively (3, 4). Although the currently approved tyrosine kinase inhibitors (TKI) for *KIT*-mutant GIST (first-line: imatinib; second-line: sunitinib; third-line: regorafenib, fourth-line: ripretinib; refs. 5–7) are typically initially effective in the treatment of unresectable disease, the emergence of secondary mutations and subsequent disease progression is a major challenge in the treatment of GIST (8–10). Patients with GIST refractory to approved TKIs typically experience rapid disease progression; median progression-free survival (PFS) and overall survival (OS) generally decrease with each additional line of treatment (11–15).

Ponatinib, a potent BCR::ABL1 inhibitor, is a third-generation TKI designed to potentially inhibit BCR::ABL1 with or without any single resistance mutation, including T315I. In the United States, ponatinib is approved for the treatment of adults with chronic phase (CP) chronic myeloid leukemia (CML) with resistance or intolerance to at least two prior kinase inhibitors; accelerated phase (AP) or blast phase (BP) CML or Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL) for whom no other kinase inhibitor is indicated; and for T315I-positive CML or Ph+ ALL (16). Ponatinib has potent preclinical activity against mutated *KIT* and platelet-derived growth factor- α (*PDGFRA*), including clinically relevant mutations found in GIST that confer resistance to available TKIs (17, 18). Detailed *in vitro* analysis has shown that ponatinib has a broad, but imperfect,

¹Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts. ²Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania. ³Pathology, Brigham and Women's Hospital, Boston, Massachusetts. ⁴Clinical Research, ARIAD Pharmaceuticals, Inc., Cambridge, Massachusetts, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. ⁵Oncology Clinical Research, Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. ⁶Medical Oncology, Portland VA Health Care System and Oregon Health & Sciences University Knight Cancer Institute, Portland, Oregon.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Current address for J. Sun: MacroGenics, Inc., Rockville, Maryland; current address for S. Zhang: EMD Serono, Rockville, Maryland; current address for J.R. Pritchard: Pennsylvania State University, Department of Biomedical Engineering, University Park, Pennsylvania; current address for J.G. Hodgson: Syros Pharmaceuticals, Cambridge, Massachusetts; current address for D. Kerstein and V.M. Rivera: Theseus Pharmaceuticals, Cambridge, Massachusetts; and current address for F.G. Haluska: Kestrel Therapeutics Inc., Wayland, Massachusetts.

Corresponding Author: Michael C. Heinrich, Knight Cancer Institute, Portland VA Health Care System and Oregon Health & Science University, R&D-19, 371 SW US Veterans Hospital Road, Portland, OR 97239. Phone: 503-494-6594; E-mail: heinrich@ohsu.edu

Clin Cancer Res 2022;28:1268–76

doi: 10.1158/1078-0432.CCR-21-2037

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 International (CC BY-NC-ND).

©2022 The Authors; Published by the American Association for Cancer Research

Translational Relevance

Ponatinib demonstrated antitumor activity in patients with advanced gastrointestinal stromal tumors (GIST), particularly in those with *KIT* exon 11 mutations. Analysis of circulating tumor DNA (ctDNA) confirmed heterogeneous resistance mutations in patients with advanced GIST previously treated with tyrosine kinase inhibitors (TKI). To the best of our knowledge, this is the first report of a clinical trial in advanced GIST in which patient enrollment was prospectively guided by mutational status. Our results demonstrate that trials evaluating TKI efficacy based on primary GIST mutations are feasible and informative. There was concordance between primary mutations detected in plasma ctDNA using beads, emulsions, amplification, and magnetics (BEAMing) and in tumor biopsies. The majority of patients had primary and/or secondary *KIT* mutations detected in plasma. The incomplete spectrum of *KIT* mutations detectable by BEAMing may be overcome by use of next-generation sequencing. Future GIST trials should continue to evaluate ctDNA using sequencing-based approaches.

inhibitory profile against various *KIT* mutations (19). Ponatinib demonstrated strong potency against *KIT* with primary mutations in exon 11 (ex11) and most secondary mutations, including mutations in the activation loop (exon 17/18) and at the T670 gatekeeper residue (exon 14). However, ponatinib had reduced activity against *KIT* with an exon 13 (V654A) secondary mutation or with an exon 9 (ex9) primary activating mutation (19).

The strong preclinical activity of ponatinib against *KIT* and *PDGFRA* supported its clinical investigation in patients with GIST. The objective of the current phase II study (NCT01874665) was to evaluate the efficacy and safety of ponatinib in patients with advanced GIST after failure of prior TKI therapy; biomarker assessments were also performed. Given the known preclinical profile of ponatinib, patients were prospectively enrolled into two cohorts based on the presence or absence of primary *KIT* ex11 mutations; ctDNA analysis was performed to explore the relationship between tumor mutation status and ponatinib activity in this heavily pretreated population.

