

# Treatment-free remission in chronic myeloid leukemia patients treated front-line with nilotinib: 10-year follow-up of the GIMEMA CML 0307 study

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## Abstract

We report the final analysis, with a 10-year follow-up, of the phase II study GIMEMA CML 0307 (NCT 00481052), which enrolled 73 adult patients (median age 51 years; range, 18-83) with newly diagnosed chronic-phase chronic myeloid leukemia to investigate the efficacy and the toxicity of front-line treatment with nilotinib. The initial dose was 400 mg twice daily; the dose was reduced to 300 mg twice daily as soon as this dose was approved and registered. The 10-year overall survival and progression-free survival were 94.5%. At the last contact, 36 (49.3%) patients were continuing nilotinib (22 patients at 300 mg twice daily, 14 at lower doses), 18 (24.7%) patients were in treatment-free remission, 14 (19.2%) were receiving other tyrosine-kinase inhibitors and four (5.5%) patients have died. The rates of major and deep molecular responses by 10 years were 96% and 83%, respectively. The median times to major and deep molecular response were 6 and 18 months, respectively. After a median duration of nilotinib treatment of 88 months, 24 (32.9%) patients discontinued nilotinib while in stable deep molecular response. In these patients, the 2-year estimated treatment-free survival was 72.6%. The overall treatment-free remission rate, calculated on all enrolled patients, was 24.7% (18/73 patients). Seventeen patients (23.3%), at a median age of 69 years, had at least one arterial obstructive event. In conclusion, the use of nilotinib front-line in chronic phase chronic myeloid leukemia can induce a stable treatment-free remission in a relevant number of patients, although cardiovascular toxicity remains of concern.

## Introduction

Nilotinib is a tyrosine-kinase inhibitor (TKI) that was in-

itially shown to be effective and well tolerated at a dose of 400 mg twice daily in patients with chronic phase (CP) chronic myeloid leukemia (CML) resistant or intolerant to

imatinib.<sup>1,2</sup> Therefore, this dose was selected to test nilotinib for the front-line treatment of CP CML in two, independent pilot studies designed in 2007 by the GIMEMA CML Working Party (CML 0307 trial) and by the MD Anderson Cancer Center (MDACC).<sup>3,4</sup> The dose of nilotinib in the GIMEMA trial was reduced to 300 mg twice daily after the approval of this dose for the front-line treatment of CP CML, according to the early results of the prospective, randomized ENESTnd trial,<sup>5</sup> which compared imatinib with two initial doses of nilotinib (400 mg twice daily and 300 mg twice daily). This study showed that the rate and depth of molecular response were greater with both doses of nilotinib than with imatinib, but that nilotinib 300 mg twice daily was as effective as and less toxic than nilotinib 400 mg twice daily. Reports updating the outcome of the patients enrolled in the ENESTnd trial confirmed, over a follow-up time extended up to 10 years, the greater efficacy of nilotinib over imatinib as a front-line treatment, with regard to the speed, rate and depth of molecular response, and also the rate of progression to advanced phase. However, there was no advantage in overall survival, which was around 90% in all arms, and the frequency of arterial obstructive events (AOE) was higher with both nilotinib doses than with imatinib.<sup>6-9</sup>

Considering the excellent overall survival obtained with TKI, the next main goals for CML patients became the avoidance of long-term adverse events and the achievement of treatment-free remission (TFR). Since a precondition for TFR is a stable deep molecular response (DMR), it is expected that the use of second-generation TKI may increase the proportion of patients eligible for TFR compared to imatinib;<sup>9</sup> however, the higher risk of adverse events may at least partially weaken this advantage. Therefore, the benefit-risk profile should be carefully evaluated for each patient.

Patients enrolled in the GIMEMA CML 0307 trial have now been followed for a minimum of 10 years, providing an academic, independent source of information on efficacy, tolerability and toxicity of nilotinib. Moreover, as TFR evolved as a safe option in selected patients, although it was not an original aim of this clinical trial, we provide here for the first-time detailed data on TFR.

## Methods

The GIMEMA CML Working Party initiated in 2007 a phase II study (NCT 00481052) with nilotinib front-line. Seventy-three adult ( $\geq 18$  years old) patients with newly diagnosed CP-CML were enrolled at 18 GIMEMA Centers in Italy between June 2007 and February 2008. The initial dose of nilotinib was 400 mg twice daily. Following the authorization in Italy in November 2011 of nilotinib at the dose of 300 mg twice daily for the front-line treatment of CP-CML,

the GIMEMA protocol was amended and then approved by local ethics committees, so that all patients still receiving the dose of 400 mg twice daily had their dose reduced to 300 mg twice daily by September 2012. Moreover, the duration of the study was extended to 10 years.

Detailed methods, and early and mid-term results have been reported previously.<sup>4,10</sup> Here, we report the final analysis of this study, with a minimum follow-up of 10 years, focusing on the rate of TFR, molecular response, survival, causes of death, as well as on the type and the severity of adverse events, particularly of AOE.

