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Updated review of nilotinib as frontline treatment for newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia

Nilotinib, a second-generation tyrosine kinase, is approved for the treatment of newly diagnosed chronic phase chronic myeloid leukemia patients. The drug showed impressive rates of cytogenetic and molecular responses with only few cases of disease progression in Phase II trials. Five years follow-up of Phase III randomized ENESTnd study demonstrated deep molecular responses, lower rate of transformation in the core treatment compared with imatinib (0.7% for nilotinib 300 mg twice daily [BID], 1.1% for nilotinib 400 mg BID and 4.2% with imatinib). ENEST1st study confirmed the activity of nilotinib in first line at the dose of 300 mg BID. Aim of this review is to provide reported evidences on the efficacy and safety of nilotinib when used for the management of early chronic phase chronic myeloid leukemia patients and to discuss about future possible discontinuation.

Keywords: chronic myeloid leukemia • chronic phase • imatinib • major molecular response • nilotinib • treatment-free remission

The treatment of chronic myeloid leukemia (CML) is dramatically changed in the last years due to the availability of tyrosine kinase inhibitors (TKIs), which act on the constitutively active protein tyrosine kinase, *BCR-ABL1*, the hallmark of this disease derived by the translocation $t(9;22)$ [1–5]. The first drug, imatinib mesylate, an inhibitor of *ABL1* and its derivative *BCR-ABL1*, as well as other tyrosine kinases provide an effective and durable therapy for CML: an 8-year follow-up of Phase III International Randomized IFN versus STI571 (IRIS) study showed complete cytogenetic response (CCyR) rate in about 87% of patients [6]. However, at the last follow-up reported, about 37% of patients did not have a long-term good outcome: 15% of patients due to acquired secondary resistance and 17% for primary resistance [7]. Different mechanisms of resistance have been identified, including gene amplification of *BCR-ABL1* transcript, decreased intracellular drug concentrations caused by drug efflux proteins (such as P-glycoprotein, Pgp) overexpression, or reduced

receptor-mediated uptake (such as OCT-1), clonal evolution, and overexpression of Src kinases involved in *BCR-ABL1*-independent activation of alternative pathways, such as Lyn and Hck [8–12]. In particular, primary or acquired resistance to imatinib is attributable to the onset of mutations in 40% of patients: amino acid substitutions in the *ABL1*-kinase domain impair the capacity of the drug to bind the critical contact point, for example, by inducing a switch from the inactive to the active conformation [13–19]. New drugs, such as nilotinib [20], dasatinib [21], bosutinib and ponatinib, have been tested in patients resistant and/or intolerant to imatinib. After the brilliant results as second-line treatment, efficacy of second-generation TKIs in newly diagnosed patients was reported, initially in Phase II trials as single arm dasatinib [22] or nilotinib [23,24]. Then, randomized Phase III trials [25,26] tested the efficacy of nilotinib or dasatinib versus imatinib and allowed the approval of those drugs for the treatment of newly diagnosed CML patients. Aim of present review is to report recent clinical

Massimo Breccia^{*1}, Matteo Molica¹ & Giuliana Alimena¹

¹Department of Cellular Biotechnologies & Hematology, Sapienza University, Rome, Italy

*Author for correspondence:

Tel.: +39 06 857 951

Fax: +39 06 4424 1984

breccia@bce.uniroma1.it

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evidences regarding efficacy and safety of nilotinib in newly diagnosed chronic phase (CP)-CML patients, with a focus on the new endpoint, the so-called treatment-free remission (TFR).