Patients and Methods

Study design

In this phase II, single-arm, open-label, multicenter study, patients were enrolled in one of two cohorts, the *KIT* ex11-positive (*KIT* ex11-pos) or the *KIT* ex11-negative (*KIT* ex11-neg) as reported by investigators based on historical tumor analysis. Tumor *KIT*/*PDGFRA* mutations were recorded for all patients enrolled in the study. If the patient did not have a genetic analysis of a tumor sample performed at a Clinical Laboratory Improvement Amendments–certified laboratory, mutational analysis of *KIT*/*PDGFRA* genes in at least 1 archival tumor specimen was performed. Patients who did not have an archival specimen were required to undergo a tumor biopsy for genetic analysis. Blood samples were obtained at baseline and at times of tumor assessment for detection of mutations in *KIT* and *PDGFRA* in circulating DNA.

All patients started on 45 mg/day ponatinib; treatment continued until the occurrence of disease progression or unacceptable toxicity. Between October 8, 2013, and May 21, 2014, a temporary clinical hold was imposed by the FDA on all ponatinib trials in

order to assess the risk of vascular occlusive events and to provide dose reduction instructions for management of these events. A total of 45 patients were enrolled prior to the hold; of those, 13 patients were censored. After the October 2013 temporary clinical hold, eligibility was amended to include only those patients who had received all three TKIs approved for GIST at that time (imatinib, sunitinib, and regorafenib); enrolled patients who had not received at least 3 prior therapies were discontinued if they had not achieved at least stable disease (SD) lasting ≥ 16 weeks during ponatinib treatment. Accordingly, patients with SD or better at the end of Cycle 6 (1 cycle = 4 weeks) had ponatinib reduced to 30 mg/day if the dose was not already reduced for other reasons; doses could be escalated to 45 mg/day upon disease progression. Reduction of ponatinib dose to as low as 15 mg/day was permitted to manage drug-related adverse events (AE) or drug–drug interactions, and the dose could be re-escalated up to 45 mg/day once events resolved. Ponatinib was supplied as oral tablets in either the 45-mg or 15-mg strengths.

This study was conducted in accordance with the ethical standards consistent with the Declaration of Helsinki and with Good Clinical Practice guidelines and all applicable regulatory requirements. The protocol and the informed consent received institutional review board/ethics committee approval. All patients signed the informed consent.

Patients

Patients ≥ 18 years of age with histologically confirmed metastatic and/or unresectable GIST who experienced progression on prior TKIs were eligible. At study initiation, patients with failure of at least one prior TKI treatment were eligible. After the October 2013 temporary clinical hold, eligibility was amended to include only those patients who had received all three approved TKIs for GIST at that time (imatinib, sunitinib, and regorafenib); enrolled patients who had not received at least three prior therapies were discontinued if they had not achieved at least SD lasting ≥ 16 weeks during ponatinib treatment. Eligible patients had Eastern Cooperative Oncology Group performance status ≤ 2 , measurable disease per modified RECIST version 1.1 (12), and adequate hepatic, renal, and pancreatic function. Patients with clinically significant, uncontrolled, or active cardiovascular disease, uncontrolled hypertension, uncontrolled hypertriglyceridemia, a bleeding disorder, ongoing or active infection, or those taking medications with risk of serious cardiac ventricular arrhythmias (e.g., torsades de pointes) were excluded, as were patients with history of chronic pancreatitis or acute pancreatitis within 1 year prior to study commencement. Women who were pregnant or lactating were not eligible. The use of approved TKIs or investigational agents within 2 weeks or six half-lives of the agent prior to start of treatment (whichever was longer) was prohibited.

Efficacy outcomes

The primary outcome measure was investigator-assessed clinical benefit rate (CBR) at 16 weeks, defined as the composite of complete response (CR), partial response (PR), and SD according to modified RECIST version 1.1 (12), in the *KIT* ex11-pos cohort. Secondary outcome measures included investigator-assessed CBR at 16 weeks in the *KIT* ex11-neg cohort and in the total patient population and objective response rate (ORR), defined as the composite of CR and PR according to modified RECIST version 1.1 (12), PFS, and OS in both cohorts and in the total patient population. Similar endpoints were used in phase II studies of regorafenib and sunitinib in patients with GIST (20, 21).

Table 1. Demographics and baseline characteristics.

