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Nilotinib 300 mg twice daily: an academic single-arm study of newly diagnosed chronic phase chronic myeloid leukemia patients

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ABSTRACT

The introduction and the extended clinical use of nilotinib in the first-line treatment of chronic myeloid leukemia have been based on company-sponsored trials. Independent confirmations are extremely important. We report an investigator-sponsored study of nilotinib 300 mg twice daily in 130 chronic myeloid leukemia patients in early chronic phase. A deep molecular response was achieved in 46% ($MR^{4.0}$) and 17% ($MR^{4.5}$) of patients at 2 years; 58% of the enrolled patients achieved a $MR^{4.0}$ at least once, with a sustained $MR^{4.0}$ in 52% of them. With a median observation of 29 months (range 24-37 months), 77% of patients were still on treatment with nilotinib. The reasons for permanent discontinuation were: 3% progression, 5% failure or suboptimal response, 8% adverse events, 1% treatment-free remission, and 5% other reasons. Thirteen thrombotic arterial events were reported in 12 patients. A prospective evaluation of metabolic effects showed an increase of fasting glucose without significant variations of glycated hemoglobin, an increase of total cholesterol (both low density lipoprotein and high density lipoprotein fractions) and a decrease of triglycerides. This study confirms a high and rapid efficacy of nilotinib 300 mg twice daily and provides detailed information on the type and incidence of non-hematologic and metabolic adverse events (*clinicaltrials.gov identifier:01535391*).

Introduction

Nilotinib is a second generation BCR-ABL1 tyrosine kinase inhibitor (TKI).[1] It has been approved for the first-line treatment of newly diagnosed, chronic phase (CP) Philadelphia chromosome-positive (Ph+), BCR-ABL1-positive (BCR-ABL1+) chronic myeloid leukemia (CML), following the report of a company-sponsored phase 3 prospective randomized trial (ENESTnd) comparing nilotinib to imatinib 400 mg once daily (OD).[2] Several updates of the study, over 6 years, have confirmed the initial findings that nilotinib was superior to imatinib for any degree of molecular response, and for the rapidity of the response.[3,4] The progression-free survival (PFS) was reported to be marginally improved and no difference in overall survival (OS) was detectable.[3,4] In the ENESTnd trial, two different nilotinib doses were tested, namely 300 mg twice daily (TD) and 400 mg TD.[2-4] The 300 mg TD dose was selected for approval because it was reported to be as effective as, but less toxic than, the 400 mg TD dose. A relevant issue was the cardiovascular toxicity, with focus on arterial occlusive events (ischemic heart disease (IHD), peripheral arterial occlusive disease (PAOD) and ischemic cerebrovascular events, ICVEs), that at 5 years were reported in 2.1% of patients in the imatinib arm, in 6.8% of patients in the nilotinib 300 mg TD arm, and in 12.6% of patients in the nilotinib 400 mg TD arm.[3]

This concern was raised and reinforced by other independent studies, for the most part retrospective and mainly in second-line treatment, reporting a significant incidence of cardio-vascular adverse events (CVAEs) during nilotinib treatment.[5-9] With a minimum observation of 24 months, the molecular response rates in a second single-arm company-sponsored study of nilotinib 300 mg TD, the ENEST1st trial, were even higher compared to the ENESTnd results, with consistent safety data.[10] There are no independent, investigator-sponsored studies of the drug in first-line treatment, with the exception of the two small pilot studies that were performed prior to the approval of nilotinib in first-line treatment, at the dose registered for second-line treatment (400 mg TD).[11-14] When the 300 mg TD dose became the standard in first-line therapy, the GIMEMA CML Working Party designed a prospective phase 3b single-arm trial of nilotinib 300 mg TD, to independently assess the efficacy and safety. Since all patients have now been followed for a minimum of two years, we herein report the results of the main analysis of response and adverse events (AEs).

Methods

A phase 3b single-arm study of nilotinib, 300 mg TD was conducted in adult patients with newly diagnosed CP BCR-ABL1+ CML (*clinicaltrials.gov identifier:01535391*). A dose increase to 400 mg TD was scheduled for suboptimal response or failure (2009 European LeukemiaNet, ELN, criteria),[15] excluding disease progression, in the absence of toxicity or relevant *BCR-ABL1* mutations. Pre-treatment with imatinib for up to 30 days was permitted. The primary endpoint was the rate of MR[4.0] at 24 months. The secondary endpoints are detailed in the *Online Supplementary Table S1*. The study was reviewed and approved by the Internal Review Board of all the participating institutions. The cut-off date for this analysis was June 30, 2015.

