Supplement to:

Phase 2 Study of Nilotinib in Pediatric Patients With Philadelphia Chromosome–Positive Chronic Myeloid Leukemia

Nobuko Hijiya, Alexey Maschan, Carmelo Rizzari, Hiroyuki Shimada, Carlo Dufour, Hiroaki Goto, Hyoung Jin Kang, Terri Guinipero, Zeynep Karakas, Francisco Bautista, Stéphane Ducassou, Keon Hee Yoo, Christian Michel Zwaan, Frédéric Millot, Paola Aimone, Alex Allepuz, Sara Quenet, Florence Hourcade-Potelleret, Sabine Hertle, and Darintr Sosothikul

Supplementary Methods

Disease classification

Patients were enrolled into 1 of 3 cohorts based on the following criteria. Patients with Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia in chronic phase (CML-CP) had < 15% blasts in peripheral blood and bone marrow, < 30% blasts plus promyelocytes in peripheral blood and bone marrow, < 20% basophils in peripheral blood, ≥ 100 × 10^9/L platelets, and no extramedullary leukemic involvement, with the exception of hepatosplenomegaly. Diagnosis of CML-CP included cytogenetic confirmation of the Ph chromosome with (9;22) translocations (standard conventional cytogenetic analysis on bone marrow; fluorescence in situ hybridization was not permitted). The newly diagnosed Ph+ CML-CP cohort included patients with CML-CP who had received a diagnosis ≤ 6 months prior to enrollment. Resistance to imatinib or dasatinib was defined by the following characteristics: increasing white blood cell or platelet count indicative of hematologic relapse or primary resistance, cytogenetic or molecular response consistent with suboptimal response or failure, progression to accelerated phase/blast crisis (AP/BC), reappearance of Ph+ bone marrow cells following achievement of a complete cytogenetic response (CCyR), increase of > 30% in Ph+ cells in peripheral blood or bone marrow, or loss of molecular response. Imatinib/dasatinib intolerance was defined as the occurrence of adverse events (AEs) that required discontinuation of therapy.

Patient eligibility criteria

Adequate renal, hepatic, pancreatic, and cardiac function was mandatory, as were normal electrolyte and blood glucose levels. Key exclusion criteria included receiving medications that strongly inhibit or induce cytochrome P450 3A4 or may prolong the QT interval, receiving myelosuppressive chemotherapy within 3 weeks prior to study entry, impaired cardiac function,

documented T315I mutation in *BCR-ABL1*, and for the imatinib/dasatinib-resistant/intolerant (R/I) cohort, prior treatment with > 1 tyrosine kinase inhibitor.

Study treatment

Nilotinib was administered orally using 50-mg, 150-mg, and 200-mg hard gelatin capsules at a dose of 230 mg/m^2 twice daily, rounded to the nearest 50-mg dose (to a maximum single dose of 400 mg) for cycles of 28 days each or until early discontinuation (discontinuation was mandatory in cases of disease progression, protocol deviations leading to a significant risk to patient safety, patient/guardian decision at any time, unacceptable toxicities, pregnancy, use of prohibited concomitant treatments, QTcF ≥ 500 ms after dose adjustment and confirmed at repeat measurements on the same day, documented episode of ventricular tachycardia or ventricular fibrillation, and complete heart block [grade III atrioventricular block or second-degree atrioventricular block Mobitz type II]). Capsules were taken whole with water or were opened and the contents dispersed in 1 teaspoon of applesauce (pureed apple) if patients experienced difficulties swallowing the intact capsules.[1] No food was consumed for 2 hours before and for ≥ 1 hour after receiving the dose. The final analysis is planned for the end of the study, when all patients will have received a total of 66 cycles of nilotinib or discontinued early.

Study endpoints and assessments

Secondary objectives were to further characterize the efficacy, safety and tolerability, and exposure to nilotinib as measured by C_{trough} and to assess whether nilotinib affects long-term outcomes (event-free survival [EFS], assessed from the date of first study drug intake to the first occurrence of loss of complete hematologic response, loss of major cytogenetic response, progression to AP/BC [from CP] or to BC [from AP], or death from any cause, on treatment), overall survival (the time between date of first study drug intake and date of death due to any

cause at any time during the study), growth, development, or maturation in pediatric patients with Ph+ CML.