The median follow-up for this analysis was 123 months (range, 111-130). The cumulative probabilities of events and survival were estimated by the Kaplan-Meier method. Unless specifically reported, all the analyses are referred to the intention-to-treat population (73 patients). Disease risk at baseline was calculated according to the Sokal<sup>11</sup> and EUTOS long-term survival (ELTS)<sup>12</sup> scores. Molecular response was evaluated on peripheral blood every 3 months until a major molecular response (MMR) was achieved and confirmed, then at least every 6 months. MMR was assessed and expressed according to the International Scale, as a *BCR-ABL1* transcript level  $\leq 0.1\%$ , corresponding to a 3-log decrease from the International Scale standard, in samples with more than 10,000 *ABL1* copies. A DMR (MR4) was defined as a *BCR-ABL1* transcript level  $\leq 0.01\%$ , corresponding to a 4-log reduction, in samples with more than 10,000 *ABL1* copies.<sup>13</sup> The response at milestones was defined according to the last version of the European LeukemiaNet (ELN) recommendations<sup>14</sup> and also according to the recent GIMEMA proposals for a TFR-oriented strategy.<sup>15</sup> Treatment discontinuation in stable DMR (defined as a MR4 or better lasting 2 years or more) aiming at TFR was not originally planned in the protocol, but it was attempted in some patients in agreement with the evolving clinical practice in recent years. For descriptive purposes, we applied the ELN requirements for treatment discontinuation.<sup>14</sup> TFR was defined as a MMR (or better) without ongoing TKI treatment.

The trial was approved by Ethics Committees of all participating Centers and it was registered in ClinicalTrials.gov (NCT00481052) and the EUDRACT (2007-000597-22) database.

## Results

### Patients' characteristics

The median age of enrolled patients was 51 years (range, 18-83); 51% of patients were males. The Sokal risk score<sup>11</sup> was low, intermediate or high in 33 (45.2%), 30 (41.1%) and ten (13.7%) patients, respectively; the ELTS risk score<sup>12</sup> was low, intermediate or high in 47 (64.4%), 22 (30.1%) and four

(5.5%) patients, respectively. Thirty-five patients (47.9%) had at least one cardiovascular risk factor. The characteristics of the patients are detailed in Table 1.

### Nilotinib dosing and discontinuation in the first 5 years

All patients started nilotinib 400 mg twice daily in the years 2007 and 2008, but in 2012, according to a protocol amendment made after the approval of nilotinib for front-line treatment of CP CML in Italy, the nilotinib dose was reduced to 300 mg twice daily in all eligible patients.

At that time, 11 (15%) patients had already permanently discontinued nilotinib because of adverse events (9 patients), progression (1 patient) or TFR (1 patient). In addition, 22 (30.1%) patients had already permanently reduced their nilotinib dose because of intolerance/adverse events (20 patients to 400 mg once daily and two patients to 400 + 200 mg daily); the median time on nilotinib 400 mg twice daily for these patients was 8 months (range, 1-54). Therefore, at the time of the protocol amendment, only 40 (54.7%) patients were still receiving nilotinib 400 mg twice daily and had a dose reduction to 300 mg twice daily; the median time on nilotinib 400 mg twice daily for these patients was 55 months (range, 49-60).

Considering the whole cohort of 73 patients, the median duration of nilotinib 400 mg twice daily prior to permanent nilotinib dose reduction or discontinuation was 51 months (range, 1-60) (Figure 1).

### Patients' disposition

The patients' disposition at the last contact is summarized in Table 2 and *Online Supplementary Figure S1*. Twenty-two patients (30.1%) were continuing nilotinib at the standard dose of 300 mg twice daily, ten patients (13.7%) at 400 mg once daily and four patients (5.5%) at 300 mg once daily. Eighteen (24.7%) patients were in TFR after nilotinib discontinuation. Fourteen (19.2%) patients were receiving other TKI (imatinib, 7 [9.5%]; dasatinib, 4 [5.4%]; bosutinib, 3 [4.1%]). Four (5.4%) patients have died. One patient was withdrawn from the study at 5 years,