The detection of a Ph chromosome and/or a *BCR-ABL1* fusion

Table 1. Patient characteristics at diagnosis.

Patients, N	130
Age, years; median (range)	50 (18 - 85)
Age ≥ 65 years, N (%)	25 (19)
Sex, male, N (%)	84 (65)
ECOG ≥ 1, N (%)	21 (16)
Hb level, g/dL; median (range)	12.0 (7.6 - 16.3)
Platelet count, 10^3/L; median (range)	396 (101 - 4093)
WBC count, 10^3/L; median (range)	41.5 (1.8 – 363.2)
Blast cells in PB, %; median (range)	1.0 (0 – 14.0)
Eosinophils in PB, %; median (range)	2.0 (0 – 10.0)
Basophils in PB, %; median (range)	2.2 (0 – 13.0)
Spleen, cm; median (range)	1 (0 - 26)
Palpable spleen, N (%)	67 (52)
Sokal score, N (%):	
Low	56 (43)
Intermediate	47 (36)
High	27 (21)
Euro score, N (%):	
Low	61 (47)
Intermediate	62 (48)
High	7 (5)
EUTOS score, N (%):	
Low	120 (92)
High	10 (8)
CCA/Ph+, present, N (%)	4 (3)
Variant translocations, present, N (%)	8 (6)
Pre-treatment, N (%):	
Imatinib	7 (5)
Hydroxyurea	69 (53)
Anagrelide	1 (1)

ECOG: performance status according to the Eastern Co-operative Oncology Group grading; Hb: hemoglobin; WBC: white blood cells; PB: peripheral blood; EUTOS: European Treatment and Outcome Study; CCA/Ph+ : clonal chromosome abnormalities in Ph-positive cells.

gene associated with consistent morphologic features were required to confirm the CML diagnosis and the chronic (CP), accelerated (AP) or blast disease phase (BP) were defined according to current ELN criteria.[16] Risk scores were calculated according to Sokal,[17] Euro,[18] and EUTOS[19] formulations. The molecular response (MR) was assessed by peripheral blood RT-PCR, according to the International Scale (IS).[20] Definitions: early molecular response (EMR), BCR-ABL1 transcript ≤ 10% at 3 months; major molecular response (MMR or MR[3.0]), BCR-ABL1 transcript ≤ 0.1%; MR[4.0] and MR[4.5], BCR-ABL1 transcript ≤ 0.01%, and ≤ 0.0032%, respectively, in samples with > 10,000, and > 32,000 ABL1 copies, respectively;[21,22] sustained MR[4.0] or MR[4.5], stable response for > 1 year with > 3 evaluable tests. Molecular tests were performed every 3 months. The cytogenetic response was assessed by chromosome banding analysis at 3, 6 and 12 months; if there were < 20 available metaphases, a fluorescence *in situ* hybridization (FISH) analysis on peripheral blood cells was accepted (complete cytogenetic response, CCyR, ≤1% of BCR-ABL1 positive nuclei, > 200 nuclei analyzed).[16] Mutational analysis of BCR-ABL1 kinase domain point mutations (Sanger Sequencing) was performed in case of progression, failure or suboptimal response.[15,23] OS, PFS, and event-free survival (EFS, or survival without treatment discontinuation) were calculated from treatment start until death (OS), until death or progression to AP or BP

(PFS), or until death, progression to AP or BP, failure on nilotinib or nilotinib treatment discontinuation for any cause, except treatment-free remission (EFS), respectively. Probabilities of OS, PFS and EFS were calculated using the Kaplan-Meier method.[24] Patients who discontinued nilotinib and patients who underwent allogeneic stem cell transplantation were not censored for OS and PFS.