Efficacy was evaluated based on molecular, cytogenetic, and hematologic response and is being assessed over the course of the study.[2] Response rates at and by designated time points are reported to provide a fuller picture of the efficacy observed; response rates by each time point are cumulative and indicate the total number of patients who achieved that response level at any time, whereas response rates at the same time point are a snapshot of the number of patients with that response at that time point. Per protocol, molecular response assessments by *BCR-ABL1* real-time quantitative polymerase chain reaction (RQ-PCR) occurred at baseline; day 28 of cycles 1, 3, and every third cycle through cycle 24 and then every 6 cycles through cycle 60; and at end of treatment. Safety and tolerability were assessed in terms of AEs, clinical laboratory evaluations, vital signs, and electrocardiogram (ECG) findings. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.03 and were coded using the Medical Dictionary for Regulatory Activities; AE data are being collected throughout the duration of the study. Blood samples were collected at designated time points over 12 cycles of nilotinib treatment for the evaluation of pharmacokinetic (PK) parameters. The acceptability and palatability of nilotinib were evaluated from data recorded in a questionnaire completed by patients, with help from parents or caregivers as needed, at specific visits: day 1 of cycle 1, day 28 of cycle 1, day 28 of cycle 12, and end of trial (if the patient discontinued before cycle 12); the method of nilotinib administration was recorded in the questionnaire. Mutational analyses were conducted at baseline and at end of treatment; additional mutational analyses were conducted at the discretion of the investigator. Samples and/or data related to PK, *BCR-ABL1* RQ-PCR and mutational assessments, ECG, and bone biomarkers were processed centrally. Safety and efficacy data are reported for all patients

based on a data cutoff of May 3, 2017, when all patients had completed 24 cycles or had discontinued study treatment early.

Efficacy parameters

Molecular response was evaluated in a central laboratory, using RQ-PCR. Peripheral blood samples were collected and analyzed for the e13a2/e14a2 *BCR-ABL1* transcript. Major molecular response was defined as *BCR-ABL1* ≤ 0.1% on the International Scale (IS), and response was confirmed by duplicate analysis of the same sample; results were reported as *BCR-ABL1:*control *ABL1* transcripts, standardized to the IS (*BCR-ABL1*^{IS}). If e13a2/e14a2 was undetectable at baseline, samples were analyzed for the presence of the e1a2 transcript. Loss of major molecular response was defined as confirmed loss of *BCR-ABL1*^{IS} ≤ 0.1% in association with a ≥ 5-fold rise in *BCR-ABL1* levels from the lowest value achieved on study treatment. This result had to be confirmed by a subsequent sample separated by at least 4 weeks or associated with confirmed loss of complete hematologic response or loss of CCyR or progression to AP/BC or CML-related death.

Cytogenetic response was determined locally and was based on the percentage of Ph+ metaphases in the bone marrow. Examination of ≥ 20 metaphases in each bone marrow sample was required.

Pharmacokinetic assessments

Blood samples for pharmacokinetic evaluation were collected on day 1, 8, 15, 22, and 28 of cycle 1, and on day 28 of cycles 3, 6, 9, and 12.

Assessment of growth, development, and sexual maturation

Height and weight were measured at baseline; day 28 of cycle 1 (± 1-day visit window); day 28 of cycles 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 (± 3-day visit window); and day 28 of cycles 15, 18, 21, and 24 (± 7-day visit window). Bone density and bone age were assessed using dual-energy x-ray absorptiometry and x-ray of the left hand and wrist, respectively. Assessments were performed at baseline, day 28 of cycle 12 (± 3-day visit window), and day 28 of cycle 24 (± 7-day visit window).

Sexual maturation was monitored by Tanner staging. Delayed puberty in girls was defined as failure to attain Tanner stage 2 (for both breast development and pubic hair) by age 13 years or absence of menarche by age 15 years or within 5 years of attaining Tanner stage 2; in boys, it was defined as failure to attain Tanner stage 2 (for both testis and pubic hair) by age 14 years. Puberty stage was only analyzed among prepubescent patients.