Long-term follow-up of Phase II trials (GIMEMA & MDACC experience) in newly diagnosed CML patients

The Italian GIMEMA CML Working Party tested nilotinib, at the dose of 400 mg twice daily (BID), as first-line treatment. The primary endpoint was the achievement of CCyR at 1 year; 73 patients were enrolled and the last median follow-up presented was at 45 months. Stratification at baseline by Sokal risk identified 45% of patients as low, 41% as intermediate and 14% as high risk. At 1 year, the cumulative CCyR rate was 100%. The cumulative rate of MMR was 96%, while the rates of MMR at 3, 6, 12, 18, 24, 30 and 45 months were 52, 66, 85, 81, 82, 71 and 97%, respectively. The cumulative incidence of MR4 (tested at least once) was 82%, while its confirmed rate was 29%. None of the patients who achieved an MMR progressed to accelerate phase/blast crisis (AP/BC). Only one patient progressed at 6 months to AP/BC: a 63-year-old female with a high Sokal risk disease in CCyR at 3 months, who developed a T315I mutation. Most of side effects reported were grade 1 or 2, manageable with temporarily dose reduction. Only two patients (3%) experienced a QTc prolongation above 450 ms, but none above 500 ms. Definitive discontinuation were recorded in four patients: three patients for recurrent episodes of amylase and/or lipase increase without evidences of pancreatitis and one patient discontinued due to atrial fibrillation. At 45 months the overall survival (OS) and failure-free survival are 97% and event-free survival (EFS) is 91% [27,28]. MD Anderson Cancer Center (MDACC) conducted an experience in newly diagnosed untreated CP-CML patients or previously treated for less than 1 month with imatinib and in a cohort of patients with previously untreated CML in AP: the primary endpoint was the achievement of MR3 at 12 months [23]. The results presented at last follow-up of 4 years showed that of 100 patients enrolled, 77% were still in study. Only two cases of progression were reported. Overall, 100% of patients obtained complete hematologic remission (CHR) in a median time of 3 weeks. The incidence of CCyR was 93% at a median time of 3 months. The cumulative incidence of MR3 was 73% (in intention to treat [ITT], 70%) and of complete molecular remission (CMR) 33% (in ITT, 32%). High rates of responses were recorded also in high Sokal risk patients. Hematological grade 3/4 adverse events were represented by anemia in 5%, neutrope-

nia in 11% and thrombocytopenia in 11% of patients, whereas grade 3/4 nonhematological events included skin rash and fatigue. Laboratory events included elevation of transaminases in 13% of patients, elevated bilirubin in 8% and elevated lipases in 6%. OS was 96% and EFS was 91% at 4 years. Transformation-free survival and failure-free survival were 97 and 78%, respectively (Tables 1 & 2; [29]).

ENESTnd study: follow-up at 5 years

The ENESTnd trial is a Phase III, international, randomized study that demonstrated the superior efficacy of nilotinib over imatinib. A recent update with 5-year follow-up was presented at last 2014 European hematology association (EHA) meeting [30]. Eight hundred and forty-six CP patients were randomized to nilotinib 300 mg BID (n = 282), nilotinib 400 mg BID (n = 281) and imatinib 400 mg once daily (n = 283). Patients were randomized according to Sokal score. Primary endpoint was the achievement of MMR ($\leq 0.1\%$ BCR-ABL^{IS}) rate at 12 months, whereas key secondary endpoints were the duration of MMR, time to MMR and CCyR, progression to AP/BC (with and without clonal evolution), EFS, progression-free survival (PFS) and OS. A recent amendment extended the follow-up at 10 years. Patients within 6 months from diagnosis were enrolled; conservative treatment with hydroxyurea or anagrelide and less than 2 weeks of imatinib was allowed. At a median follow-up of 5 years, 59.9% of patients in the nilotinib 300 mg BID arm, 61–9% in the nilotinib 400 mg BID arm and 49.8% of patients treated with imatinib remained in the core treatment. The rate of progression on treatment to advanced phases of disease was also significantly lower for nilotinib compared with imatinib: it was 0.7% for nilotinib 300 mg BID (p = 0.003), 1.1% for nilotinib 400 mg BID (p = 0.008) and 4.2% for imatinib. Intention-to-treat analysis including patients who discontinued treatment, but remained on study, showed a progression rate of 3.5, 2.1 and 7.4%, respectively. All the few events that occurred in the last years of follow-up were in high Sokal risk patients and all patients had a ratio >10% at 3 months of therapy. In case of suboptimal response or treatment failure, dose escalation was allowed only for imatinib (to 800 mg) but not for nilotinib. At a median follow-up of 5 years, the cumulative incidence of MR3 was superior for nilotinib 300 mg BID (77%) and nilotinib 400 mg BID (77%) compared with imatinib (60%). Cumulative incidence of deep molecular response (MR4.5) again was higher for nilotinib 300 mg BID (54%) and nilotinib 400 mg BID (52%) compared with imatinib (31%). Rates of MR4.5 were consistently higher in patients treated with nilotinib across all Sokal