The rates of molecular and cytogenetic response "at" milestones were calculated by dividing the number of responders at that timepoint by the number of all enrolled patients. The time to response was calculated from treatment start until the first achievement of the response. The cumulative probability of response was calculated taking into consideration the presence of competing risks (failure, progression or death).[25,26]

The AEs were graded according to the NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0). Lipid modifications were graded according to adapted American Association of Clinical Endocrinologists (AACE) criteria,[27] and glucose abnormalities according to adapted American Diabetes Association (ADA) criteria.[28]

Results

Baseline characteristics

One hundred and thirty patients were enrolled between December 2011 and November 2012 at 32 GIMEMA Clinical Centers. The baseline characteristics of the patients are shown in Table 1. The median age was 50 years. Sixty-five percent were males. High-risk patients were 21% by Sokal, 5% by EURO, and 8% by EUTOS scores. Clonal chromosome abnormalities in Ph+ cells were reported in 3% of patients.

Patient disposition

The median follow-up was 29 months (range 24-37 months). The patient disposition at the last contact is shown in Table 2. Overall, 77% of patients were still on nilotinib (80% at 2 years), mainly at the initial 300 mg TD dose. In 6 patients the nilotinib dose was escalated to 400 mg TD due to the absence of an optimal response: 4 patients are still on treatment, 2 of them with an optimal response and 2 without significant improvements, while 2 patients definitively discontinued nilotinib as a result of treatment failure. Twenty-eight patients permanently discontinued nilotinib, of whom 11 (8%) were due to failure. Four of these patients progressed to AP or BP after 1, 4, 6, and 19 months, respectively; all these patients were alive at last contact. One of these patients, a 41 year old with an

intermediate Sokal score, developed a lymphoid blast crisis with *T315I* mutation and was treated with conventional chemotherapy followed by allogeneic stem cell transplantation (alloSCT). A second patient, a 46 year old with an intermediate Sokal score, progressed to AP with *V280A* mutation and was treated with dasatinib. The third of these patients, a 32 year old with a low Sokal score, progressed to AP with clonal evolution and myelodysplastic features, without *BCR-ABL1* mutations, and was submitted to alloSCT. The fourth and final patient, a 43 year old with a low Sokal score, progressed to a myeloid blast phase without *BCR-ABL1* mutations and is on second-line treatment with dasatinib (*Online Supplementary Table S2*). Eleven patients (8%) discontinued nilotinib for toxicity, one patient (1%) decided to discontinue the treatment because of stable deep molecular response, achieving a treatment-free remission (duration of nilotinib treatment and duration of stable MR[4.0] before discontinuation, 35 and 23 months, respectively), and 5 patients (4%) discontinued for other reasons, including withdrawal of informed consent and pregnancy. Two patients (2%) were lost to follow-up. Overall, 3

Figure 1. Cumulative incidence of deep molecular response. (A) MR[4.0]: the 2-year estimated cumulative incidence of MR[4.0] was 51% (95% CI, 42-60%), B) MR[4.5]: the 2-year estimated cumulative incidence of MR[4.5] was 24% (95% CI, 16-31%). The cumulative probability of achieving a response was calculated under consideration of competing risks, defined as failure followed by treatment change, progression or death.

Table 2. Patient disposition at last contact.

Patients, N	130	
Still on nilotinib, N (%)	100	(77%)
600 mg	89	(68%)
< 600 mg	7	(5%)
800 mg	4	(3%)
Discontinued nilotinib, N (%)	28	(22%)
Progression to advanced phase	4	(3%)
Failure or suboptimal response	7	(5%)
Adverse events	11	(8%)
Treatment-free remission	1	(1%)
Other causes[a]	5	(4%)
Lost to follow-up, N (%)	2	(2%)

