Supplementary Material

Supplementary Methods

Primary and secondary endpoints

The primary objective was to assess the efficacy of nilotinib, details of which have been previously reported.[1]

Secondary objectives were to further characterize the efficacy, safety and tolerability of nilotinib by the assessment of long-term outcomes including event-free survival (EFS; assessed from the date of first study drug intake to the first occurrence of loss of complete hematologic response [CHR], loss of major cytogenetic response [MCyR], progression to accelerated phase [AP]/ blast crisis [BC] [from chronic phase] 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, and sexual maturation in pediatric patients with Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML).

Assessments for efficacy

Molecular response was assessed by determining the level of *BCR*::*ABL1* transcripts by real-time quantitative polymerase chain reaction (RQ-PCR); analysis was conducted by a central laboratory at baseline, day 28 of cycles 1 and 3, every three cycles to cycle 24, every six cycles thereafter to cycle 66, and at the end of treatment if discontinued early. Major molecular response (MMR) was defined as at least 3.0 log reduction of BCR::ABL1 transcript from standardized baseline value or *BCR*::*ABL1* ≤0.1% on the International Scale (IS); response was confirmed by duplicate analysis of the same sample and results were reported as *BCR*::*ABL1:*control *ABL1* transcripts, standardized to the IS (*BCR::ABL1*^{IS}). If e13a2/e14a2 transcripts were undetectable at baseline, samples were analyzed for the presence of the e1a2 transcript. Loss of MMR was defined as a confirmed loss of ≥3.0 log reduction in *BCR::ABL1*

transcript levels compared to standardized baseline value or a 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 four weeks or associated with confirmed loss of CHR or loss of complete cytogenetic response (CCyR) or progression to AP/BC or CML-related death. Molecular response MR^4 and $MR^{4.5}$ were defined as a *BCR::ABL1*^{IS} transcript level ≤0.01% and ≤ 0.0032%, respectively. Cytogenetic response was determined based on the percentage of Ph+ metaphases in the bone marrow (examination of ≥ 20 metaphases in each bone marrow sample were required) and was assessed at baseline, day 28 of cycle 6, every six cycles to cycle 24, then every 12 cycles to cycle 60, cycle 66 and at the end of treatment if discontinued early. A CCyR was defined as 0% Ph+ metaphases and a MCyR was defined as 0 to 35% Ph+ metaphases.

Hematologic response was assessed by complete blood count (including white blood cell [WBC] count, full differential count, red blood cell count, hematocrit, hemoglobin, and platelet count) at baseline, every seven days of cycle 1, and at day 28 of cycles 2 to 12, every three cycles up to cycle 24, then every six cycles afterwards to cycle 66, and at the end of treatment if discontinued early. A CHR was defined as the presence of the following for ≥4 weeks (i.e., present at least as two visits 4-weeks apart with no intermediate visit showing no CHR): WBC count <$10\times10^{9/}$L, platelet count <$450\times10^{9/}$L, basophils <5%, no blasts and promyelocytes in peripheral blood, myelocytes and metamyelocytes <5% in peripheral blood, and no evidence of extramedullary disease, including spleen and liver.

Mutational analyses were conducted at baseline and at the end of treatment; additional mutational analyses were conducted at the discretion of the investigator. Sample related to

mutational assessment were processed centrally. Quantification of the additional chromosomal abnormalities was performed and analyzed locally.

Assessment of growth, development, and sexual maturation

Height and body weight were measured at baseline, at every cycle up to cycle 12, every three cycles up to cycle 24 and every six cycles thereafter, and at the end of treatment if discontinued early. Bone density and bone age were assessed using dual-energy x-ray absorptiometry (lumbar spine and whole body, except for the head) and x-ray of the left hand and wrist, respectively. Assessments were performed at baseline and at day 28 of every twelfth cycle, and at the end of treatment.

Sexual maturation was monitored by Tanner staging[2,3] and was assessed at baseline and every six cycles. 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.