Table 1. Long-term follow-up of Phase II and III trials in newly diagnosed chronic myeloid leukemia patients treated with nilotinib.

Reference	CML phase	Number of patients	Response rate (%)			Ref.
			CCyR	MR3	MR4	
MDACC [†]	Early CP and AP	100	100	95	66 [†]	[29]
GIMEMA [‡]	Early CP	73	92	82	25 [‡]	[28]
ENESTnd [§]	Early CP	Nilotinib 300 mg BID (282)	87	77	54 [§]	[30]
		Nilotinib 400 mg BID (281)	85	77	52 [§]	
		Imatinib 400 mg (283)	77	60	31 [§]	
ENEST1st	Early CP	Nilotinib 300 mg BID	88	63.5	36.3 [¶]	[31]

[†]In best response at 4 years follow-up. CMR was considered as ratio <0.0032%. Nilotinib was used at 400 mg BID.
[‡]In ITT analysis beyond 30 months follow-up. MR4 was defined as a *BCR-ABL/ABL* ratio <0.01% IS. Nilotinib was used at 400 mg BID.
[§]In ITT analysis on the whole population on core treatment at 5 years follow-up. MR4.5 was defined as ratio <0.032% IS.
[¶]In ITT analysis.
 BID: Twice daily; CCyR: Complete cytogenetic response; CML: Chronic myeloid leukemia; CP: Chronic phase; ITT: Intention to treat.

risks. According to BCR/ABL ratio at 3 months, low Sokal risk patients that did not achieve the endpoint of an early molecular response (EMR, ratio <10% at 3 months of therapy) were 7.2% with nilotinib compared with 20.6% with imatinib; intermediate risk patients were 7.7% with nilotinib 300 mg BID compared with 30.4% with imatinib and high Sokal risk patients were 14.3% with nilotinib 300 mg BID compared with 55.7% with imatinib. A pooled landmark analysis presented that stratifying patients according to Sokal risk and molecular response obtained at 3 months showed that in terms of long-term outcome, patients with low Sokal risk not achieving a BCR-ABL ratio <10% at 3 months did not have worse outcome, with PFS and OS being similar to those of patients achieving EMR after 3 months. Indeed, a difference in long-term outcome at 5 years with worse PFS and OS was shown for patients with intermediate and high Sokal risk not achieving EMR. Estimated OS rate (including data from follow-up after discontinuation) at 5 years was higher for nilotinib 300 mg BID

(93.7%, $p = 0.64$) and nilotinib 400 mg BID (96.2%, $p = 0.21$) compared with imatinib (91.7%). Taken into account only deaths related to CML, a significant difference was reported for nilotinib (97.7%) compared with imatinib (93.8%). Grade 3/4 myelosuppression registered as anemia was 4, 5 and 6% respectively for the three arms. Grade 3/4 neutropenia was 12, 11 and 22%, respectively and thrombocytopenia was 10, 12 and 9% for nilotinib 300 mg BID, nilotinib 400 mg BID and imatinib, respectively. Grade 3/4 laboratory abnormalities were lower for nilotinib 300 mg BID (9% lipase elevations, 4% total bilirubin elevations and 7% glucose elevation) compared with nilotinib 400 mg BID (10% lipase, 9% total bilirubin and 7% glucose elevation) and imatinib (4% lipase and alanine transaminase [ALT], <1% total bilirubin and <1% glucose elevation). Glucose elevation of any grade was reported in 50, 53 and 31% of patients in the nilotinib 300 mg BID, nilotinib 400 mg BID and imatinib arms, respectively. Total cholesterol elevations were reported in 28, 27 and 4% of patients in the nilotinib

Table 2. Long-term outcomes reported in chronic myeloid leukemia patient treated with nilotinib upfront.