**Table 1.** Patients' characteristics.

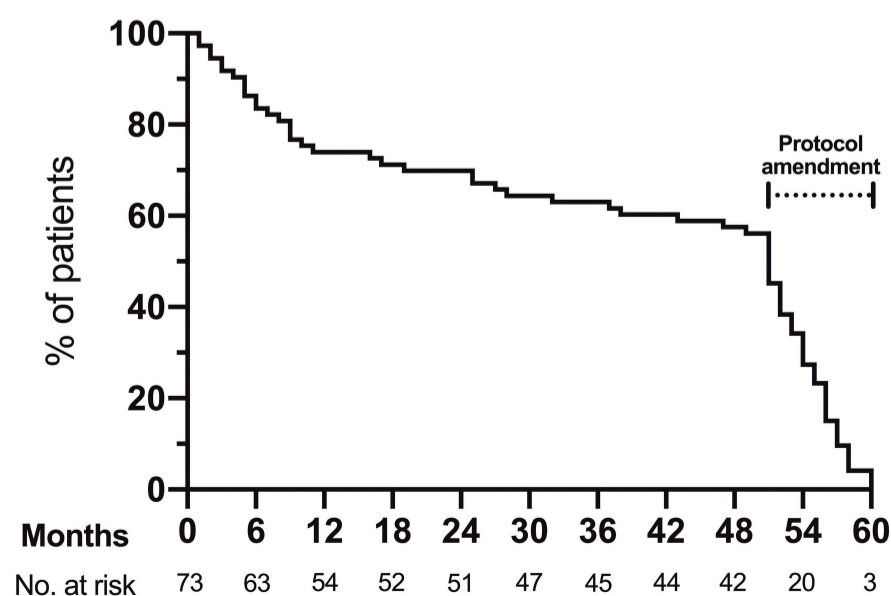
Patients	N=73	
Males / females, N	37 / 36	
Age in years, median (range)	51 (18-83)	
Risk score, N (%)	Sokal	ELTS
Low	33 (45.2)	47 (64.4)
Intermediate	30 (41.1)	22 (30.1)
High	10 (13.7)	4 (5.5)
Transcript type, N (%)		
e14a2	32 (43.8)	
e13a2	29 (39.7)	
e13a2/e14a2	12 (16.4)	
Cardiovascular risk factors, N (%)	35 (47.9) <sup>a</sup>	
Smoking	16 (21.9)	
Hypertension	14 (19.2)	
Body mass index $\geq 30$	13 (17.8)	
Diabetes mellitus	6 (8.2)	
Hypercholesterolemia	4 (5.5)	
Prior cardiovascular event	2 (2.7)	
Follow-up, months; median (range)	123 (111-130)	

<sup>a</sup>Patients with at least one cardiovascular risk factor. ELTS: EUTOS long-term risk score.

while still on nilotinib 400 twice daily, for administrative reasons.

### Survival

Overall, four patients have died (1 in blast phase, 3 in CP). The 10-year overall survival (Figure 2) and progression-free survival were 94.5% (95% confidence interval: 86-97.9). In detail: one patient died 9 months after beginning treatment, at the age of 63, following a blast phase progression carrying a T315I mutation (before ponatinib became available); two patients, aged 75 and 78, died in a condition of progressive cerebral deterioration without any specific cardiovascular or cerebral event, after 32 months (still on nilotinib) and 121 months (57 months after nilotinib discontinuation and while on dasatinib) of follow-up, re-



**Figure 1.** Duration of treatment with nilotinib 400 mg twice daily in the first 5 years of the study. All patients started nilotinib at the dose of 400 mg twice daily between 2007 and 2008. Following the authorization in Italy in November 2011 of nilotinib at the dose of 300 mg twice daily for the front-line treatment of chronic phase chronic myeloid leukemia, the GIMEMA protocol was amended, so that the dose in all patients still receiving the dose of 400 mg twice daily was reduced to 300 mg twice daily by September 2012. The median duration of nilotinib 400 mg was 51 months (interquartile range, 25-56).



spectively; one patient died 68 months after the start of nilotinib, at the age of 90, due to congestive heart failure, as a complication of a myocardial infarct that happened 4 months previously (while still on nilotinib).

### Response

The cumulative rates of MMR and MR4 by 10 years were 96% and 83%, respectively. The median time to MMR and MR4 were 6 and 18 months, respectively.<sup>10</sup> At 3 months, 66 (90.4%) patients had a *BCR-ABL1* transcript level <10% (*Online Supplementary Table S1*); at 6 months 61 (83.5%) patients had a *BCR-ABL1* <1%; at 12 months 55 (75.3%) patients had a *BCR-ABL1* ≤0.1%; and at 24 months 33 (45.2%) patients had a *BCR-ABL1* ≤0.01%. Response at milestones according to the ELN and GIMEMA recommendations are reported in *Online Supplementary Table S2*. Overall, of the 36 (49.3%) patients continuing nilotinib at the last evalu-

ation, 22 (30.1%) and 14 (19.2%) were in DMR and MMR, respectively.

Treatment failure leading to permanent nilotinib discontinuation occurred in three (4.1%) patients. In one patient (high Sokal risk; intermediate ELTS risk) it occurred early, following a blast phase progression carrying a T315I mutation at 6 months. In the other two patients (both low Sokal and ELTS risk) it was a very late event: these patients had a confirmed loss of molecular response, with a *BCR-ABL1* transcript level >1% at 101 and 110 months after the start of treatment, without detectable kinase domain mutations; one patient was on nilotinib 300 mg twice daily, the other on 300 mg once daily.

During the 10 years of the study, 29 (39.7%) patients have had a permanent reduction in the dose of nilotinib to 400 or 300 mg once daily: 17 patients held a stable DMR (11 subsequently attempted TFR); 11 maintained a stable MMR; and one patient lost the molecular response (with a *BCR-ABL1* level >1%) and was switched to another TKI.