Height SDS was evaluated in a linear mixed-effects model, to assess changes in the slope parameter; height SDS was used as the response variable and time (based on 6-month intervals) as the explanatory variable. Further model-based analysis was performed to assess changes in slope, considering covariates (baseline pubertal stage, pubertal status over time, and sex) and their interaction with time. For the purposes of the mixed-model analysis, the distinction between prepubertal and pubertal was defined as attainment of Tanner stage 2 for breast or genitalia in girls and boys, respectively.[4]

Supplementary Tables and Figures

Table S1. AEs reported in >20% patients

Preferred term, n (%)	R/I cohort N=33		ND cohort N=25		All patients N=58	
	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4
Patients with ≥1 AE	33 (100)	20 (60.6)	25 (100)	18 (72.0)	58 (100)	38 (65.5)
Headache	13 (39.4)	1 (3.0)	15 (60.0)	0	28 (48.3)	1 (1.7)
Increased blood bilirubin[a]	16 (48.5)	3 (9.1)	16 (64.0)	4 (16.0)	32 (55.2)	7 (12.1)
Pyrexia	13 (39.4)	0	9 (36.0)	1 (4.0)	22 (37.9)	1 (1.7)
ALT increased	10 (30.3)	4 (12.1)	11 (44.0)	3 (12.0)	21 (36.2)	7 (12.1)
Nausea	8 (24.2)	0	10 (40.0)	0	18 (31.0)	0
Rash	7 (21.2)	3 (9.1)	11 (44.0)	2 (8.0)	18 (31.0)	5 (8.6)
AST increased	8 (24.2)	1 (3.0)	9 (36.0)	1 (4.0)	17 (29.3)	2 (3.4)
URT infection	10 (30.3)	1 (3.0)	7 (28.0)	0	17 (29.3)	1 (1.7)
Pain in extremity	9 (27.3)	0	7 (28.0)	0	16 (27.6)	0
Vomiting	7 (21.2)	0	8 (32.0)	1 (4.0)	15 (25.9)	1 (1.7)
Diarrhea	7 (21.2)	0	5 (20.0)	0	12 (20.7)	0
Nasopharyngitis	5 (15.2)	0	7 (28.0)	0	12 (20.7)	0

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CML, chronic myeloid leukemia; CP, chronic phase; ND, newly diagnosed CML-CP; R/I, imatinib/dasatinib resistant/intolerant CML-CP; URT, upper respiratory tract. A patient with multiple severity grades for an AE is only counted once under the maximum grade. A patient with multiple occurrences of an AE under one cohort is counted only once in the AE category for that cohort. MedDRA version 23.0, CTCAE version 4.03. [a]Includes blood bilirubin increased, hyperbilirubinemia, bilirubin conjugated increased and blood bilirubin unconjugated increase.

Table S2. AEs suspected to be study drug-related reported in ≥ 20% patients in any cohort

Preferred term, n (%)	R/I cohort N=33		ND cohort N=25		All patients N=58	
	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4
Patients with ≥1 AE	29 (87.9)	16 (48.5)	24 (96.0)	17 (68.0)	53 (91.4)	33 (56.9)
Increased blood bilirubin[a]	16 (48.5)	3 (9.1)	15 (60.0)	4 (16.0)	31 (53.4)	7 (12.1)
ALT increased	10 (30.3)	4 (12.1)	10 (40.0)	3 (12.0)	20 (34.5)	7 (12.1)
AST increased	8 (24.2)	1 (3.0)	8 (32.0)	0	16 (27.6)	1 (1.7)
Headache	7 (21.2)	1 (3.0)	8 (32.0)	0	15 (25.9)	1 (1.7)
Rash	6 (18.2)	3 (9.1)	7 (28.0)	2 (8.0)	13 (22.4)	5 (8.6)
Nausea	4 (12.1)	0	5 (20.0)	0	9 (15.5)	0
Vomiting	2 (6.1)	0	5 (20.0)	1 (4.0)	7 (12.1)	1 (1.7)
Fatigue	0	0	5 (20.0)	0	5 (8.6)	0

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase CML, chronic myeloid leukemia; CP, chronic phase; ND, newly diagnosed CML-CP; R/I, imatinib/dasatinib resistant/intolerant CML-CP. AEs by preferred terms are presented in descending order of frequency in the "All patients" group. A patient with multiple severity grades for an AE is only counted under the maximum grade. A patient with multiple occurrences of an AE under one cohort is counted only once in the AE category for that cohort. MedDRA version 23.0, CTCAE version 4.03.