Characteristics	<i>KIT</i> exon 11-positive (n = 30)	<i>KIT</i> exon 11-negative (n = 15)	Total (N = 45)
Median age (range), years	62 (40–81)	56 (24–77)	59 (24–81)
Sex, male, n (%)	19 (63)	7 (47)	26 (58)
Race, ^a white, n (%)	29 (97)	15 (100)	44 (98)
Median time since diagnosis (range), years	6 (1–30)	7 (1–25)	6 (1–30)
ECOG performance status, n (%)			
0	17 (57)	6 (40)	23 (51)
1	12 (40)	9 (60)	21 (47)
2	1 (3)	0	1 (2)
Primary tumor site, n (%)			
Stomach	10 (33)	5 (33)	15 (33)
Small intestine	7 (23)	7 (47)	14 (31)
Esophagus	2 (7)	0	2 (4)
Other/unknown	11 (37)	3 (20)	14 (31)
Number of target lesions, n (%)			
1	0	0	0
2	7 (23)	5 (33)	12 (27)
3	12 (40)	3 (20)	15 (33)
4	8 (27)	5 (33)	13 (29)
5	3 (10)	2 (13)	5 (11)
Number of prior anticancer regimens, n (%)			
≤2	6 (20)	2 (13)	8 (18)
3–5	16 (53)	10 (67)	26 (58)
≥6	8 (27)	3 (20)	11 (24)
Median (range)	4 (1–10)	5 (2–7)	4 (1–10)
Number of prior approved TKIs, n (%)			
1	2 (7)	1 (7)	3 (7)
2	10 (33)	6 (40)	16 (36)
3	18 (60)	8 (53)	26 (58)
Among patients with <i>KIT</i> exon 11-negative tumors (n = 15)			
<i>KIT</i> exon 9 mutations	NA	7 (47)	7 (16)
<i>KIT</i> exon 13 mutations	NA	1 (7)	3 (7)
<i>KIT</i> exon 17 mutations	NA	2 (13)	5 (11)
Wild-type <i>KIT</i> and <i>PDGFRA</i>	NA	6 (40)	6 (13)
Among patients with wild-type <i>KIT</i> tumors (n = 6)			
SDHB-intact	NA	2 (13)	2 (4)
SDHB-negative	NA	1 (7)	1 (2)
Unknown (tissue not available for testing)	NA	3 (20)	3 (7)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; SDHB, succinate dehydrogenase complex iron sulfur subunit B.

^aRace was unknown in one patient in cohort A.

Tumor response was assessed by CT or magnetic resonance imaging every two cycles for the first six cycles and every three cycles thereafter. PFS was defined as the time from start of ponatinib administration to objective disease progression or death due to any cause. OS data were collected every 6 months following discontinuation for up to 24 months after enrollment of the last patient.

Correlative assessments

The association between *KIT* mutations detected using ctDNA and antitumor activity of ponatinib was assessed as an exploratory outcome. Blood was drawn for detection of *KIT* mutations in ctDNA (liquid biopsy) at baseline and at the time of tumor assessment. Samples were analyzed for *KIT* mutations using beads, emulsions, amplification, and magnetics (BEAMing) digital polymerase chain reaction technology (Sysmex Inostics, Inc.; refs. 22, 23), which allowed detection of seven primary (exons 9, 11, 13) and 20 secondary (exons 13/14, 17/18) mutations (ref. 24; Supplementary Table S1).

Safety outcomes

Safety was assessed by routine physical examination, laboratory evaluations, electrocardiograms, and echocardiograms as defined prospectively in the protocol. The laboratory evaluations included serum analyses performed at baseline and prespecified time points for the following labs: serum chemistry, fasting serum lipid panel, hemoglobin A1c (HbA1c), C-reactive protein, cardiac troponin (cTn), and N-terminal pro-brain natriuretic peptide (NT-proBNP). AEs were assessed throughout the study and categorized by the U.S. National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Statistical analysis

For CBR and ORR, 95% exact confidence intervals (CI) were calculated. For PFS and OS, medians were estimated using the Kaplan–Meier method. Descriptive statistics were used to summarize exposure to ponatinib over time, AE incidence rates, and the incidence of occurrence of overall toxicity, categorized by toxicity grades.

Determination of sample size

The planned enrollment for the study was 45 patients (30 in the *KIT* ex11-pos cohort and 15 in the *KIT* ex11-neg cohort). For the primary analysis population (*KIT* ex11-pos cohort), an exact binomial test determined that a one-stage design with at least 30 patients with the ex11 mutation could distinguish a favorable true CBR of 30% from a null rate of 10% with more than 80% power and a one-sided type 1 error rate of 0.025. Ponatinib would be considered promising in this population if at least seven of 30 patients experienced clinical benefit.

Data availability

The data sets, including the redacted study protocol, redacted statistical analysis plan, and individual participant data supporting the results reported in this article, will be made available within three months from initial request, to researchers who provide a methodologically sound proposal. The data will be provided after de-identification, in compliance with applicable privacy laws, data protection, and requirements for consent and anonymization.

Results

Demographics and baseline characteristics

A total of 45 patients were enrolled and initiated ponatinib treatment at three centers in the United States between June 5, 2013, and October 1, 2014. The demographics and baseline characteristics of patients (30 with GIST harboring *KIT* ex11 mutations and 15 with GIST without *KIT* ex11 mutations) are summarized in **Table 1**. Of the 15 patients with *KIT* ex11-neg tumors, seven cases harbored *KIT* ex9 mutations, one harbored a primary *KIT* exon 13 mutation, one harbored a primary *KIT* exon 17 mutation, and six were wild type at the *KIT* and *PDGFRA* loci. Of the six patients with wild-type *KIT* tumors, two patients had tumors that were succinate dehydrogenase complex iron sulfur subunit B (SDHB)-intact (wild-type) and one was SDHB-negative by IHC; no tissue was available for testing in tumors from three patients. Overall, patients were heavily pretreated (with 82% having undergone treatment with at least three prior anticancer regimens) and typically had multiple lesions (with 73% having three or more lesions).

