**Supplementary Content** (online only)

## Study Investigators

In addition to the authors, the following investigators enrolled patients included in the current analysis: Philippe Schafhausen (Germany), Yuri Shatokhin (Russia), Michele Baccarani (Italy), Irina Krylova (Russia), Delong Liu (United States), Gert Ossenkoppele (The Netherlands), Martin Muller (Germany), Emilio Lanari Zubiaur (Argentina), Tamara Zagoskina (Russia), Rainer Ordemann (Germany), Herman Liu (Hong Kong), Kimmo Porkka (Finland), Jeff Lipton (Canada), Yongquiang Zhao (China), Li Yu (China), Helio Pinczowski (Brazil), Jorge Milone (Argentina), Jane Apperley (United Kingdom), Vinitha Philip (South Africa), Judy Sutherland (Canada), Anatoly Golenkov (Russia), Josef Thaler (Austria), Luke Akard (United States), Ming Yao (Taiwan), David Gomez (Mexico), Juan Navarro (Peru), Lugui Qiu (China), Kenny Galvez (Columbia), Yeow Tee Goh (Singapore), Juan Luis Steegmann (Spain), Matthew Seftel (Canada), David Snyder (United States), Raymond Liang (Hong Kong), Henrik Hjorth-Hansen (Norway), Bengt Simonsson (Sweden), Kerry Taylor (Australia), Paul Ruff (South Africa), Nicolas Novitzky (South Africa), Vernon Louw (South Africa), Guillermo Quintero (Columbia), Maria Zanichelli (Brazil), Eduardo Yanez (Chile), Miguel Tezanos Pinto (Argentina), and Steven O'Brien (United Kingdom).

## Extended Methods

### *Patients*

The study design and eligibility criteria have been previously described [1-3]. The current analysis included patients aged ≥18 years with Philadelphia chromosome–positive (Ph+) chronic phase (CP) chronic myeloid leukemia (CML) and resistance to prior imatinib ≥600 mg/day or intolerance to any dose of imatinib, with no previous exposure to other tyrosine kinase inhibitors [1]. Additional key enrollment criteria included an Eastern Cooperative Oncology Group Performance Status score of 0 or 1; adequate bone marrow (only required for imatinib-resistant patients), hepatic, and renal function; ≥7 days since any prior antiproliferative treatment except for hydroxyurea and anagrelide; and ≥3 months post-allogeneic hematopoietic stem cell transplant. All patients provided written informed consent prior to study enrollment.

### *Study Design*

This was a phase 1/2, open-label, multicenter, 2-part study of bosutinib in patients with Ph+ leukemias. Part 1 was a dose-escalation study that determined a recommended phase 2 dose of bosutinib 500 mg/day in patients with CP CML [1]. Part 2, described in this report, evaluated the efficacy and safety of continuous oral daily dosing of bosutinib at this dose. Dose escalation was allowed for lack of efficacy (no complete hematological response [CHR] by week 8 or no complete cytogenetic response [CCyR] by week 12) in the absence of grade 3/4 treatment-related toxicity. Doses could be held or reduced by 100-mg increments to a minimum dose of 300 mg/day based on the severity and duration of treatment-related toxicities. Treatment could continue until disease progression

(defined as transformation to accelerated/blast phase CML, increased white blood cell count [ie, doubling occurring over ≥1 month with the second count >$20\times10^9$/L and confirmed ≥1 week later], or loss of previously attained major cytogenetic response [MCyR] or any hematological response), unacceptable toxicity (including intolerance to bosutinib ≤300 mg/day), or withdrawal of consent. Long-term follow-up after treatment discontinuation consisted of in-person contact or a telephone call every 3 months for 2 years to determine patient-reported progression, initiation of new anticancer treatment, and survival. Further therapy was at the discretion of the treating physician.

The protocol was approved by the central or institutional review board for each study site, and the study was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki.

***Efficacy and Safety Analyses***

Patients recruited in Part 1 were further analyzed along with patients from Part 2 for both efficacy and long-term safety. The primary endpoint of Part 2 was the rate of MCyR at week 24 in patients with imatinib-resistant CP CML and has been previously reported [1]; thus, only cumulative endpoints are reported in the current manuscript. Key secondary and exploratory efficacy endpoints included cumulative cytogenetic, hematologic, and molecular response; time to and duration of response; response by baseline Bcr-Abl kinase domain mutation; progression-free survival (PFS); and overall survival (OS).