[a]Includes blood bilirubin increased, hyperbilirubinaemia, bilirubin conjugated increased and blood bilirubin unconjugated increase.

Table S3. MedDRA preferred terms reported in ≥1 patient within each AESI group term

AESI group term	R/I cohort N=33	ND cohort N=25
Blood cholesterol increased	• Blood cholesterol increased (n=2) • High density lipoprotein increased (n=1) • Hypercholesterolemia (n=1)	• Blood cholesterol increased (n=2) • High density lipoprotein increased (n=1) • Hypercholesterolemia (n=1) • Low density lipoprotein increased (n=1)
Blood glucose increased	• Blood glucose increased (n=2)	• Blood glucose increased (n=1)
Fluid retention	• Weight increased (n=1) • Lip swelling (n=1)	• Weight increased (n=3) • Eye swelling (n=1) • Oedema peripheral (n=1)
Growth retardation	• Growth retardation (n=1) • Short stature (n=1) • Growth hormone deficiency (n=1)	• Growth retardation (n=1)
Hepatotoxicity	• Blood bilirubin increased (n=12) • Alanine aminotransferase increased (n=10) • Aspartate aminotransferase increased (n=8) • Hyperbilirubinemia (n=4) • Gamma-glutamyl transferase increased (n=4) • Bilirubin conjugated increased (n=1) • Blood bilirubin unconjugated increased (n=1) • Drug-induced liver injury (n=1) • Hepatomegaly (n=1) • Hypoalbuminemia (n=1) • Jaundice (n=1)	• Alanine aminotransferase increased (n=11) • Blood bilirubin increased (n=10) • Aspartate aminotransferase increased (n=9) • Hyperbilirubinemia (n=8) • Gamma-glutamyl transferase increased (n=1) • Bilirubin conjugated increased (n=1) • Blood bilirubin unconjugated increased (n=1) • Hepatic enzyme increased (n=1)
Thrombocytopenia	• Thrombocytopenia (n=1)	• Platelet count decreased (n=5) • Thrombocytopenia (n=3)
QT prolongation	• Electrocardiogram QT prolonged (n=5)	• Electrocardiogram QT prolonged (n=2) • Syncope (n=1)
Rash	• Rash (n=7) • Rash maculo-papular (n=5) • Rash papular (n=2) • Rash pruritic (n=1) • Rash follicular (n=1) • Rash macular (n=1)	• Rash (n=11) • Rash maculo-papular (n=3) • Rash papular (n=1) • Rash pruritic (n=1) • Drug eruption (n=1)

AE, adverse event; AESI, adverse event of special interest CML, chronic myeloid leukemia; CP, chronic phase; ND, newly diagnosed CML-CP; R/I, imatinib/dasatinib resistant/intolerant CML-CP.

MedDRA version 23.0, CTCAE version 4.03. MedDRA preferred terms under each group term are listed; preferred terms are presented in descending order.

Table S4. AEs leading to discontinuation

Preferred term, n (%)	R/I cohort N=33	ND cohort N=25	All patients N=58
Patients with ≥1 AE	6 (18.2)	8 (32.0)	14 (24.1)
Increased blood bilirubin[a]	3 (9.1)	3 (12.0)	6 (10.3)
Rash	1 (3.0)	1 (4.0)	2 (3.4)
Increased ALT	0	1 (4.0)	1 (1.7)
Anemia	1 (3.0)	0	1 (1.7)
Increased AST	0	1 (4.0)	1 (1.7)
Autoimmune thyroiditis	0	1 (4.0)	1 (1.7)
Bone pain	1 (3.0)	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 maculo-papular	0	1 (4.0)	1 (1.7)

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase CML, chronic myeloid leukemia; CP, chronic phase; ND, newly diagnosed CML-CP; R/I, imatinib/dasatinib resistant/intolerant CML-CP. AEs by preferred terms are presented in descending order of frequency in the "All patients" group. MedDRA version 23.0, CTCAE version 4.03. [a]Includes blood bilirubin increased, hyperbilirubinaemia, bilirubin conjugated increased and blood bilirubin unconjugated increase.