Patient disposition

At study termination (data cutoff: August 1, 2016), median follow-up was 14.7 months (range, 0.4–34.3) in the *KIT* ex11-pos cohort and 13.6 months (0.6–32.0) in the *KIT* ex11-neg cohort. Two patients (4.4%) continued ponatinib treatment after study termination at the discretion of their physician. Primary reasons for discontinuation included progressive disease (50% *KIT* ex11-pos cohort; 67% *KIT* ex11-neg cohort) and AEs (20% *KIT* ex11-pos; 13% *KIT* ex11-neg cohort; Supplementary Table S2).

Efficacy

Tumor response data are summarized in **Table 2**, which excludes two patients who were discontinued from the study per FDA request as they had not already responded to ponatinib, nor had they been treated with all other TKIs approved for GIST. Clinical benefit at 16 weeks was reported in 10 of 28 patients (36%) in the *KIT* ex11-pos cohort (primary outcome, **Fig. 1**) and 3 of 15 patients (20%) in the *KIT* ex11-neg cohort, with a CBR of 30% (13/43) in the total patient population.

In patients with *KIT* ex11-pos tumors treated with two and three prior TKIs, CBR at 16 weeks was 44% and 33%, respectively. Clinical benefit was not experienced by patients with *KIT* ex9-positive tumors.

Table 2. Investigator-reported tumor response.

Endpoint	<i>KIT</i> exon 11-positive <i>n</i> = 28 ^a	<i>KIT</i> exon 11-negative <i>n</i> = 15	Total <i>N</i> = 43 ^a
Response at 16 weeks, <i>n</i> (%)			
CBR at 16 weeks, <i>n</i> (%)	10 (36)	3 (20)	13 (30)
(95% CI)	(19–56)	(4–48)	(17–46)
CR	0	0	0
PR	1 (4)	0	1 (2)
SD	9 (32)	3 (20)	12 (28)
PD	9 (32)	7 (47)	16 (37)
Not evaluable ^b	9 (32)	5 (33)	14 (33)
Best response, <i>n</i> (%)			
ORR (CR + PR), <i>n</i> (%)	2 (7)	0	2 (5)
(95% CI)	(1–24)	(0–22)	(1–16)
CR	0	0	0
PR	2 (7)	0	2 (5)
SD	14 (50)	7 (47)	21 (49)
PD	5 (18)	5 (33)	10 (23)
Not evaluable ^b	7 (25)	3 (20)	10 (23)

Abbreviation: PD, progressive disease.

^aExcludes two patients who were discontinued from study per FDA request.

^bNot evaluable group are patients whose tumor was not evaluable but who remained on study.

PR as best response was reported in two patients, both with *KIT* ex11-pos tumors, resulting in an ORR of 7% in the *KIT* ex11-pos cohort, and 0% in the *KIT* ex11-neg cohort. Best response data for individual patients are summarized in **Fig. 2**. In the patient with SDHB-negative GIST as assessed by IHC, the best response was SD; total time on treatment at study termination was 29.4 months; the patient continued ponatinib 45 mg/day after study termination.

Kaplan-Meier plots of PFS and OS are shown in Supplementary Fig. S1. Median PFS was 4.0 months (95% CI, 2–9) in the *KIT* ex11-pos cohort and 2.0 months (2–18) in the *KIT* ex11-neg cohort; median OS was 14.7 months (95% CI, 8–30) and 14.3 months (3–not reached), respectively.

Association of *KIT* mutations detected in ctDNA with antitumor activity of ponatinib

At the time of ctDNA analyses (March 2, 2015), a total of 105 plasma samples from 37 patients were analyzed (Supplementary Table S3); 28 patients had baseline and postbaseline samples. In 100% (17/17) of cases in which the primary *KIT* mutation detected in the tumor was included in the BEAMing panel, the same mutation was also detected in the plasma (Supplementary Fig. S2). In the 12 cases in which the primary *KIT* mutation detected in the tumor was not included in the BEAMing panel, no primary mutations were detected in the plasma.