**Table 2.** Patients' disposition.

Patients	N=73
On nilotinib, N (%)	36 (49.3) <sup>a</sup>
300 mg twice daily	22 (30.1)
400 mg once daily	10 (13.7)
300 mg once daily	4 (5.5)
In TFR after nilotinib front-line, N (%)	18 (24.7)
Alive in CP, on treatment with other TKI, N (%)	14 (19.2)
Imatinib 2 <sup>nd</sup> line	6 (8.2)
Imatinib 3 <sup>rd</sup> line	1 <sup>b</sup>
Dasatinib 2 <sup>nd</sup> line	2 (2.7)
Dasatinib 3 <sup>rd</sup> line	2 (2.7) <sup>c</sup>
Bosutinib 2 <sup>nd</sup> line	1
Bosutinib 3 <sup>rd</sup> line	2 (2.7) <sup>d</sup>
Deaths, N (%)	4 (5.5)
Off-study for administrative reasons, N	1 <sup>e</sup>

<sup>a</sup>Fifteen out of 36 patients had the European LeukemiaNet minimal requirements for treatment discontinuation but never attempted TFR.

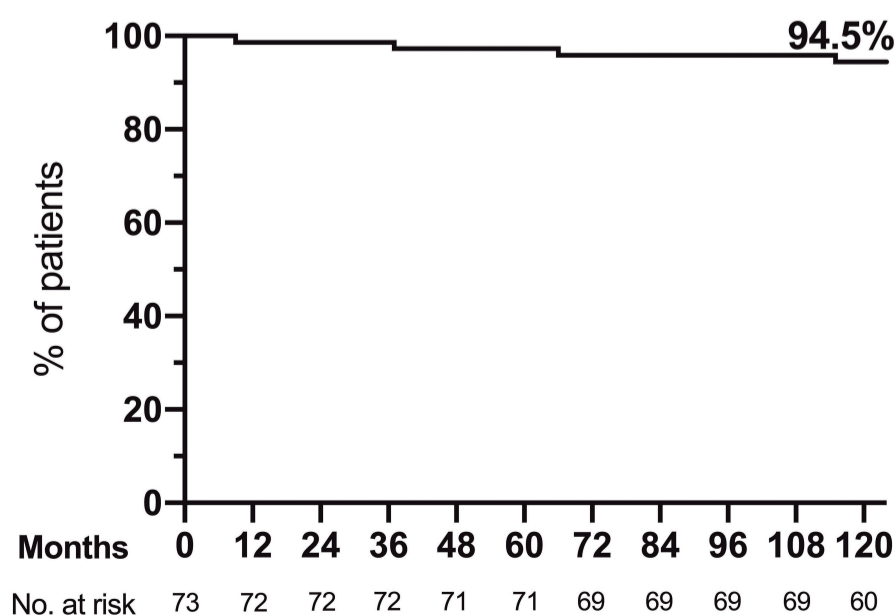
<sup>b</sup>After dasatinib. <sup>c</sup>After imatinib. <sup>d</sup>One patient after imatinib and one after dasatinib. <sup>e</sup>Study extension not approved by local Ethics Committee; at the end-of-study visit (2012) the patient was continuing nilotinib. TFR: treatment-free remission; CP: chronic phase.

### Treatment-free remission

Treatment discontinuation in stable DMR, aiming at TFR, was not planned in the protocol. Therefore, the decision for treatment discontinuation and its management reflected the evolving clinical practice in the participating Italian centers over the last decade.

During the study 36 (49.3%) patients met the ELN minimal requirements for treatment discontinuation (nilotinib treatment duration >4 years and MR4 or better lasting >2 years), but not all of these patients attempted TFR.

Overall, 24 (32.8%) patients (13 females, 11 males) discontinued nilotinib while in stable DMR (Table 3). In 18 patients the decision to stop nilotinib was specifically made aiming at TFR; in the remaining six, the occurrence of an adverse event prompted discontinuation of nilotinib. The median age at treatment discontinuation was 62 years (range, 29-80). The Sokal score was low, intermediate or high in 14, seven and three patients, respectively; the ELTS score was



**Figure 2.** Ten-year overall survival.

low, intermediate or high in 16, seven and one patient, respectively. In these patients, the median duration of nilotinib treatment prior to discontinuation was 88 months (range, 25-117); the median interval from first detection of MR4 to treatment discontinuation was 74 months (range, 7-110) and the median follow-up after treatment discontinuation was 34 months (range, 7-98). The time from starting nilotinib to the TFR attempt is reported in Figure 3A.

Of the 24 patients who discontinued nilotinib, 18 (75%) maintained a stable TFR up to the last evaluation (17 patients in stable DMR and 1 patient in stable MMR). Six (25%) patients had a confirmed loss of MMR (after 3, 3, 6, 7, 13 and 23 months) and restarted therapy (nilotinib 4 patients, imatinib 1, dasatinib 1); they all regained the MMR (4 of them obtained a DMR again). The estimated treatment-free survival at 24 months was 72.6% (95% confidence interval: 48.3-86.9%) (Figure 3B). Considering the whole cohort of enrolled patients, the rate of stable TFR was 24.7% (18 out of 73 patients). TFR rates according to response at the ELN and GIMEMA milestones are reported in *Online Supplementary Table S3*.