Reference	Number of patients	Outcome (%)			Ref.
		OS	PFS	EFS	
MDACC (at 4 years)	100	96	98	95	[29]
GIMEMA (after 36 months)	73	97	97	91	[28]
ENESTnd (at 5 years)	Nilotinib 300 mg BID (282)	93.7	98	Nr	[30]
	Nilotinib 400 mg BID (281)	96.2	97.7	Nr	
	Imatinib 400 mg (283)	91.7	95.2	Nr	
ENEST1st (at 2 years)	Nilotinib 300 mg BID	98.5	99	NR	[31]

BID: Twice daily; EFS: Event-free survival; OS: Overall survival; PFS: Progression-free survival.

300 mg BID, nilotinib 400 mg BID and imatinib arms, respectively (Table 3). Seventeen patients with a normal glycemic status at baseline became diabetic at the last follow-up of 5 years in both nilotinib arms. With longer follow-up there was minimal change in the occurrence of nonhematological grade 3/4 adverse events registered, the most common being nausea, rash and headache with nilotinib (Table 1 & Table 2; [30]).

ENEST1st: an interim analysis

ENEST1st was a Phase III, not randomized, single arm trial that tested nilotinib at the dose of 300 mg BID in newly diagnosed CML patients in chronic phase. The primary endpoint was the achievement of MR4 at 18 months, with standardized responses evaluated in EUTOS laboratories. A total of 1086 patients were enrolled at 305 sites in 26 countries and the first interim analysis at 2 years of follow-up was presented for 820 patients. Of these, 19.6% were previously treated with imatinib but for a period of less than 1 month. For the first time in a sponsored trial, patients were classified also according to EUTOS score: 9% of patients were recognized as having a high risk, whereas 18.8% of patients were classified as having a high risk according to Sokal score. Of 820 patients, 658 remained in treatment after 2 years, the main reason for discontinuation being occurrence of adverse events. Dose interruption for a median duration of 15 days was needed in 38% of patients, whereas 46% of patients required dose reduction or temporarily discontinuation mainly for adverse events. The primary endpoint at 18 months was reached

by 36.3% of patients in ITT analysis with 34.6% of patients by 24 months. As a cumulative incidence at 24 months, 80% of patients achieved an MR3, 55% an MR4 and 39% of patients an MR4.5. Cumulative incidence of MR4 was different according to EUTOS score: 57% of the low-risk and 38% of the high-risk patients. Cumulative incidence of CCyR after 24 months was 88%, without difference for low- or high-risk category according to EUTOS risk (89 and 85%, respectively). A total of 7 patients (0.85%) progressed to blast crisis and 12 patients died for reasons not related to CML. More frequent side effects reported were rash, pruritus and alopecia, with only low rates being grade 3/4. Neutropenia and thrombocytopenia of grade 3/4 occurred in 4.5 and 6.1% of patients, respectively. The most frequent new biochemical abnormalities occurring of grade 3/4 were lipase elevation (7.1%), total bilirubin increase (3.3%) and transaminases elevation (2.5%) (Table 1 & Table 2; [31]).

Cardiac safety profile of nilotinib as reported in sponsored trials

Even if nilotinib has the potential to prolong QTc interval, only few patients experienced significant QTc interval prolongation and prolongation >500 ms was reported at low rate [32]. In the ENESTnd trial [33], patients were excluded at baseline if they had known uncontrolled or past significant cardiac disease, left ventricular ejection fraction <45% or QTcF interval >450 ms. Prospective assessment of QTc evaluated with Fridericia formula was performed during the study:

Table 3. Safety of nilotinib versus imatinib in firstline treatment.