Analysis of ctDNA revealed a high degree of tumor heterogeneity, with at least two (and up to eight) unique secondary *KIT* mutations detected in 35% (13/37) of all patients and 54% (13/24) of patients in the *KIT* ex11-pos cohort (**Fig. 3**). In order to understand the heterogeneity and dynamics of resistance mutations post-ponatinib treatment, mutation dynamics in individual patients and the dynamics of the most common secondary mutations [V654A (exon 13), N822K, Y823D, D820A/G (all in exon 17/18)] were assessed (Supplementary Figs. S3 and S4). Although V654A was only detectable at baseline in one patient, it frequently emerged during treatment with ponatinib, becoming detectable in eight additional patients (range, 60–480 days of

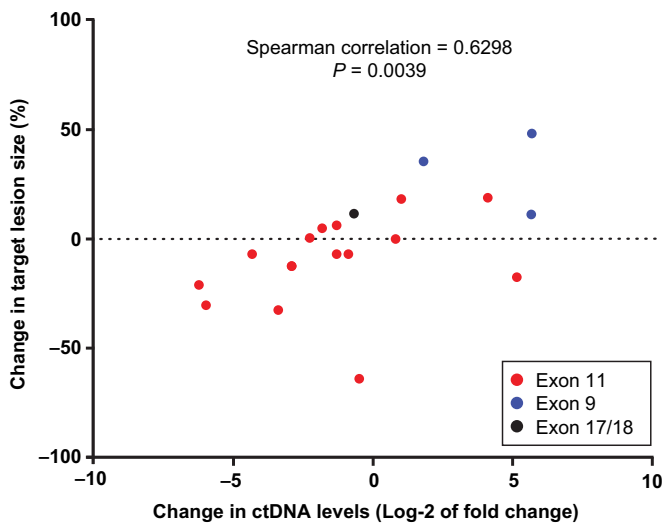


Figure 4.

Association between changes in target lesion size and *KIT*-mutated ctDNA levels ($n = 19$). Target lesion size (sum of all target lesions) and levels of primary mutation (in ctDNA) were compared at baseline and first post-baseline visits.

AEs

Treatment-emergent AEs reported in $\geq 20\%$ of patients and serious treatment-emergent AEs reported in $\geq 2\%$ of patients are included in **Table 3**. Three patients had arterial occlusive events [AOE; myocardial ischemia ($n = 1$), cerebrovascular accident ($n = 1$), and peripheral artery stenosis ($n = 1$)]. Two patients had venous thromboembolic events [pulmonary embolism ($n = 2$); in one of these patients, deep vein thrombosis was also reported].

Deaths within 30 days of the last dose and treatment-related deaths at any time were reported in six patients: hepatic failure ($n = 1$), pneumonia ($n = 1$), pulmonary embolism ($n = 1$), respiratory failure

($n = 1$), neoplasm progression ($n = 2$); deaths due to pneumonia and pulmonary embolism were considered possibly related to ponatinib treatment by the investigator. Hepatic failure was considered not related to treatment by the investigator; this patient with hepatic failure received ponatinib at a dose of 45 mg.

Discussion

In this phase II study in patients with advanced GIST who progressed on prior TKIs, ponatinib showed evidence of clinical activity in patients with activating mutations in *KIT* ex11. Clinical benefit (43% CBR) was observed in patients with primary *KIT* ex11 mutations and secondary exon 17/18 mutations detected at baseline. Ponatinib had no clinical activity in patients with primary mutations in *KIT* ex9. Consistent with the observed clinical activity, ctDNA levels of the most common exon 17 and exon 18 mutations typically decreased during ponatinib treatment, whereas ctDNA levels of *KIT* ex9 primary and V654A (exon 13) secondary mutations generally increased during treatment, the latter of which may represent a key acquired resistance mechanism. Associations between *KIT* mutation status assessed using ctDNA and ponatinib antitumor activity correlated with data from preclinical models that demonstrated that ponatinib has potent activity against secondary mutations such as N822K, Y823D, and D820A/G but not against V654A (19). ctDNA analysis indicated the presence of at least two secondary mutations in 54% of patients with *KIT* ex11 mutations. The reduced activity of ponatinib in *KIT* ex11-negative patients could be attributed to the presence of other mutations such as *KIT* ex9. Previous preclinical studies demonstrated limited activity of ponatinib in ex9 mutant models (19). In addition, the lack of activity in patients with *KIT* ex9 mutations is not unique and was previously reported in the phase III trial of nilotinib versus imatinib, in which nilotinib demonstrated reduced efficacy compared with imatinib (25). These findings suggest that the presence of a *KIT* ex11 mutation may serve as a response biomarker for ponatinib. Furthermore, the presence of *KIT* ex9 mutation may serve as a marker of resistance to ponatinib. However, additional studies are needed because the observed resistance to ponatinib could be due to the presence of other mutations, such as *KIT* exon 17 or exon 13 mutations.

The safety profile of ponatinib in this study is consistent with that reported in leukemia studies (26, 27), with the exception that myelosuppression occurred less frequently in patients with GIST. Based on

Table 3. Treatment-emergent AEs.