#### Arterial obstructive events

Overall, 17 (23.3%) patients developed AOE: 15 had a single AOE while two had multiple AOE during the follow-up (Figure 4, Table 4, *Online Supplementary Table S4*). In detail, the first-occurring AOE was peripheral arterial disease (PAD) in seven (9.5%) patients, a coronary syndrome in five (6.8%; 4 acute and 1 chronic), carotid stenosis in three (4.1%), ischemic stroke in one and a transient ischemic attack in one. These events were symptomatic in 13/17 (76.5%) patients, while in four patients the AOE (3 carotid stenosis, 1 PAD) were diagnosed only after routine screening examinations, while still asymptomatic.

The median age at the time of the first AOE was 69 years (range, 49-88), after a median duration of nilotinib treatment of 64 months (range, 24-113). The dose of nilotinib at the time of the AOE was 400 mg twice daily in 6/17 patients (4 PAD; 2 coronary syndrome), 300 mg twice daily in five (2 PAD; 2 carotid stenosis; 1 ischemic stroke), and 400 mg once daily in four patients (2 with coronary syndrome; 1 PAD; 1 transient ischemic attack); moreover, two patients were already in TFR (1 coronary syndrome; 1 carotid stenosis).

The management of AOE included angioplasty with stent insertion in 5/17 patients, amputation of a lower limb in three, vascular surgery in three, and medical treatment alone in six. Nine of 15 (60%) patients still on nilotinib permanently discontinued nilotinib due to the occurrence of the AOE; the remaining six patients continued nilotinib at a lower dose, but two of them experienced additional AOE.

Regarding the management of the four asymptomatic patients diagnosed after routine screening examinations,

**Table 3.** Characteristics of patients attempting treatment-free remission.

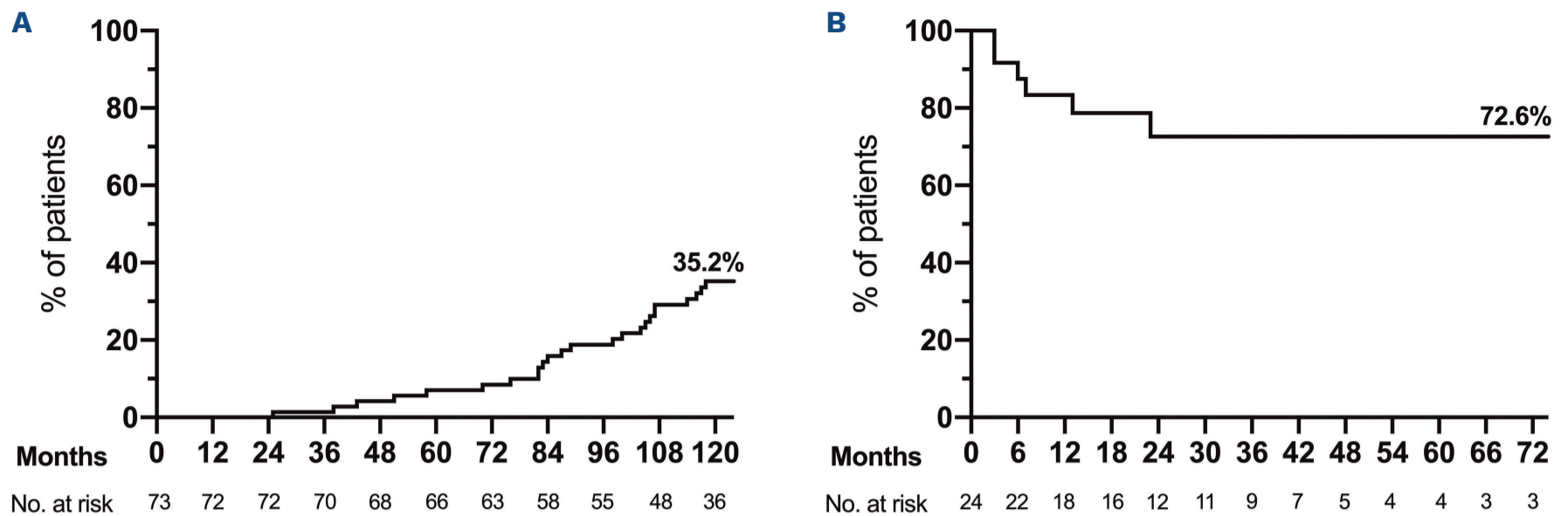
Patients attempting TFR	24/73 (32.8%) <sup>a</sup>	
Age in years (median)	62 (29-80)	
Males / females, N	11 / 13	
Risk score, N (% <sup>b</sup> )	Sokal	ELTS
Low	14/33 (42.4)	16/47 (34)
Intermediate	7/30 (23.3)	7/22 (31.8)
High	3/10 (30)	1/4 (25)
Transcript type, N (% <sup>c</sup> )		
e13a2	9/32 (28.1)	
e14a2	13/29 (44.8)	
e13a2/e14a2	2/12 (16.7)	
Duration of nilotinib treatment prior to D/C, months; median (range)	88 (25-118)	
Interval from achievement of MR4 to treatment D/C, months; median (range)	74 (7-110)	
ELN 2020 requirements for treatment D/C, N (%)		
minimal (stop allowed) <sup>d</sup>	21/24 (87.5) <sup>f</sup>	
optimal (stop recommended for consideration) <sup>e</sup>	20/24 (83.3) <sup>g</sup>	
Follow-up after treatment D/C, months; median (range)	34 (7-98)	
Patients with confirmed loss of MMR in TFR, N (%)	6/24 (25%)	
Patients in TFR at last evaluation in patients attempting TFR, N (%)	18/24 (75) <sup>h</sup>	
in the whole cohort, N (%)	18/73 (24.7) <sup>h</sup>	