Side effect	Nilotinib 300 mg BID	Nilotinib 400 mg BID	Imatinib
Skin rash	38%	45%	19%
Headache	32%	36%	23%
Nausea	22%	31%	41%
Diarrhea	19%	23%	46%
Muscle spasms	12%	12%	34%
Ischemic heart disease	3.9%	8.7%	1.8%
Ischemic cerebrovascular events	1.4%	3.2%	0.4%
Peripheral artery disease	2.5%	2.5%	0
Anemia (grade 3/4)	4%	5%	6%
Thrombocytopenia (grade 3/4)	10%	12%	9%
Neutropenia (grade 3/4)	12%	11%	22%
Elevated lipase (grade 3/4)	9%	10%	4%
Elevated total bilirubin (grade 3/4)	4%	9%	<1%
Elevated glucose (grade 3/4)	7%	7%	<1%
Elevated total cholesterol (grade 3/4)	0	1%	0

BID: Twice daily.

26% of patients had QTcF increased above 30 ms from baseline in either nilotinib arms as compared with 18% of patients in imatinib arm, but increases above 60 ms were uncommon (less than 1% of patients in all arms). Between 3 and 6 months of therapy occurred the highest mean values of changes in QTcF interval: 10.4, 12.4 and 7.9 ms in nilotinib 300 mg BID, nilotinib 400 mg BID and imatinib arm, respectively. No episodes of torsade de point or sudden death or QTcF interval prolongation >500 ms in none of the 3 arms were recorded and none of the patients enrolled discontinued the treatment for QT prolongation. At the last follow-up of 5 years, cardiovascular events were recorded in 64 patients; of these, 35 patients experienced an ischemic heart disease event after a median treatment duration of 18 months: 11 patients on nilotinib 300 mg BID, 24 patients on nilotinib 400 mg BID and 5 patients on imatinib arm. Overall, 14 patients experienced an ischemic cerebrovascular events: 4 patients with nilotinib 300 mg BID, 9 with nilotinib 400 mg BID and 1 patient with imatinib [31]. Peripheral artery diseases (PAODs) were recorded in 14 patients: 7 patients with nilotinib 300 mg and 7 patients with nilotinib 400 mg BID. In the ENEST1st trial, ischemic heart disease events were registered in 31 patients (3.8%), ischemic cerebrovascular conditions in 4 patients (0.5%) and PAODs in 13 patients (1.6%) [33].

PAOD reported with nilotinib

Hypercholesterolemia was described as *in vivo* metabolic effect occurring during nilotinib treatment and was associated with the occurrence of PAOD [34]. An increased low-density lipoprotein (LDL) fraction in 13 out of 31 patients who had switched from imatinib to nilotinib for suboptimal response was also recently reported [35]. Increased cholesterol LDL level and concomitant reduction of triglycerides were also observed in a group of 27 patients treated either as first or second line with the drug [36]. In particular, an increased proportion of patients with nonoptimal LDL cholesterol level (from 48.1 to 88.9% by 12 months) were reported, leading to start of cholesterol-lowering drug in 22.2% of subjects. Indeed, the proportion of patients with low levels of high-density lipoprotein cholesterol decreased from 40.7 to 7.4% by 12 months. A significant decrease in triglycerides was also observed. The authors calculated also the global cardiovascular risk that worsened in 11.1% of patients due to diabetes or occlusive arterial events. Several observations relating PAOD were published until now, which reported on only retrospective analyses or single cases. Aichberger *et al.* [37] referred at first on three cases out of 24 patients treated with nilotinib who suffered from PAOD: median time of its occurrence was