Treatment-emergent AEs reported in $\geq 20\%$ of patients ($N = 45$)	Any grade, n (%)	Grades 3-5, n (%)
Rash	27 (60)	2 (4)
Fatigue	24 (53)	3 (7)
Abdominal pain	22 (49)	4 (9)
Headache	20 (44)	0
Constipation	19 (42)	0
Myalgia	18 (40)	0
Hypertension	17 (38)	4 (9)
Peripheral edema	16 (36)	1 (2)
Decreased appetite	15 (33)	1 (2)
Dry skin	15 (33)	1 (2)
Increased blood alkaline phosphatase	13 (29)	2 (4)
Cough	12 (27)	0
Vomiting	11 (24)	3 (7)
Nausea	11 (24)	2 (4)
Diarrhea	10 (22)	2 (4)
Increased aspartate aminotransferase	10 (22)	1 (2)
Pyrexia	10 (22)	0
Serious treatment-emergent AEs reported in ≥ 2 patients ($N = 45$)	n (%)	
Abdominal pain	4 (9)	
Pneumonia	3 (7)	
Small intestine obstruction	3 (7)	
Fatigue	2 (4)	
Nausea	2 (4)	
Pulmonary embolism	2 (4)	
Vomiting	2 (4)	

experience in phase I and phase II clinical studies, the starting dose of ponatinib used was 45 mg/day in order to maximize potential efficacy in this heavily pretreated patient population. To reduce the potential risk of AOE's due to long-term exposure, the ponatinib dose was reduced after six cycles of treatment to 30 mg/day in patients with SD or better, if it had not already been reduced for other reasons. In this heavily pretreated population, ponatinib showed modest response and clinical benefit rates with limited durability of response. Based on the dose-dependent toxicities observed in this study, lower dosing (30 mg) was used in the POETIG phase II trial in pretreated patients with GIST (NCT03171389; ref. 28).

To the best of our knowledge, this is the first report of a clinical trial in advanced GIST in which patient enrollment was prospectively guided by mutational status and ctDNA analysis was incorporated to better understand acquired resistance to ponatinib. Our results support the utility of this approach and demonstrate that clinical trials evaluating the efficacy of TKIs based on primary tumor mutation in GIST are feasible and informative. ctDNA analysis by BEAMing further defined the primary and secondary resistance landscape in this trial. There was concordance between primary mutations detected in ctDNA using BEAMing and in tumor biopsies. BEAMing detected primary and/or secondary *KIT* mutations in plasma from the majority of patients enrolled; the incomplete spectrum of *KIT* mutations detectable by BEAMing may be overcome by use of next-generation sequencing approaches (29–31). Future GIST trials should continue to evaluate ctDNA using sequencing-based approaches. The utility of ctDNA analysis in the management of patients with GIST was demonstrated in a study that included 243 patients with GIST. In comparison with analysis of tumor mutations, plasma ctDNA analysis had a positive predictive value of 100%. ctDNA analysis provides a rapid, noninvasive approach to characterize current mutations, thereby allowing tailored, personalized therapeutic strategies based on resistance mutations (32).

The trial had several limitations. Although patient enrollment was guided by *KIT* ex11 mutation status, grouping all *KIT* ex11–negative patients in one cohort posed challenges in interpreting the lack of activity due to the heterogeneity of the clinical impact of various mutations on response to treatment. In addition, most patients included in the study were white (98%), which may impact the broad applicability of the findings. Further mutational analysis is needed to mechanistically define the limited activity of ponatinib in *KIT* ex11–negative patients and to determine whether this was solely due to the presence of *KIT* ex9 mutation or combinations of other relevant mutations. Another key limitation of the trial is the limited number of patients with *KIT* exon 13 and exon 17 mutations.

The emergence of two or more secondary mutations in a large proportion of patients suggests a high degree of underlying tumor heterogeneity and indicates that effective treatment approaches for GIST may require earlier use of pan-*KIT* inhibitors or combination therapies that can suppress development of resistance mutations. Although ponatinib will not be developed further in GIST due to the risk/benefit profile, future studies in GIST should focus on informative study designs, based on strong preclinical data with robust correlatives together with broad coverage of known resistance mutations.

References

1. Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, et al. Diagnosis of gastrointestinal stromal tumors: A consensus approach. *Hum Pathol* 2002;33:459–65.

Authors' Disclosures

S. George reports grants from ARIAD during the conduct of the study; grants from Blueprint Medicines, Deciphera, Daiichi Sankyo, Pfizer, Bayer, Merck, Eisai, and Springworks, personal fees from Blueprint Medicines, Deciphera, Immunium, and Kayothera, and other support from WCG outside the submitted work. M. von Mehren reports personal fees and other support from Deciphera, Novartis, and Blueprint, and personal fees from Exelixis during the conduct of the study; grants from Novartis outside the submitted work. J.A. Fletcher reports a patent for Imatinib licensed to Novartis. S. Zhang reports other support from ARIAD and EMD Serono outside the submitted work. J.R. Pritchard reports other support from ARIAD Pharmaceuticals during the conduct of the study; grants from Theseus Pharmaceuticals, personal fees from Theseus Pharmaceuticals, Moma Therapeutics, Third Rock Ventures, and Takeda Pharmaceuticals, and other support from Theseus Pharmaceuticals and Moma Therapeutics outside the submitted work. D. Kerstein reports other support from ARIAD Pharmaceuticals during the conduct of the study and other support from Theseus Pharmaceuticals outside the submitted work. V.M. Rivera reports other support from ARIAD Pharmaceuticals and other support from Theseus Pharmaceuticals outside the submitted work. F.G. Haluska reports other support from ARIAD Pharmaceuticals during the conduct of the study. M.C. Heinrich reports grants from ARIAD during the conduct of the study; personal fees and other support from MolecularMD, personal fees from Novartis, and grants and personal fees from Blueprint Medicines and Deciphera Pharmaceuticals outside the submitted work; in addition, M.C. Heinrich has a patent for Treatment of GIST issued, licensed, and with royalties paid from Novartis. No disclosures were reported by the other authors.