<sup>a</sup>In 18 patients the decision to stop nilotinib was specifically made aiming at treatment-free remission (TFR); in the remaining six, the occurrence of an adverse event prompted nilotinib discontinuation.

<sup>b</sup>Percent of patients in the same Sokal or ELTS risk group. <sup>c</sup>Percent of patients in the same transcript type group. <sup>d</sup>Nilotinib duration >4 years and duration of deep molecular response (MR4) or better >2 years. <sup>e</sup>Nilotinib duration >5 years and duration of MR4 or better >3 years. <sup>f</sup>Details of the three patients without the European Leukemia-Net (ELN) minimal criteria for stopping treatment: one patient (male, age 70 years, ELTS low, transcript type e14a2) stopped nilotinib after 25 months because of an adverse event; while being in stable MR4 for 18 months; the patient maintained the TFR. Another patient (male, 76 years, ELTS intermediate, transcript type e13a2) stopped nilotinib after 38 months because of an adverse event, while being in stable MR4 for 24 months; he soon lost MMR (at 3 months). The third patient (female, 66 years, ELTS intermediate, transcript type e14a2) stopped nilotinib after 43 months because of an adverse event, while being in stable MR4 for only 7 months; despite this she maintained the TFR.

<sup>g</sup>Details of the additional patient without the ELN optimal criteria for stopping treatment: the patient (female, 70 years, ELTS low, transcript type e13a2) stopped nilotinib aiming at TFR after 51 months, with a stable MR4 lasting 48 months; she maintained the TFR. <sup>h</sup>In 15 patients the decision to stop nilotinib was specifically made aiming at the TFR; three patients discontinued nilotinib following an adverse event. CP: chronic phase; TKI: tyrosine kinase inhibitor; TFR: treatment-free remission; ELTS: EUTOS long-term risk score; D/C: discontinuation; ELN: European LeukemiaNet; MMR: major molecular response.

two patients were switched to imatinib, one reduced the dose of nilotinib and then entered the TFR phase, and one was already in TFR.



**Figure 3. Treatment-free remission.** (A) Cumulative incidence of attempted treatment-free remission (TFR). Twenty-four out of 73 patients attempted TFR while in stable deep molecular response (DMR). (B) Treatment-free survival in 24 patients discontinuing nilotinib in stable DMR. Eighteen patients maintained the TFR (17 with a stable DMR and 1 with a stable major molecular response), while six patients lost the major molecular response and resumed treatment with a tyrosine kinase inhibitor.

With a median follow-up after AOE of 47 months (range, 1-101), two patients have died: one patient (90 years old) died from congestive heart failure, 4 months after an acute coronary syndrome; the other one (78 years old) died for reasons unrelated to the previous AOE. At the last contact, 4/17 (24.6%) patients who suffered from an AOE were in stable TFR.

#### Other adverse events

Hematologic toxicity, hepatic and pancreatic laboratory abnormalities (increases in aspartate aminotransferase, alanine aminotransferase, bilirubin, amylase, and lipase) as well as other well-known nilotinib-related clinical adverse events (skin rash, pruritus and muscle and joint pain) developed early during the study,<sup>4</sup> with no new events recorded in patients continuing nilotinib in the long-term. Recurrent grade 3 or grade 4 elevation of lipase levels, without clinical pancreatitis, led to permanent discontinuation of nilotinib in three patients, after 9, 17 and 27 months of treatment.

Other common emerging adverse events during the follow-up were hypercholesterolemia (in 28 [38.3%] patients), hypertension (in 7 [9.6%] patients) and diabetes (in 4 [5.5%] patients); none of these events was severe, but in most instances a specific therapy was started.

## Discussion

The prospective, long-term, 10-year observation of newly diagnosed CP-CML patients who were treated front-line in the GIMEMA CML 0307 trial with nilotinib 400 mg twice daily and then continued with lower doses of nilotinib provides an academic independent confirmation of the efficacy and the cardiovascular toxicity of this TKI.