short and several pathways involvement were hypothesized. Following this first observation, 11 out of 176 patients were identified in a retrospective study [38]: higher median age, long time from start of treatment and metabolic alterations, such as increased cholesterol level, were reported as main predisposing factors for the occurrence of events. A retrospective large analysis was then conducted including patients enrolled in the main trials (IRIS, TOPS and ENESTnd): relative risk of PAOD resulted to be increased in the cohort of patients treated with nilotinib when compared with patients receiving imatinib or in the cohort of patients with no exposition to TKIs. Moreover, 87% of patients suffering from PAOD had predisposing risk factors, such as diabetes, dyslipidaemia, hypertension, advanced age or smoking [39]. A cross-sectional study reported on the importance of ankle-brachial index, which measured the ratio between ankle and brachial blood pressure as a factor possibly identifying patients at risk of atherosclerotic events: ankle-brachial index was <0.9 in 24 out of 129 patients treated with different drugs and 17 of these were treated with nilotinib. A significant association with increased cholesterol level and other risk factors was observed [40].

Kinetics of molecular response & BCR-ABL mutation status in the ENESTnd trial

MMR, primary endpoint of the study, was defined according to the International Scale (IS, BCR-ABL transcript level of 0.1% or less in peripheral blood on RQ-PCR assay). Molecular monitoring was performed at baseline, each month for the first 3 months and then every three months; mutational analysis was performed by long-range PCR amplification of BCR-ABL and direct sequencing at baseline and then only for the occurrence of fivefold increase in transcript level, or in case of failure to achieve MMR at 12 months, loss of MMR and at the end of treatment [41]. Rapid decline of BCR-ABL ratio in the nilotinib arms compared with imatinib was observed and median BCR-ABL levels on nilotinib at 6 months was similar to those obtained on imatinib at 18 months (0.19% for both nilotinib arms vs 0.17% for imatinib at respective times). Median time to reach MMR was shorter with nilotinib compared with imatinib (6 and 8 months with nilotinib 300 mg BID and 400 mg BID, respectively, compared with 10 months with imatinib). Also kinetics of deep molecular response was rapidly reached with nilotinib as compared with imatinib: MR4.5 was 11% after the first year with nilotinib 300 mg BID, 10% with nilotinib 400 mg BID and less than 1% with imatinib. MMR was lost by seven patients on nilotinib 300 mg BID arm, six patients on nilotinib 400 mg BID and seven patients in the imatinib arm: progression to

advanced phase of disease was detected in two patients on nilotinib 400 mg BID arm. Three patients in the imatinib arm and two patients on nilotinib 400 mg BID arm developed mutations (E255V, Y253H/T315I). Indeed, five out of seven patients treated with nilotinib 300 mg BID and four out of six patients on nilotinib 400 mg BID regained MMR continuing the same drug. Newly detectable mutations during treatment were detected in 12 patients on nilotinib 300 mg BID, 11 patients on nilotinib 400 mg BID and 22 patients on imatinib. Y253H, E255K, F359V, E459K and T315I mutations detected in the nilotinib arms were poorly sensitive to the drug. T315I mutation occurred in four patients on nilotinib 300 mg BID, in two patients with nilotinib 400 mg BID and in three patients on imatinib. One patient on nilotinib 300 mg BID with E459K mutation, two patients on nilotinib 400 mg BID with Y253H/T315I and E255V mutations and seven patients on imatinib (with M244V, Y253H, Y253H/F359I, M351T, F359I and two patients with E459K) progressed to blast phase. These results indicated that molecular responses were faster and deeper with nilotinib and the incidence of new mutations was lower with nilotinib compared with imatinib [41].

TFR as new endpoint in CML

TFR endpoint in CML is based on the hypothesis that it is possible to discontinue TKIs in some patients after the achievement of a deep molecular response. After the first attempts with imatinib in the STIM trial, in which after discontinuation of imatinib the overall probability to maintain CMR at 36 months was 39% [42], several other trials showed the feasibility of discontinuation of the drug, such as the Australian Twister study, the A-STIM, the STIM2 and the Euro-SKI study. The results of these studies showed that TFR ranged between 40 and 60% and that some clinical baseline features were predictive for relapse after discontinuation, such as high Sokal risk, low median duration of imatinib treatment and lack of previous exposure to IFN [43–46]. The French CML group reported the first pivotal trial of second-generation TKIs discontinuation in patients treated for at least 36 months and with a stable undetectable molecular disease for at least 24 months. Primary endpoint was to establish survival without loss of MMR, which defined the criterion for molecular relapse and need for resumption of therapy. After a minimum follow-up of 12 months, 42 patients were included in the trial: only 2 patients had been treated upfront, while the majority had received dasatinib or nilotinib due to imatinib intolerance. The 12-month probability to remain in stable MMR was 58.3% (55.8% according to STIM trial criteria and 44% according to TWISTER crite-