[a]*details of other causes: pregnancy, 2 patients, withdrawal of informed consent, 1 patient, low compliance, 1 patient, alcohol abuse, 1 patient.*
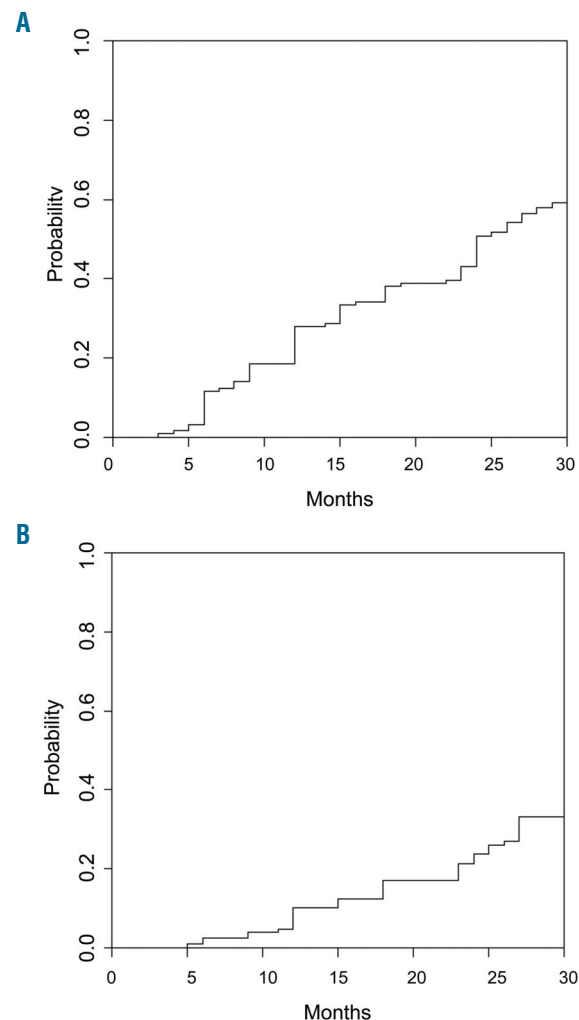
patients underwent alloSCT, 2 after transformation to AP (previously described) and 1 after resistance to all TKIs, including ponatinib, without progression and without detectable *BCR-ABL1* mutations.

Responses and outcome

According to the intention-to-treat (ITT) principle, 70% of patients were in CCyR at 6 months, and 77% at 12 months. The 3-month EMR was achieved in 80% of patients (ITT). The rates of MMR, MR[4.0], and MR[4.5] at 3, 6, 12, 18, and 24 months are shown in Table 3. At 24 months, according to the ITT principle, 65% of patients were in MMR, 46% were in MR[4.0], and 17% were in MR[4.5]. Overall, 75/130 patients (58%) achieved a MR[4.0] at least once and 39/75 (52%) achieved a sustained MR[4.0]; 40/130 patients achieved a MR[4.5] and 11/40 (28%) a sustained MR[4.5]. The cumulative probability of achieving MR[4.0] and MR[4.5] is shown in Figure 1; after two years of treatment the probabilities were 51% (95% CI, 42-60%) and 24% (95% CI, 16-31%), respectively. The duration of observation is still too short for a detailed analysis of the stability of deep molecular response.

Only 1 patient died, due to the worsening of a pre-existing comorbidity (chronic obstructive pulmonary disease: a worsening of general clinical conditions, followed by a switch to imatinib with a stable response, was observed). As shown in Figure 2, the 30-month OS was 99% (95% CI 91-100%), the 30-month PFS was 96% (95% CI 89-98%) and the 30-month EFS (survival without treatment discontinuation, except for treatment-free remission) was 76% (95% CI 67-83%).

Safety

A grade 3-4 thrombocytopenia and neutropenia were recorded in 8% and 9% of patients. Hematologic AEs caused early permanent treatment discontinuation in 2

patients. Twenty-five AEs listed under a comprehensive definition of cardiovascular AEs (CVAEs) were reported in 21 patients (16%): arterial thrombosis, 13 events (Table 4); venous thrombosis, 4 events (1 deep venous thrombosis, 3 superficial thrombophlebitis); QTc prolongation, 3 events (only 1 case > 500 msec); arrhythmias, 3 events (2 atrial fibrillations, 1 atrial-ventricular blockade); congestive heart failure, 1 event; acute pericarditis, 1 event. They were reported as a grade 3 event in nine cases, and as a grade 4 event in one case. The treatment was temporarily discontinued for 9 events in 8 patients (36% of CVAEs, 6% of all patients), and it was permanently discontinued for 8 events (32% of CVAEs, 6% of all patients). A medical treatment without hospitalization was administered for 13 events; hospitalization was required for 8 events. Major surgery was performed in 2 patients: substitution of the femoral head and amputation of the right lower limb, respectively. One case of myocardial infarction was treated with coronary stents. No patient died of toxicity. The characteristics of patients with (N = 12) or without (N = 118) arterial thrombotic events are shown in Table 5. The patients with high or very high cardiovascular risk according to the European Guidelines on cardiovascular disease prevention in clinical practice,[29] retrospectively assessed, had a significantly higher probability of arterial thrombotic events (*Online Supplementary Figure S1*). Among the other non-hematologic and non-cardiovascular AEs, only fatigue (17%

Table 3. Molecular response at milestones.