These features were already highlighted in the ENESTnd

trial, the regulatory, company-sponsored study that compared front-line treatment with nilotinib 400 mg or 300 mg twice daily and imatinib 400 mg once daily.<sup>5-9</sup> In that trial, 281 and 282 newly diagnosed CP CML patients (median age 47 years in both groups) were assigned to receive nilotinib 400 or 300 mg twice daily, respectively. In both arms, 37%, and 28% of the patients had low and high Sokal risk scores, respectively. The 10-year overall survival was 87.6% in the 300 mg twice daily group and 90.3% in the 400 mg twice daily group. At 10 years, the cumulative probability of MR4 was 68.3% in the 400 mg twice daily arm and 69.5% in the 300 mg twice daily arm. Based on a retrospective evaluation of the patients who might have met the criteria for treatment discontinuation (defined as MR4.5 for more than 1 year), over the whole 10-year period, 48.6% and 47.3% of patients would have been eligible for treatment discontinuation in the 400 mg twice daily and 300 mg twice daily group, respectively.<sup>9</sup>

The GIMEMA CML 0307 trial was initiated before the ENESTnd study and almost at the same time as the phase II MDACC trial of nilotinib 400 mg twice daily in front-line.<sup>3</sup> Interestingly, an update of the data of the MDACC trial was published recently.<sup>16</sup> One hundred and twenty-two patients were enrolled: the patients had a median age of 51 years and 68% and 7% had a low and high Sokal risk score, respectively. At the last follow-up (median 78.3 months), 51% of patients were still on nilotinib, with more than 50% at daily doses of 400 to 150 mg. Six of the 122 patients had progressed to blast phase. The 5-year overall survival was 93%. At 10 years, the cumulative probability of achieving MR4 and MR4.5 was 82% and 75%, respectively. Overall, these data are quite similar to the data of this GIMEMA CML 0307 study, highlighting the efficacy of nilotinib in terms of molecular response.

Of note, our study is the first to report extensive data of TFR based on all enrolled patients. The rate of treatment



**Table 4.** Arterial obstructive events.

Patients experiencing AOE	N = 17/73 (23.3%)
Cardiovascular risk factors <sup>a</sup> at CML diagnosis in patients with AOE, N (%):	
0	4 (23.5)
1	7 (41.2)
≥2	6 (35.3)
Duration of NIL treatment prior to first AOE, months; median (range)	64 (24-113)
Treatment at AOE:	
Nilotinib 400 mg twice daily	6 (35.3)
Nilotinib 300 mg twice daily	5 (29.4)
Nilotinib 400 mg once daily	4 (23.5)
Treatment-free remission	2 (11.7)
Age in years at first AOE, median (range)	69 (49-88)
Type of first AOE, N (%)	
Peripheral arterial disease	7 (41.1)
Coronary events	5 (29.4)
Carotid stenosis	3 (17.6)
Stroke	1 (5.9)
Cerebral transient ischemic attack	1 (5.9)
Management of AOE, N (%)	
Angioplasty + stent	5 (29.4)
Amputation	3 (17.6)
Vascular surgery	3 (17.6)
Medical	6 (35.3)
Permanent nilotinib discontinuation	9 (52.9) <sup>b</sup>
Follow-up after AOE, median (range), months	47 (1-101)
Patients with additional AOE during the follow-up	2 <sup>c,d</sup>
Deaths from AOE, N	1 <sup>c</sup>

<sup>a</sup>Smoking, arterial hypertension, obesity, diabetes mellitus, dyslipidemia, prior ischemic event <sup>b</sup>Two additional patients were already in treatment-free remission (TFR) at the time of an arterial obstructive event (AOE). <sup>c</sup>One patient had three AOE, namely peripheral arterial disease, stroke and myocardial infarction; the patient died at the age of 90 due to congestive heart failure, 4 months after the infarct, while continuing nilotinib at a low dose <sup>d</sup>One patient had multiple coronary events but continued on a low dose of nilotinib until he discontinued the treatment in stable deep molecular response, aiming at TFR.

discontinuation aiming at TFR was 32.8%, a “real-life” rate that is considerably lower than the theoretical estimated rates from the ENESTnd trial (48.6% and 47.3%).<sup>9</sup> Physicians’ and/or patients’ cautiousness in years when TFR was not yet widely accepted as a standard clinical practice may have hampered treatment discontinuation in some eligible patients. In our patients who attempted TFR, the estimated 24-month treatment-free survival was 72.6%, similar to that documented in a large Italian retrospective analysis of patients discontinuing TKI,<sup>17</sup> but apparently higher than that in prospective studies of TFR.<sup>18,19</sup> In the ENEST-freedom study,<sup>18</sup> which enrolled patients treated front-line with nilotinib, the 96-week treatment-free survival was 50.9%. Even if no direct comparison between these studies is possible, the longer nilotinib

treatment duration (median 88 vs. 43.5 months) as well as the longer duration of DMR (median 74 vs. 30 months) in our study may have favored this better outcome. Indeed, in the EURO-SKI study, in patients receiving imatinib front-line, longer treatment duration (odds ratio per year 1.14) and longer DMR duration (odds ratio per year 1.13) were associated with an increasing probability of maintenance of MMR at 6 months.<sup>19</sup>