Table S5. Abnormal growth by cohort (analysis restricted to patients who were prepubertal or did not complete pubertal development prior to study entry)

Decrease from baseline	R/I cohort N=25		ND cohort N=20		All patients N=45	
	N1	n (%)	N1	n (%)	N1	n (%)
1 main percentile line	24	7 (28.0)	19	7 (35.0)	43	14 (31.1)
2 main percentile lines	24	1 (4.0)	19	4 (20.0)	43	5 (11.1)
3 main percentile lines	24	2 (8.0)	19	1 (5.0)	43	3 (6.7)

CML, chronic myeloid leukemia; CP, chronic phase; N1, number of patients with both a baseline and post-baseline value; ND, newly diagnosed CML-CP; R/I, imatinib/dasatinib resistant/intolerant CML-CP.

Decrease from baseline of 1 (respective 2 or 3) SDS category corresponds to a decrease of one (respective 2 or 3) main percentile lines (95^{th}, 90^{th}, 75^{th}, 50^{th}, 25^{th}, 10^{th} and 5^{th} percentiles). One patient in each cohort was excluded due to not having post-baseline height measurement.

Table S6: Mixed effect model for height SDS over time by cohort, according to covariates

Time*covariate	Estimate	Standard Error	p-value
R/I cohort			
Time*chronological age at baseline	0.0118	0.0042	0.0056
Time*gender	0.0497	0.0249	0.0474
Time*pubertal status at baseline	0.0355	0.0251	0.1589
Time*pubertal status over time	0.0266	0.0196	0.1753
ND cohort			
Time*chronological age at baseline	0.0198	0.0108	0.0760
Time*gender	0.0102	0.0276	0.7118
Time*pubertal status at baseline	0.0544	0.0361	0.1339
Time*pubertal status over time	0.1259	0.0460	0.0069