Authors' Contributions

S. George: Conceptualization, resources, writing—original draft, writing—review and editing. **M. von Mehren:** Conceptualization, resources, writing—original draft, writing—review and editing. **J.A. Fletcher:** Conceptualization, resources, methodology, writing—original draft, writing—review and editing. **J. Sun:** Conceptualization, resources, methodology, writing—original draft, writing—review and editing. **S. Zhang:** Conceptualization, resources, methodology, writing—original draft, writing—review and editing. **J.R. Pritchard:** Conceptualization, resources, methodology, writing—original draft, writing—review and editing. **J.G. Hodgson:** Conceptualization, resources, methodology, writing—original draft, writing—review and editing. **D. Kerstein:** Conceptualization, resources, methodology, writing—original draft, writing—review and editing. **V.M. Rivera:** Conceptualization, resources, writing—original draft, writing—review and editing. **F.G. Haluska:** Conceptualization, project administration, writing—review and editing. **M.C. Heinrich:** Conceptualization, resources, writing—original draft, writing—review and editing.

Acknowledgments

This study is sponsored by Takeda Development Center Americas, Inc. M.C. Heinrich received partial salary support from a Merit Review grant from the U.S. Department of Veterans Affairs (11OBX005358-01) and a grant from the NCI (R21CA263400).

We thank all of the patients and their families, and the investigators and staff at all clinical sites, for their participation in the study. The authors acknowledge Duprane Young, PhD, of Peloton Advantage, an OPEN Health company, and Meenakshi Subramanian of Evidence Scientific Solutions for medical writing support for the development of this manuscript under the direction of the authors, which was funded by Takeda Pharmaceuticals, USA, Inc., and complied with the Good Publication Practice 3 ethical guidelines (Battisti WP, et al. *Ann Intern Med* 2015;163:461–4).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 14, 2021; revised December 15, 2021; accepted January 25, 2022; published first January 28, 2022.

2. Miettinen M, Lasota J. Gastrointestinal stromal tumors—definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch* 2001;438:1–12.