Cytogenetic response assessments were performed every 3 months through 2 years, and every 6 months thereafter during treatment. Additionally, peripheral blood was collected at weeks 1, 2, 3, 4, 8, and 12 for analysis of complete blood cell count and Bcr-Abl transcript-levels (performed monthly), and thereafter was collected on the same schedule as cytogenetic response assessments. Treated patients evaluable for cytogenetic and hematologic response must have had an adequate baseline assessment for the respective response. The definition of CHR was standard [1]; hematologic response was required to be confirmed and to last for ≥4 weeks, with peripheral blood and/or bone marrow documentation. Patients with a baseline hematologic response were considered responders if they maintained the response for ≥5 weeks. Cytogenetic response [4] was determined using standard cytogenetics (G-band karyotype) with ≥20 metaphases counted for post-baseline assessments; if <20 metaphases were available post-baseline, fluorescence in situ hybridization (FISH) analysis of bone marrow aspirate with ≥200 cells for the presence of Bcr-Abl fusion gene was used. MCyR included partial cytogenetic response (PCyR; 1%-35% Ph+ metaphases) and CCyR (0% Ph+ metaphases; <1% if using FISH). Patients with a baseline cytogenetic response were considered responders if they maintained the response for ≥4 weeks. Molecular response was assessed at a central laboratory (Quest Diagnostics) using non-nested real time polymerase chain reaction (PCR) for the ratio of Bcr-Abl to Abl transcripts. Major molecular response (MMR) was categorized as a ≥3-log reduction from standardized baseline, and also included complete molecular response (CMR; undetectable Bcr-Abl transcript with a PCR sensitivity of ≥5 logs). To be considered a responder for MMR/CMR, the patient should also have had detectable Bcr-Abl transcript levels at

baseline or any time post-baseline, and have achieved/maintained a CCyR. Additionally, patients with a cytogenetic assessment not showing CCyR on the same day of molecular assessment were not considered to have an MMR/CMR at that time point. MMR was not assessed using the International Scale as it was not widely available when the study was initiated. Because of logistical constraints, patients enrolled in China, India, Russia, and South Africa could not be evaluated for molecular response; treated patients not from these 4 countries were evaluable for molecular response.

Time to response was analyzed using cumulative incidence adjusted for the competing risk of treatment discontinuation without response and calculated from the start date of therapy to the first date of attained/maintained response (confirmed response for hematologic response and unconfirmed response for cytogenetic and molecular responses). Duration of response was calculated from the first date of response until confirmed loss of response, treatment discontinuation due to disease progression or death, or death occurring up to 30 days following the last dose. PFS was calculated for the all-treated population from the start date of therapy until treatment discontinuation due to disease progression (as assessed by the investigator) or death, or death occurring up to 30 days following the last dose. OS was calculated for the all-treated population from the start date of therapy to the date of death due to any cause, with patients censored at the last contact (patients were followed for 2 years after treatment discontinuation). For time-to-event endpoints except OS, patients were censored at the last assessment visit for those not known to have the respective endpoint. Efficacy endpoints were summarized

using descriptive statistics, cumulative incidence, the Kaplan-Meier method, response rates, and confidence intervals.

AEs were reported at each study visit through 30 days after the last bosutinib dose; physical examinations, vital signs, and laboratory tests were also performed routinely. Toxicities were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. All patients who received ≥1 dose of bosutinib were included in the safety population.

**Supplementary Table S1. Patient demographic and baseline clinical characteristics.**

| Parameter | Imatinib-resistant (n = 200) | Imatinib-intolerant (n = 88) | Total (n = 288) |
|---|---|---|---|
| Median (range) age, y | 51 (18-86) | 54.5 (23-91) | 53 (18-91) |
| Male sex, n (%) | 116 (58) | 37 (42) | 153 (53) |
| ECOG Performance Status,[a] n (%) | | | |
| 0 | 156 (78) | 64 (74) | 220 (77) |
| 1 | 44 (22) | 22 (25) | 66 (23) |
| 2 | 0 | 1 (1) | 1 (<1) |
| Median (range) duration of disease, y | 4.0 (0.1-15.1) | 2.8 (0.1-13.6) | 3.6 (0.1-15.1) |
| Treatment history, n (%) | | | |
| No. of previous therapies | | | |
| 1 | 128 (64) | 65 (74) | 193 (67) |
| 2 | 72 (36) | 23 (26) | 95 (33) |
| Previous imatinib | 200 (100) | 88 (100) | 288 (100) |
| Median (range) duration of prior imatinib, y | 2.5 (0.4-8.8) | 1.5 (<0.1-8.3) | 2.2 (<0.1-8.8) |
| Reason for stopping imatinib | | | |
| Disease progression/inadequate response | 186 (98) | 0 | 186 (67) |
| AE | 0 | 87 (99) | 87 (31) |
| Regimen completed | 3 (2) | 0 | 3 (1) |
| Other[b] | 0 | 1 (1) | 1 (<1) |
| Missing[c] | 11 | 0 | 11 |
| Previous IFN | 72 (36) | 23 (26) | 95 (33) |
| Previous stem cell transplant | 6 (3) | 2 (2) | 8 (3) |
| Bcr-Abl mutation, n (%) | | | |
| Assessed patients | 153 | 59 | 212 |
| At least 1 mutation | 73 (48) | 6 (10) | 79 (37) |
| Country,[d] n | | | |
| United States | | | 56 |
| Russia | | | 44 |
| China | | | 30 |
| Italy | | | 22 |
| Germany | | | 17 |
| South Korea | | | 17 |
| Brazil | | | 13 |
| Netherlands | | | 13 |