BCR-ABL1[IS]	≤ 0.1%	≤ 0.01%	≤ 0.0032%
3 months	23%	2%	0
6 months	53%	12%	2%
12 months	57%	28%	7%
18 months	63%	31%	11%
24 months	65%	46%	17%

'BCR-ABL1[IS]': BCR-ABL/ABL% ratio, according to the International Scale. The response rates were calculated by dividing the number of patients with that response at that time by the total number of enrolled patients (N=130).
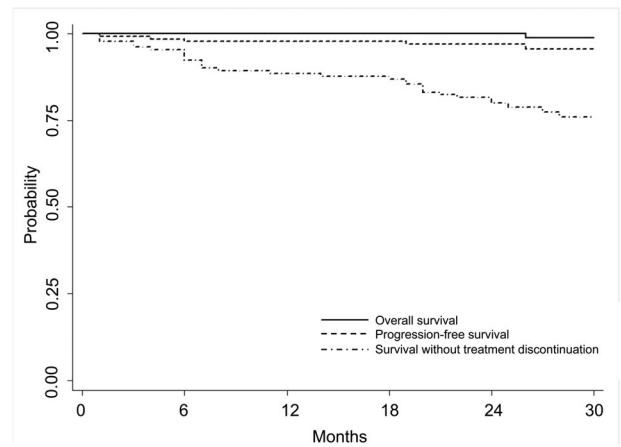

Figure 2. Outcome of all the 130 enrolled patients. The estimated 30-month overall survival was 99% (95% CI, 91-100%), the 30-month estimated progression-free survival was 96% (95% CI, 89-98%), the estimated 30-month survival without treatment discontinuation was 76% (95% CI, 67-83%).

Table 4. List of arterial thrombotic adverse events by year, all grades, irrespective of causality.

Type of event	1st year*	2nd year*	3rd year*	Total	Permanent treatment discontinuation	Temporary treatment discontinuation
Lower limbs[a]	1	1	1	3	1	1
Coronary[b]	2	2	2	6	4	1
Carotid[c]	1	0	0	1	0	1
Optic	0	1	0	1	0	0
Avascular necrosis of the femoral head	1	1	0	2	1	0
Total	5	5	3	13[+]	6	3

*Duration of nilotinib treatment: ≤12 months, 130 patients; 13-24 months; 115 patients; > 24 months, 67 patients; a Amputation, 1 case; b Myocardial infarction, 1 case, stable or unstable angina, 4 cases; troponin increase after atrial fibrillation, 1 case; c Transient ischemic attack, 1 case; + The 13 events were observed in 12 patients (one patient had two events): the number of patients remaining on nilotinib was 6 (three patients assuming 600 mg daily, three patients assuming 300 mg daily).

Table 5. Characteristics of patients with and without arterial thrombotic adverse events.

Variables	Patients WITH events N = 12	Patients WITHOUT events N = 118	P
At baseline			
Age, years, median (range)	62 (49-85)	47 (18-81)	0.002
Sex, male, N (%)	7 (58)	77 (65)	0.753
Body mass index, N (%)			
< 25	6 (50)	69 (58)	0.162
25-29.9	3 (25)	35 (30)	
30-34.9	2 (17)	10 (8)	
≥ 35	1 (8)	4 (3)	
ECOG ≥ 1, N (%)	4 (33)	17 (14)	0.104
Smokers, N (%)	4 (33)	22 (19)	0.257
Arterial hypertension, N (%)	4 (33)	24 (20)	0.287
Diabetes, N (%)	3 (25)	9 (8)	0.082
HbA1c, %, median (range)	6.2 (4.7-7.0)	5.9 (3.6-8.7)	0.809
Total cholesterol, mg/dL, median (range)	170 (126-315)	168 (81-260)	0.425
LDL, mg/dL, median (range)	111 (57-211)	103 (36-186)	0.692
HDL, mg/dL, median (range)	36 (15-75)	36 (11-79)	0.435
High or very high EURO CV score, N (%)	9 (75)	28 (24)	< 0.001
On treatment[a]			
HbA1c, %, median (range)	6.4 (5.2-8.2)[b]	5.6 (3.8-8.7)[c]	0.023
Total cholesterol, mg/dL, median (range)	206 (140-272)	210 (107-328)	0.754
LDL, mg/dL, median (range)	128 (55-183)	133 (42-215)	0.598
HDL, mg/dL, median (range)	49 (29-90)	55 (10-103)	0.414