Statistical analysis

A formal power-based calculation was not performed. A minimum of 50 patients (including a minimum of 15 patients with newly diagnosed Ph+ CML-CP and 15 with Ph+ CML-CP R/I to imatinib/dasatinib) were to be enrolled in the study. Enrollment minimums were selected based on operational and feasibility criteria and in alignment with the minimum numbers required by health authorities.

Supplemental Table 1. AEs related to bilirubin elevations

MedDRA preferred term, n (%)	Resistant/intolerant imatinib/dasatinib (n = 33)		Newly diagnosed (n = 25)		All patients (N = 58)	
	All grade	Grade 3/4	All grade	Grade 3/4	All grade	Grade 3/4
Total	17 (51.5)	3 (9.1)	15 (60.0)	4 (16.0)	32 (55.2)	7 (12.1)
Blood bilirubin increased	12 (36.4)	2 (6.1)	8 (32.0)	0	20 (34.5)	2 (3.4)
Hyperbilirubinemia	4 (12.1)	0	8 (32.0)	4 (16.0)	12 (20.7)	4 (6.9)
Bilirubin conjugated increased	2 (6.1)	1 (3.0)	2 (8.0)	0	4 (6.9)	1 (1.7)
Blood bilirubin unconjugated increased	1 (3.0)	0	2 (8.0)	0	3 (5.2)	0

Supplemental Table 2. AEs leading to discontinuation of study treatment

Patients, n (%)	Resistant/intolerant imatinib/dasatinib (n = 33)	Newly diagnosed (n = 25)	All patients (N = 58)
Any AE leading to discontinuation	5 (15.2)[a]	6 (24.0)[b]	11 (19.0)
AEs related to bilirubin elevations	3 (9.1)	2 (8.0)	5 (8.6)
Hyperbilirubinemia	1 (3.0)	2 (8.0)	3 (5.2)
Blood bilirubin increased	2 (6.1)	0	2 (3.4)
Rash	1 (3.0)	1 (4.0)	2 (3.4)
ALT increased	0	1 (4.0)	1 (1.7)
Anemia	1 (3.0)	0	1 (1.7)
AST increased	0	1 (4.0)	1 (1.7)
Decreased appetite	1 (3.0)	0	1 (1.7)
Headache	1 (3.0)	0	1 (1.7)
Hyperamylasemia	0	1 (4.0)	1 (1.7)
Keratosis pilaris	1 (3.0)	0	1 (1.7)
Malaise	1 (3.0)	0	1 (1.7)
Nausea	1 (3.0)	0	1 (1.7)
Pain in extremity	1 (3.0)	0	1 (1.7)
Pancreatic enlargement	0	1 (4.0)	1 (1.7)
Platelet count decreased	0	1 (4.0)	1 (1.7)
Rash maculopapular	0	1 (4.0)	1 (1.7)

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

[a] Of the 5 patients in the resistant/intolerant cohort with AEs leading to discontinuation, 1 patient experienced decreased appetite, headache, pain in extremity, malaise, and nausea, 1 experienced rash and increased blood bilirubin, 1 experienced anemia and increased blood bilirubin, 1 experienced keratosis pilaris, and 1 experienced hyperbilirubinemia.

[b] Of the 6 patients in the newly diagnosed cohort with AEs leading to discontinuation, 1 patient experienced rash, increased ALT, and increased AST, 1 experienced hyperamylasemia and pancreatic enlargement, and the other 4 each experienced only 1 AE leading to discontinuation (hyperbilirubinemia in 2 patients, decreased platelet count in 1 patient, and rash maculopapular in 1 patient).

References to the Online Supplement

1. Yin OQ, Rudoltz M, Galetic I, et al. Effects of yogurt and applesauce on the oral bioavailability of nilotinib in healthy volunteers. *J Clin Pharmacol*. 2011;51(11):1580-1586.

2. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood*. 2013;122(6):872-884.