ria). No progressions to advanced phase of disease were revealed. Analysis of variables associated with stable MMR showed that only treatment started for imatinib intolerance was associated with a low probability of relapse, but the analysis was impaired due to the small sample size. After relapse, the same drug was restarted in all patients, except one, resulting in an undetectable disease in 13 out of 15 evaluable patients. At a median time of 16 months, 18 patients remained in stable MMR without therapy and, among them, 7 had stable undetectable BCR-ABL transcript [47]. Several ongoing studies will clarify clinical baseline features of patients that, if treated with nilotinib or dasatinib first line, will have the potential to stop treatment. In particular with nilotinib, the ENEST freedom study is ongoing: after 2 years of induction therapy and achievement of MR4.5, patients enter the study and receive 1 year of consolidation with the same drug. After 1 year of treatment, patients with persistent MR4.5 will try to discontinue: the threshold to restart the drug will be the loss of MR3. Another large trial, called ENEST Path, is ongoing: patients previously treated with imatinib that achieved CCyR but not MR4 are allowed to enter the study and switch to nilotinib 300 mg BID for 2 years. If at the end of this period they will reach a deep molecular response, will be randomized to discontinuation or to continue for another year with the same drug and then will have access to discontinuation. This trial will attest if long duration of treatment may be responsible for a different rate of relapse after discontinuation. Only the results of these ongoing trials will clarify the potentiality of nilotinib as a drug that could be used as inhibitor to reach discontinuation without relapse.

Discussion & conclusion

Deep molecular responses continued to increase over time in nilotinib arms as proven in the last follow-up of Phase III trials indicating that this drug could become the standard of care for frontline treatment. The last follow-up at 5 years confirmed a low incidence of progressions as well as a low incidence of grade 3/4 hematological and nonhematological adverse events compared with imatinib. In the last follow-up of ENESTnd study, it was reported that nilotinib 300 mg BID is clearly associated with increased rate of fasting glucose levels, cholesterol and cardiovascular events. Primary endpoint of ENEST1st study was the achievement of MR4 at 18 months using nilotinib 300 mg BID, but the study was also associated with investigational substudies for the characterization of stem cell compartment and other genomic aspects. Long-term follow-up of patients treated with imatinib first line emphasized some critical issues: the need to achieve a molecular response because it is associated with higher EFS and PFS; the

prompt identification of nonoptimal response or resistant patients, due to the availability of more potent and selective drugs, because it should drive to a quick change to a second therapeutic strategy. At present, it is not clear if all patients have to start with second-generation TKIs or have to early switch, after 3 months, from imatinib for lack of efficacy (patients not achieving an EMR, ratio <10%). Nilotinib upfront offers several advantages, such as early reduction of molecular residual disease burden and consequently significant reduction of progression rate, higher rate of deep molecular responses that can allow to plan a future discontinuation of therapy. On the other hand, nilotinib could be associated with specific safety profile (increased glycaemia, total cholesterol level and increased rate of cardiovascular events), which imposed a selection of patients at baseline, excluding subjects at high risk of possible cardiovascular complications. It is possible to identify patients considered at high risk, considering the European School of Cardiology modifiable and unmodifiable criteria: patients with previously cardiovascular events, affected by diabetes, obesity, dyslipidemia, severe renal impairment, who smoke, are considered at high risk to develop cardiovascular disease and with higher probability of related death [48]. These patients should be excluded from nilotinib treatment or strictly monitored during therapy. Standardization of molecular response