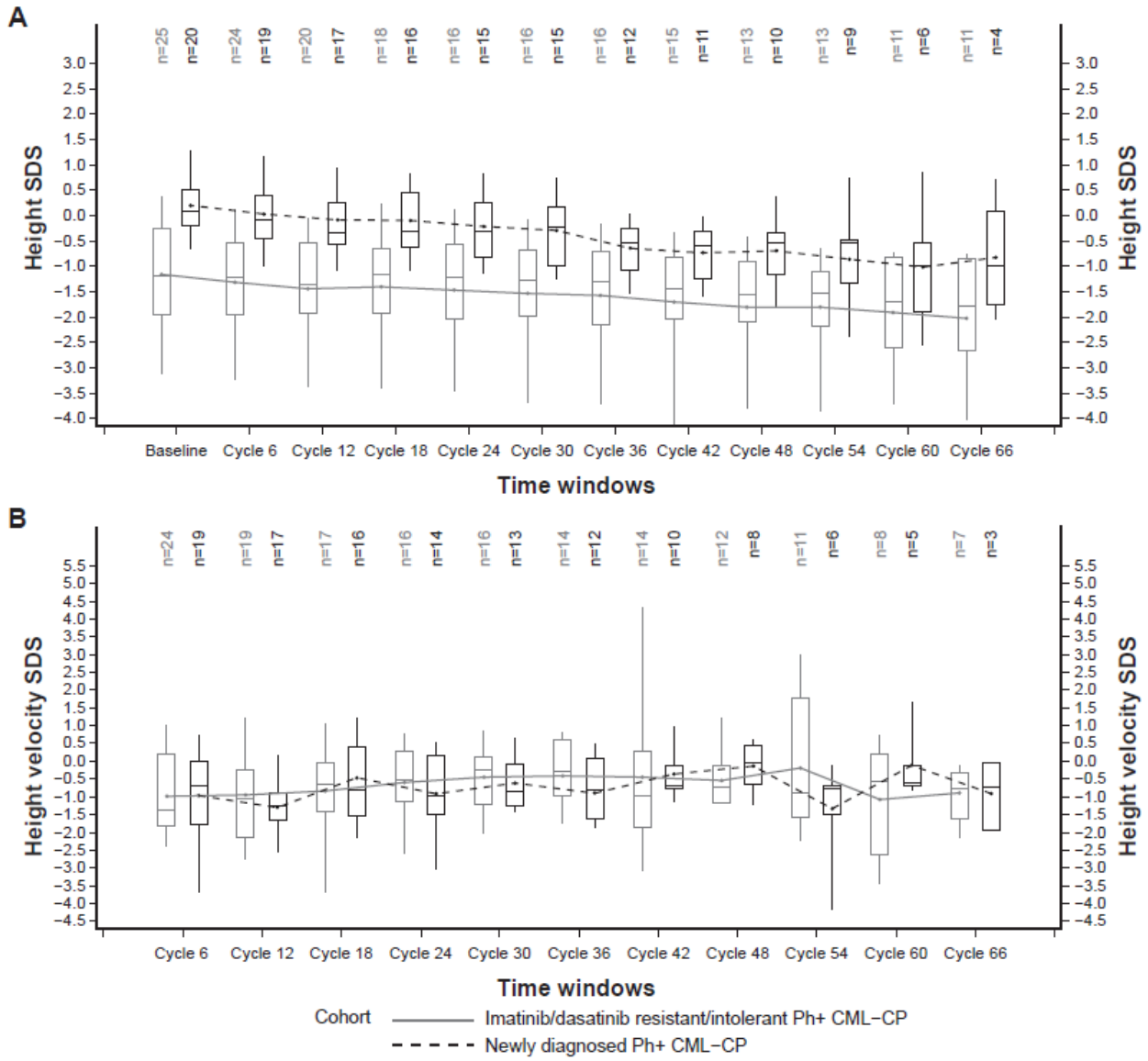
CML, chronic myeloid leukemia; CP, chronic phase; ND, newly diagnosed CML-CP; R/I, imatinib/dasatinib resistant/intolerant CML-CP; SDS standard deviation scores. p-value was compared with two-sided type I error of 0.1. Pubertal patients are those who achieved Tanner stage 2.

Table S7. Bone age SDS by timepoint and cohort

Cycle	Baseline	12	24	36	48	60	66
R/I cohort							
n	31	25	15	14	11	7	6
Median (range)	-0.51 (-4.5, 1.2)	-0.56 (-4.5, 2.0)	-0.87 (-4.3, 0.9)	-0.56 (-3.5, 1.8)	-0.92 (-2.6, 0.9)	-0.53 (-2.1, 1.9)	-0.13 (-3.4, 1.2)
ND cohort							
n	25	21	15	11	8	3	0
Median (range)	0.11 (-2.6, 2.6)	0.08 (-2.5, 2.6)	0.00 (-1.0, 1.5)	0.09 (-2.7, 1.8)	0.20 (-2.1, 1.5)	0.44 (-1.0, 0.9)	-

CML, chronic myeloid leukemia; CP, chronic phase; ND, newly diagnosed CML-CP; R/I, imatinib/dasatinib resistant/intolerant CML-CP; SDS, standard deviation scores.