3. Wasag B, Debiec-Rychter M, Pauwels P, Stul M, Vranckx H, Oosterom AV, et al. Differential expression of KIT/PDGFRα mutant isoforms in epithelioid and mixed variants of gastrointestinal stromal tumors depends predominantly on the tumor site. *Mod Pathol* 2004;17:889–94.
4. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* 2003;21:4342–9.
5. Qinlock [package insert]. Waltham, MA: Deciphera Pharmaceuticals, LLC; 2020.
6. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: Soft tissue sarcoma v1.2021. Pennsylvania: National Comprehensive Cancer Network; 2020. Available from: https://www.nccn.org/professionals/physician_gls/pdf/sarcoma.pdf.
7. Blay JY, Serrano C, Heinrich MC, Zalberg J, Bauer S, Gelderblom H, et al. Ripretinib in patients with advanced gastrointestinal stromal tumours (INVICTUS): A double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol* 2020;21:923–34.
8. Corless CL. Gastrointestinal stromal tumors: What do we know now? *Mod Pathol* 2014;27(Suppl 1):S1–16.
9. Schaefer IM, Marino-Enriquez A, Fletcher JA. What is new in gastrointestinal stromal tumor? *Adv Anat Pathol* 2017;24:259–67.
10. Heinrich MC, Maki RG, Corless CL, Antonescu CR, Harlow A, Griffith D, et al. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. *J Clin Oncol* 2008;26:5352–9.
11. Blanke CD, Demetri GD, von Mehren M, Heinrich MC, Eisenberg B, Fletcher JA, et al. Long-term results from a randomized phase II trial of standard-versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumors expressing KIT. *J Clin Oncol* 2008;26:620–5.
12. Demetri GD, Reichardt P, Kang YK, Blay JY, Rutkowski P, Gelderblom H, et al. Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): An international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013;381:295–302.
13. Montemurro M, Gelderblom H, Bitz U, Schutte J, Blay JY, Joensuu H, et al. Sorafenib as third- or fourth-line treatment of advanced gastrointestinal stromal tumour and pretreatment including both imatinib and sunitinib, and nilotinib: A retrospective analysis. *Eur J Cancer* 2013;49:1027–31.
14. Italiano A, Cioffi A, Coco P, Maki RG, Schoffski P, Rutkowski P, et al. Patterns of care, prognosis, and survival in patients with metastatic gastrointestinal stromal tumors (GIST) refractory to first-line imatinib and second-line sunitinib. *Ann Surg Oncol* 2012;19:1551–9.
15. Rutkowski P, Bylina E, Klimczak A, Switaj T, Falkowski S, Kroc J, et al. The outcome and predictive factors of sunitinib therapy in advanced gastrointestinal stromal tumors (GIST) after imatinib failure - one institution study. *BMC Cancer* 2012;12:107.
16. Iclusig [package insert]. Cambridge, MA: Takeda Pharmaceutical Company Limited; 2020.
17. Gozgit JM, Wong MJ, Wardwell S, Tyner JW, Loriaux MM, Mohemmad QK, et al. Potent activity of ponatinib (AP24534) in models of FLT3-driven acute myeloid leukemia and other hematologic malignancies. *Mol Cancer Ther* 2011;10:1028–35.
18. Lierman E, Smits S, Cools J, Dewaele B, Debiec-Rychter M, Vandenberghe P. Ponatinib is active against imatinib-resistant mutants of FIP1L1-PDGFRα and KIT, and against FGFR1-derived fusion kinases. *Leukemia* 2012;26:1693–5.
19. Garner AP, Gozgit JM, Anjum R, Vodala S, Schrock A, Zhou T, et al. Ponatinib inhibits polyclonal drug-resistant KIT oncoproteins and shows therapeutic potential in heavily pretreated gastrointestinal stromal tumor (GIST) patients. *Clin Cancer Res* 2014;20:5745–55.
20. Wagner AJ, Severson PL, Shields AF, Patnaik A, Chugh R, Tinoco G, et al. Association of combination of conformation-specific KIT inhibitors with clinical benefit in patients with refractory gastrointestinal stromal tumors: A phase 1b/2a nonrandomized clinical trial. *JAMA Oncol* 2021;7:1343–50.
21. Ben-Ami E, Barysaukas CM, von Mehren M, Heinrich MC, Corless CL, Butrynski JE, et al. Long-term follow-up results of the multicenter phase II trial of regorafenib in patients with metastatic and/or unresectable GI stromal tumor after failure of standard tyrosine kinase inhibitor therapy. *Ann Oncol* 2016;27:1794–9.
22. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. *Nat Med* 2008;14:985–90.
23. Dressman D, Yan H, Traverso G, Kinzler KW, Vogelstein B. Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. *Proc Natl Acad Sci U S A* 2003;100:8817–22.
24. KIT Gene. Catalogue of Somatic Mutations in Cancer (COSMIC), 2019. Available from: <https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=KIT>.
25. Blay JY, Shen L, Kang YK, Rutkowski P, Qin S, Nosov D, et al. Nilotinib versus imatinib as first-line therapy for patients with unresectable or metastatic gastrointestinal stromal tumours (ENESTg1): A randomised phase 3 trial. *Lancet Oncol* 2015;16:550–60.
26. Cortes JE, Kantarjian H, Shah NP, Bixby D, Mauro MJ, Flinn I, et al. Ponatinib in refractory Philadelphia chromosome-positive leukemias. *N Engl J Med* 2012;367:2075–88.
27. Cortes JE, Kim DW, Pinilla-Ibarz J, le Coutre P, Paquette R, Chuah C, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. *N Engl J Med* 2013;369:1783–96.
28. Falkenhorst J, Hamacher R, Reichardt P, Ivanyi P, Kasper B, Hohenberger P, et al. Lower-dosing ponatinib in pre-treated GIST: Results of the POETIG phase II trial [abstract 11536]. *J Clin Oncol* 2020;38:11536.
29. Li B, Bai J, Zhu L, Zhang S, Wang G, Dai P, et al. Application of next generation sequencing (NGS) in gastrointestinal stromal tumor (GIST) [abstract]. *J Clin Oncol* 2020;38:e13675.
30. Vanden Bempt I, Vander Borgh S, Sciot R, Spans L, Claeu S, Brems H, et al. Comprehensive targeted next-generation sequencing approach in the molecular diagnosis of gastrointestinal stromal tumor. *Genes Chromosomes Cancer* 2021;60:239–49.
31. Zhou Y, Zhang X, Wu X, Zhou Y, Zhang B, Liu X, et al. A prospective multicenter phase II study on the efficacy and safety of dasatinib in the treatment of metastatic gastrointestinal stromal tumors failed by imatinib and sunitinib and analysis of NGS in peripheral blood. *Cancer Med* 2020;9:6225–33.
32. Arshad J, Roberts A, Ahmed J, Cotta J, Pico BA, Kwon D, et al. Utility of circulating tumor DNA in the management of patients with GI stromal tumor: Analysis of 243 patients. *JCO Precis Oncol* 2020:66–73.