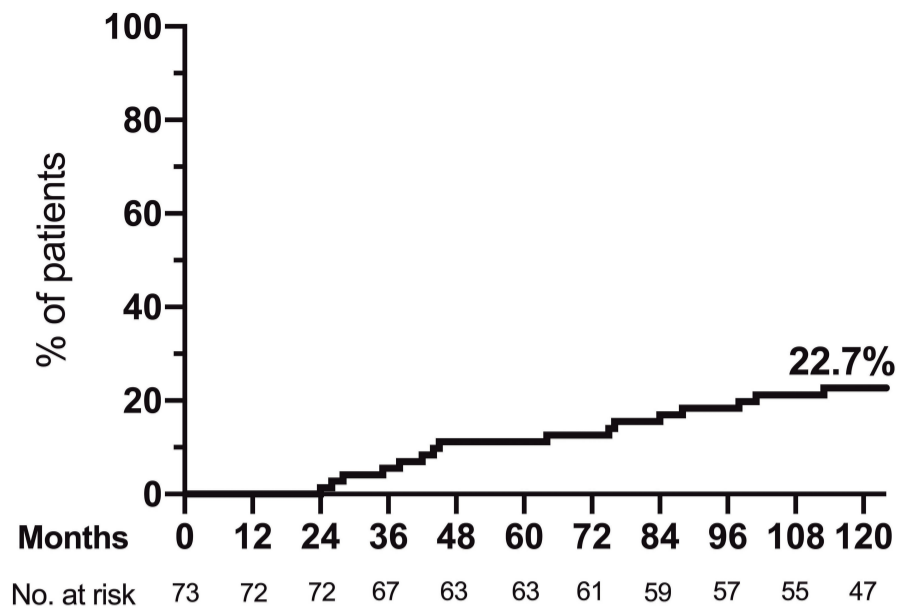
In this GIMEMA study, considering all enrolled patients, the proportion of patients who did not relapse molecularly and remained treatment-free was 24.7%. Since not all eligible patients made an attempt at TFR, the potential proportion of patients in TFR at 10 years could have been even higher. While awaiting the results of prospective trials in newly diagnosed patients specifically designed to assess the TFR rates, our data are in line with the current expectation that 20-30% of CML patients treated with second-generation TKI in front-line can reach a stable TFR.

Regarding long-term tolerability and safety, it is worth noting that after 10 years of follow-up 49.3% of patients were still on treatment with nilotinib, with only a minority of patients (19.2%) receiving other TKI. No new safety issues have emerged, but the 10-year rate of AOE was 23.3% (17.8% considering only symptomatic events). We observed AOE almost exclusively (all but one) in elderly patients (median age 69 years) and/or in patients with pre-existing cardiovascular risk factors (76.4%). Older age, the presence of cardiovascular risk factors, the high initial dose of nilotinib, and the unawareness of nilotinib cardiovascular toxicity in the first phase of the study may all have contributed to the high incidence of AOE.

Mortality from AOE was low, with only one patient (90 years old) who may have died from later consequences of an AOE. However, morbidity was relevant, as almost two-thirds of patients with AOE needed an invasive treatment (angioplasty with stenting, vascular surgery, or, in a few cases, limb amputation).

The rate of AOE in our study was similar to that observed in the nilotinib arms of the ENESTnd study (23.5% with 400 mg twice daily and 16.5% with 300 mg twice daily)<sup>14</sup> but higher compared to that in the MDACC trial, in which ischemic adverse events were reported in 8.2% of patients, with a surprisingly low rate of PAD (<1%).<sup>16</sup> This difference from our study may in part be explained by a shorter follow-up (median 78.3 vs. 123 months) but other factors (patients’ baseline characteristics, management of risk factors, dose reductions, AOE screening and reporting) could be involved; in any case, the small numbers do not allow the statistical significance of this difference to be determined.

As expected,<sup>14</sup> our study confirms the cardiovascular toxicity of nilotinib in the long-term, which is higher than that observed with imatinib in the ENESTnd study (3.5%)<sup>9</sup>



**Figure 4. Cumulative incidence of arterial obstructive events.** Seventeen patients had at least one arterial obstructive event. Two patients had multiple events (only the first event is reported in the graph).

or in other trials.<sup>20,21</sup> Studies with dasatinib, bosutinib and ponatinib in front-line have a shorter follow-up,<sup>22-24</sup> preventing any suitable long-term comparison of the AOE rates.

Taken together, the long-term results of the GIMEMA CML 0307 study show that the use of nilotinib front-line is capable of inducing a stable TFR in a relevant number of patients. This approach is however associated with AOE. It is likely that the number of CML patients who can obtain TFR may increase, and that the number of cardiovascular complications may decrease, through a more accurate selection of patients according to age and individual cardiovascular risk factors, and through careful dose adaptation over time.

### Disclosures

GG has received honoraria from Novartis and Incyte; FC has received honoraria from Novartis, Celgene, Pfizer, and In-

cyte; GR has received honoraria from Novartis, Celgene, Pfizer, and Incyte; the remaining authors declared no competing financial interests.

### Contributions

GG analyzed the data; GG and MBa wrote the first draft of the manuscript; all authors contributed to the design of the study, to the collection of the data, and to the final report.

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### Data-sharing statement

The original protocol is available upon request. Individual participant data will not be shared.

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