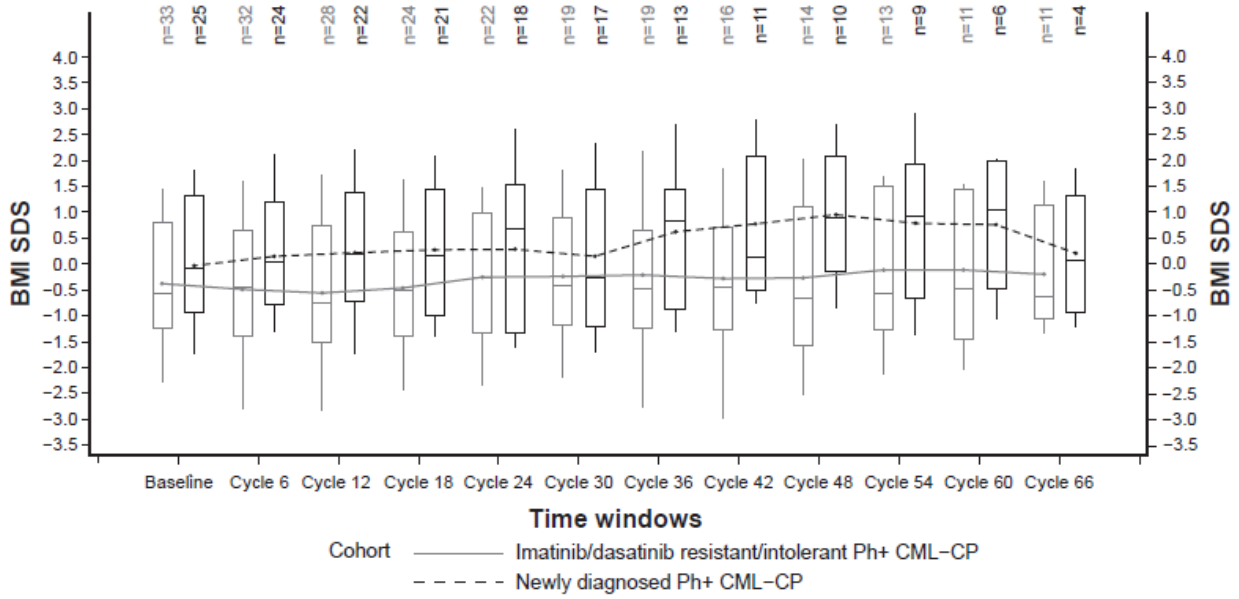
Figure S1. Height SDS (A) and height velocity SDS (B) over time by cohort in patients who had not completed puberty at baseline



CML, chronic myeloid leukemia; CP, chronic phase; ND, newly diagnosed; Ph+, Philadelphia chromosome-positive; R/I, resistant or intolerant; SD, standard deviation; SDS, standard deviation scores.