ECOG, Eastern Cooperative Oncology Group; AE, adverse event; IFN, interferon.
Percentages may not total 100% due to rounding.
[a]ECOG Performance Status was missing for 1 imatinib-intolerant patient.
[b]Patient wished to become pregnant.
[c]When the study was initiated, the reason for stopping imatinib was not part of the data collected; therefore, these data are missing for some patients.

[d]All countries with ≥10 patients enrolled are shown. Additional countries enrolling patients included Argentina (n = 8), Australia (n = 6), Austria (n = 2), Canada (n = 9), Chile (n = 1), Columbia (n = 3), Spain (n = 5), Finland (n = 3), Great Britain (n = 4), Hong Kong (n = 4), Hungary (n = 7), India (n = 8), Mexico (n = 2), Norway (n = 1), Peru (n = 2), Singapore (n = 2), Sweden (n = 1), Taiwan (n = 2), South Africa (n = 6).

**Supplementary Table S2. Treatment discontinuations.**

| Reason, n (%) | Imatinib-resistant (n = 200) | Imatinib-intolerant (n = 88) | Total (n = 288) |
|---|---|---|---|
| Discontinued treatment | 108 (54) | 51 (58) | 159 (55) |
| AE[a] | 33 (17) | 33 (38) | 66 (23) |
| Thrombocytopenia | 6 (3) | 8 (9) | 14 (5) |
| Increased ALT | 4 (2) | 3 (3) | 7 (2) |
| Diarrhea | 4 (2) | 2 (2) | 6 (2) |
| Neutropenia | 1 (1) | 4 (5) | 5 (2) |
| Rash | 1 (1) | 3 (3) | 4 (1) |
| Vomiting | 2 (1) | 2 (2) | 4 (1) |
| Increased AST | 3 (2) | 1 (1) | 4 (1) |
| Disease progression[b] | 35 (18) | 6 (7) | 41 (14) |
| Unsatisfactory response/lack of efficacy[c] | 17 (9) | 4 (5) | 21 (7) |
| Patient request | 11 (6) | 7 (8) | 18 (6) |
| Death | 5 (3) | 0 | 5 (2) |
| Lost to follow-up | 2 (1) | 0 | 2 (1) |
| Investigator request | 1 (1) | 0 | 1 (<1) |
| Other[d] | 4 (2) | 3 (3) | 7 (2) |

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CML, chronic myeloid leukemia; CHR, complete hematologic response; MCyR, major cytogenetic response; CCyR, complete cytogenetic response.

[a]Individual AEs leading to treatment discontinuation in ≥3 patients are shown in the table.

[b]Disease progression included transformation to accelerated or blast phase CML, loss of confirmed CHR or MCyR, and increased white blood cell count (ie, doubling over ≥1 month, with a second count $>20\times10^9$/L confirmed ≥1 week later).

[c]Lack of efficacy included failure to reach CHR by week 8 or CCyR by week 12 or as reported by investigator.

[d]For imatinib-resistant patients, 'other' included no cytogenetic response at week 48 (n = 1), non-compliance (n = 1), T315I mutation (n = 1), and loss of CCyR, investigator/patient request, and increasing transcript levels (n = 1). For imatinib-intolerant patients, 'other' included transplant (n = 2) and non-compliance (n = 1).

## References

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3. Khoury HJ, Cortes JE, Kantarjian HM, et al. Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. Blood 2012;119(15):3403-3412.
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