ECOG: performance status according to the Eastern Cooperative Oncology Group grading; HbA1c: glycated hemoglobin; EURO CV score: cardiovascular risk according to the European Guidelines on cardiovascular disease prevention in clinical practice, version 2012 (31). LDL: low density lipoprotein; HDL: high density lipoprotein. [a]Data at 6 months (the results do not change considering other milestones, e.g. 3 months or 12 months); [b]66% of patients with HbA1c > 6.5% at 6 months were diabetic at baseline; [c]50% of patients with HbA1c > 6.5% at 6 months were diabetic at baseline.

grade 1-2 and 1% grade 3), bone and muscle and joint pain (22% grade 1-2 and 1% grade 3), and skin rash (29% grade 1-2 and 1% grade 3) were reported in more than 10% of patients. Grade 3-4 laboratory abnormalities were as follows: grade 3 transaminase increase 2% (permanent treatment discontinuation, 1 case), grade 3 bilirubin increase 5%, grade 3 and grade 4 lipase increase 12% and 2%, respectively, and grade 3 amylase increase 1% (no pancreatitis).

According to adapted ADA criteria, and considering the maximum grade reached by each patient while on study, 47%, 11%, 5% and 6% of patients experienced a grade 1 (101-125 mg/dl), grade 2 (126-150 mg/dl), grade 3 (151-200 mg/dl) and grade 4 (>200 mg/dl) hyperglycemia, respectively; 29% of patients had an increase of fasting glucose at 1 year, compared to baseline levels (P<0.001). According to adapted ADA criteria, 47% of patients had a grade 1 (5.7-6.4%), 10% a grade 2 (6.5-6.9%), 3% a grade 3 (7-7.9%) and 5% a grade 4 (≥8%) glycated hemoglobin (HbA1c), respectively, (maximum grade reached on our study); no significant increase of glycated hemoglobin has been observed from baseline. According to the AACE criteria, 40 patients (31%) experienced a borderline (200-239 mg/dL) and 54 (42%) a high-risk (≥240 mg/dL) hypercholesterolemia (maximum grade reached on our study). A significant increase of cholesterol, both in LDL and HDL fractions (P<0.001), and a significant reduction of triglycerides (P<0.001) were detected at 1 year from baseline. The serum glucose, HbA1c and lipid concentrations at each milestone are shown in Figure 3 and *Online Supplementary Table S3.*

Discussion

The introduction and the extended clinical use of second

generation TKIs is becoming a very important issue in first-line therapy for CML.[2-4,10,30-38] They offer a treatment choice that must be weighed for short- and long-term efficacy and toxicity, and for cost-efficacy.[16,39,40] The information on nilotinib is limited to data coming from two company-sponsored studies, of which one was designed to compare nilotinib and imatinib, and the other to confirm the rate of deep molecular response on nilotinib.[2-4,10] There are no data from independent, investigator-sponsored, studies. This GIMEMA trial was made possible thanks to the support of Novartis Oncology Italy, who provided the drug free of charge and a small unrestricted support. The study was designed, conducted and analyzed by GIMEMA, so that this is the first study providing company-independent data on the treatment of newly diagnosed CP CML patients with nilotinib 300 mg TD. The study has some strengths and some limitations. The strengths are the independence and the involvement of several clinical centers all over Italy. The major limitation is the short time of observation, with a minimum of 2 years, a median of 2.5 years, and a maximum of 3 years. The ENESTnd and the ENEST1st trials were reported with a minimum observation period of 6 and 2 years, respectively.[4,10]

Two major issues are important in the treatment of CML. One issue is the rapidity[31,41-44] and the depth[45] of the molecular response. We found that EMR was achieved less frequently than in both ENEST trials, but that MMR (MR[3.0]), MR[4.0] and MR[4.5] rates were in the same range of ENEST1st, and even higher than in ENESTnd (*Online Supplementary Table S4*).[2-4,10] The significance of these differences cannot be assessed because the three studies are different, with different age distribution and proportion of high-risk (Sokal) patients (28% in ENESTnd, 18% in ENEST1st, 21% in this study), with different enrolment criteria and different guidelines for dose reduction or treatment discontinuation.