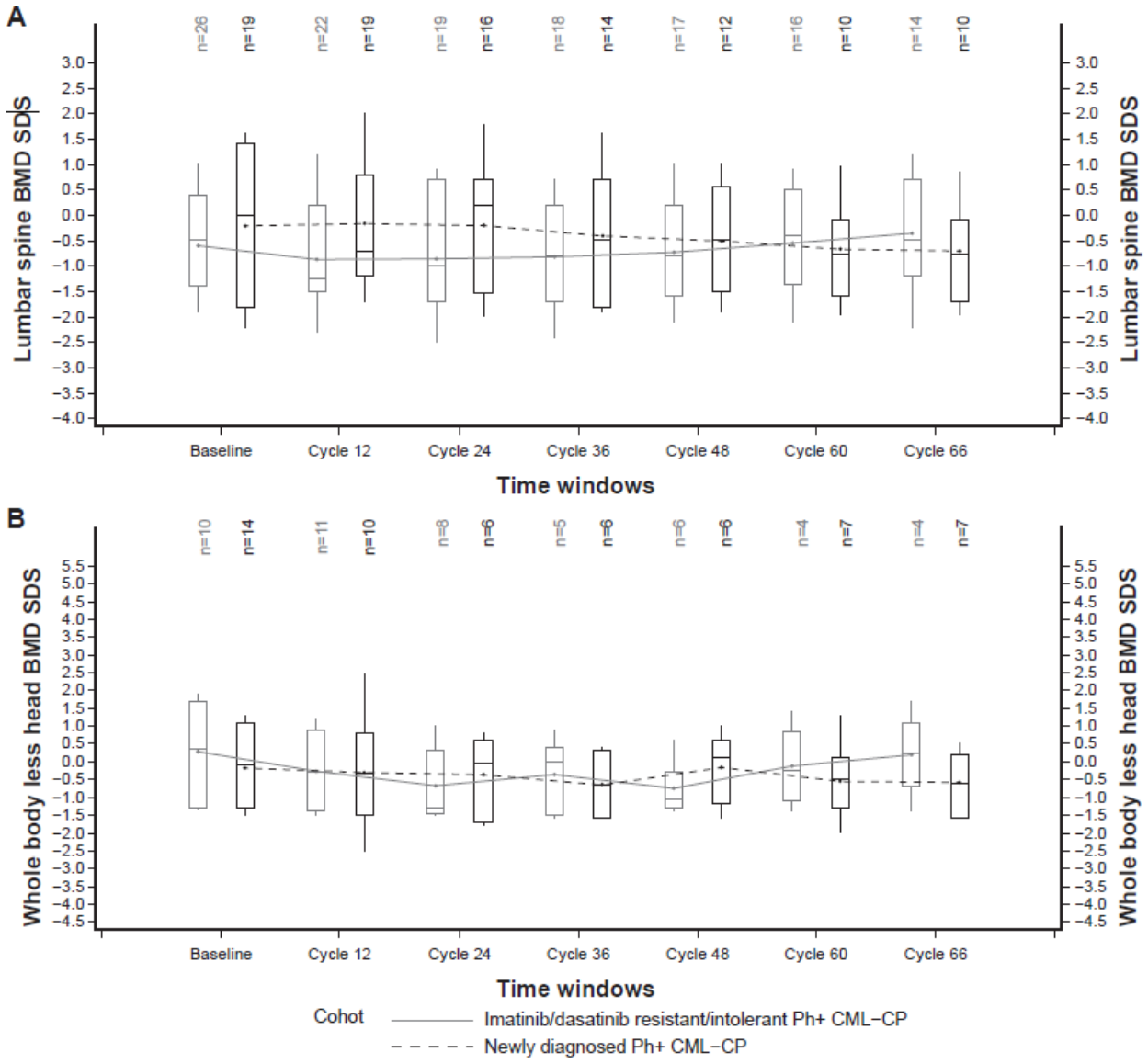
Plot shows boxes (25th–75th percentiles) with median as horizontal line. The dots in the boxes and joining lines represent mean values. Whiskers extend to 10th and 90th percentiles. Values outside this range are not displayed.

Figure S2. BMI SDS over time by cohort



BMI, body mass index; CML, chronic myeloid leukemia; CP, chronic phase; ND, newly diagnosed; Ph+, Philadelphia chromosome-positive; R/I, resistant or intolerant; SD, standard deviation; SDS, standard deviation scores. Plot shows boxes (25^{th}–75^{th} percentiles) with median as horizontal line. The dots in the boxes and joining lines represent mean values. Whiskers extend to 10^{th} and 90^{th} percentiles. Values outside this range are not displayed.

Figure S3. Lumbar spine (A) and whole body (except for the head) (B) bone mineral density SDS by timepoint and cohort



BMD, bone mineral density; CML, chronic myeloid leukemia; CP, chronic phase; ND, newly diagnosed; Ph+, Philadelphia chromosome-positive; R/I, resistant or intolerant; SD, standard deviation; SDS, standard deviation scores.

Plot shows boxes (25^{th}–75^{th} percentiles) with median as horizontal line. The dots in the boxes and joining lines represent mean values. Whiskers extend to 10^{th} and 90^{th} percentiles. Values outside this range are not displayed.

Supplementary References

1. Hijiya N, Maschan A, Rizzari C, et al. Phase 2 study of nilotinib in pediatric patients with Philadelphia chromosome-positive chronic myeloid leukemia. *Blood*. 2019;134(23):2036-2045.

2. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child*. 1969;44(235):291-303.

3. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child*. 1970;45(239):13-23.

4. Hijiya N, Maschan A, Rizzari C, et al. A phase 2 study of nilotinib in pediatric patients with CML: long-term update on growth retardation and safety. *Blood Adv*. 2021;5(14):2925-2934.