It should be noted that the molecular response rates have been calculated in two ways and reported as response rates "at" and "by" different time points, where the rate "at" the time point gives the actual proportion of patients who are in that response at the given point, and the rate "by" the same time point gives the cumulative proportion of patients achieving the response at least once before the given time point. The estimated cumulative incidence of response (response "by") helps to make a comparison between two treatments, as in the ENESTnd study,[2-4] but overestimates the actual proportion (response "at") of the patients who are in that response at that time, which is important for clinical decisions. The response rates "at" each time point are frequently lower than the values "by" the same time points.

The second major issue is the so-called cardiovascular toxicity, that includes different events, with different physiopathologic mechanisms and different clinical relevance: myocardial infarction, atrial fibrillation, superficial throm-bophlebitis, arterial thrombosis, congestive heart failure, stroke, and many others. The incidence, the severity and the consequences of these complications are difficult to assess and to compare, because they may depend on different variables, including not only the patients characteristics, the baseline cardiovascular risk, the prior treatments and the drug dose,[46-48] but also the definition of the events, and, importantly, the accuracy of the event reports, that depends on the retrospective or prospective nature of the data collection. When the GIMEMA study was designed, the cardiovascular toxicity was not yet pointed out, apart from QTc prolongation, and the cardiovascular risk at baseline was not routinely assessed. However, the cardiovascular toxicity was revealed as soon as patient enrolment began, so that the identification and the reporting of CVAEs, in facts, became prospective. However, monitoring, prophylaxis, and the treatment of CVAEs were left to local investigators, because it was not possible to provide guidelines. Several reports indicated a possible metabolic non-target effect of
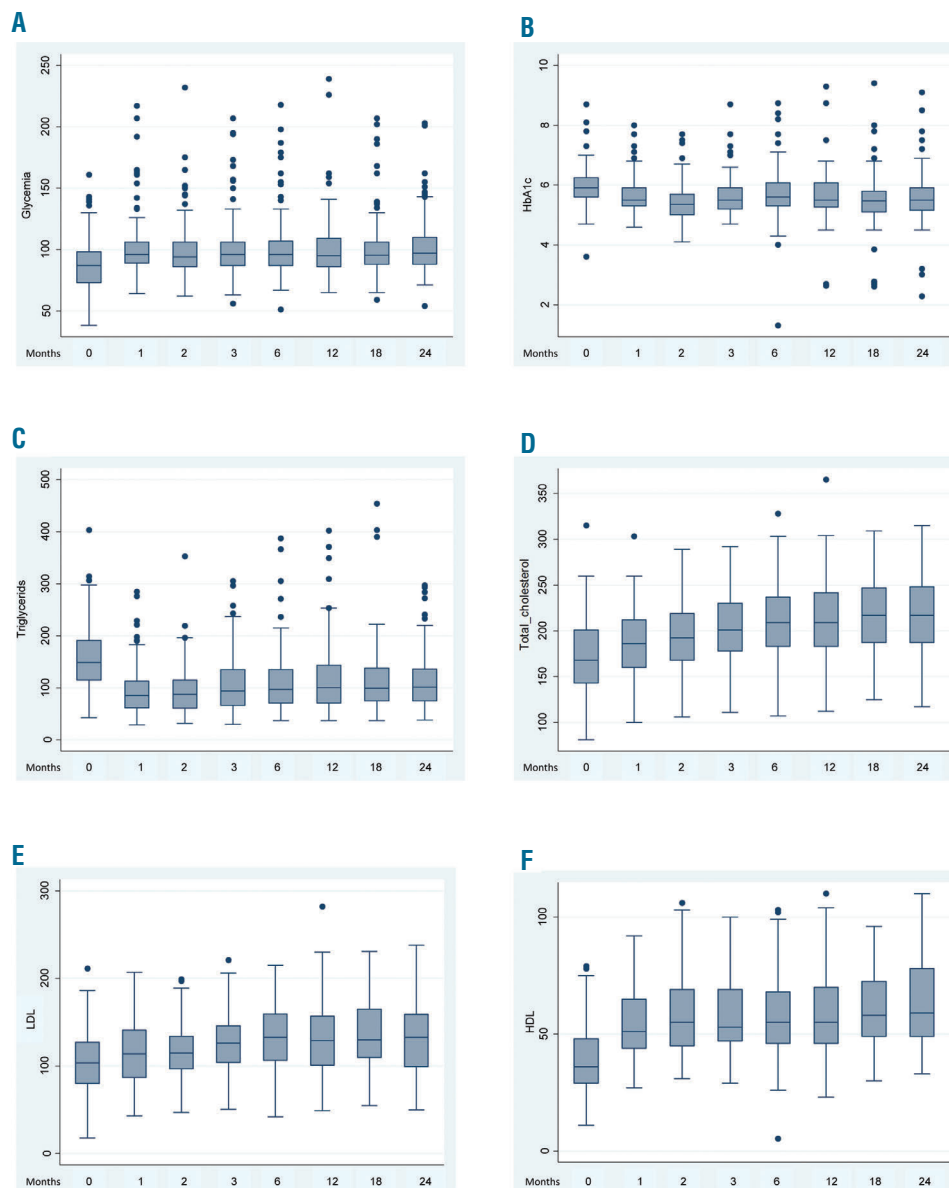

Figure 3. Metabolic effects of nilotinib by time. (A) Distribution of fasting glucose at milestones; (B) Distribution of glycated hemoglobin at milestones; (C) Distribution of triglycerides at milestones; (D) Distribution of total cholesterol at milestones; (E) Distribution of low density lipoprotein at milestones; (F) Distribution of high density lipoprotein at milestones.

nilotinib, potentially related to CVAEs; importantly, in our study, fasting glucose, glycated hemoglobin and serum lipids were prospectively assessed. Moreover, to evaluate the clinical impact of metabolic effects, we decided to classify the abnormalities according to specific criteria, as recommended by ADA and AACE guidelines.[27,28]

With these caveats, it is possible to describe, but not to compare, the 2-year incidence of major CVAEs in the present trial and in the ENEST1st one:[10] ischemic heart events were 4.6% and 3.4% respectively; arterial thrombotic events were 4.6% (including one case of retinal artery occlusion and two cases of avascular necrosis of the femoral head) and 1.9%, respectively; arrhythmias were 2.3% and 0.7%, respectively; congestive heart failure was reported in 0.8% and 0.3%, respectively. The incidence of QTc prolongation (2.3% in the GIMEMA study) and of venous thrombosis (3.1%) was not reported in the ENEST1[st] study. With a median follow-up of 29 months, the number of reported arterial thrombotic events was higher during the first and the second year, if compared to the third year of treatment, but considering the number of patients on nilotinib treatment, the yearly incidence was comparable (Table 4). A linear increase of the cumulative incidence of CVAEs over time was also described in the 5-year update of the ENESTnd trial; the yearly incidence of CVAE continues unabated after 5 years and may even increase.[3] In our study the cumulative probability of developing a CVAE was 7 % (95% CI, 4-13%) at 1 year and 13 % (95% CI, 8-20%) at 2 years (*data not shown*). We confirmed a significant increase of fasting glucose level during nilotinib treatment, but we were not able to demonstrate any significant increase of glycated hemoglobin. Similar results were reported in the ENESTnd trial, in a sub-analysis including only patients with normal glycemic status at baseline:[49] the majority of patients with hyperglycemia did not meet the glycated hemoglobin criteria for diabetes. The patients with arterial thrombotic events had a higher incidence of cardiovascular risk factors at baseline (in particular, a higher incidence of diabetes was observed), and were elderly (Table 5, *Online Supplementary Figure S1*). The exact pathogenesis of fasting glucose alterations during nilotinib is still controversial and probably related to insulin resistance: both in the ENESTnd study[49] and in the ENIGMA 2 study[50] an increase in insulin production and a decrease of fasting C-peptide were observed, with increased levels of HOMA-IR and HOMA-β values.

In conclusion, this independent study highlights the therapeutic efficacy of nilotinib, confirming the rates, the velocity, and the depth of molecular response; moreover, it confirms that the risk of cardiovascular toxicity, including several different events, is higher in patients with high cardiovascular risk, requiring specific measures of prophylaxis and monitoring.

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