and identification of cutoff for deep response will allow us to clearly define patients who reach the moment to try TFR. Ongoing studies will allow the scientific community to understand the characteristics of patients candidate from baseline to future discontinuation. In this prospective, nilotinib was proven to be more effective to reduce the Ph⁺ stem cell compartment when used in newly diagnosed patients, if compared with imatinib [49]. Better characterization of stem cell compartment in CML allows the identification of multiple pathways to explore TFR and possible new endpoint of complete eradication of drug resistance. In this light nilotinib could be associated with other drugs (ongoing trials associating the drug to ruxolitinib, an anti-JAK2 inhibitor, or to IFN), in order to improve not only the rate of deep molecular responses but also to target stem cells and its environment.

Financial & competing interests disclosure

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Executive summary

- Nilotinib is a second-generation, highly selective, rationally designed tyrosine kinase inhibitor, less susceptible to the development of point mutations due to the binding affinity for *ABL* and with improved capacity to reduce Ph⁺ stem cell compartment.
- US FDA approved nilotinib as treatment for newly diagnosed chronic myeloid leukemia patients in chronic phase at the dose of 300 mg twice daily, in June 2010.
- Long-term results of ENESTnd trial showed the continued superiority of nilotinib compared with imatinib, with improved deep molecular responses.
- Low rate of progression to blastic phase was confirmed at follow-up of 5 years.
- Low incidence of hematologic toxicity but increased rate of biochemical abnormalities (hyperglycemia, hypercholesterolemia) and increased rate of cardiovascular events were reported during nilotinib treatment.
- The preliminary results of ENEST1st, still ongoing, confirmed the superior and faster molecular responses obtained with nilotinib 300 mg twice daily.
- Ongoing trials are testing the possibility to discontinue nilotinib after the achievement of deep and stable molecular response.

References

Papers of special note have been highlighted as:

•• of considerable interest

- 1 Goldman JM, Melo JV. Chronic myeloid leukemia—advances in biology and new approaches to treatment. *N. Engl. J. Med.* 349(15), 1451–1464 (2003).
- 2 Hantschel O, Superti-Furga G. Regulation of the c-Abl and Bcr-Abl tyrosine kinases. *Nat. Rev. Mol. Cell Biol.* 5, 33–44 (2004).
- 3 Faderl S, Talpaz M, Estrov Z, Kantarjian H. Chronic myelogenous leukemia: biology and therapy. *Ann. Intern. Med.* 131, 207–219 (1999).
- 4 Quintas-Cardama A, Cortes J. Molecular biology of bcr-abl1 positive chronic myeloid leukaemia. *Blood* 113(8), 1619–1630 (2009).
- 5 Melo JV, Deininger MW. Biology of chronic myelogenous leukaemia-signaling pathways of initiation and transformation. *Hematol. Oncol. Clin. North Am.* 18(3), 545–568 (2004).

- 6 O'Brien SG, Guilhot F, Goldman JM *et al.* International randomized study of interferon versus STI571 (IRIS) 7-year follow-up: sustained survival, low rate of transformation and increased rate of major molecular response (MMR) in patients (pts) with newly diagnosed chronic myeloid leukemia in chronic phase (CMLCP) treated with imatinib (IM). *Blood* 112, Abstract 186 (2008).
- 7 Deininger MW, O'Brien SG, Guilhot F *et al.* International randomized study of interferon vs STI571 (IRIS) 8-year follow-up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. *Blood* 114, Abstract 1126 (2009).
- **Follow-up IRIS study at 8 years.**
- 8 Apperley JF. Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia. *Lancet Oncol.* 8(11), 1018–1029 (2007).
- 9 Apperley JF. Part II: management of resistance to imatinib in chronic myeloid leukemia. *Lancet Oncol.* 8(12), 1116–1128 (2007).
- 10 Melo JV, Chuah C. Resistance to imatinib mesylate in chronic myeloid leukaemia. *Cancer Lett.* 249(2), 121–132 (2007).
- 11 Milojkovic D, Apperley JF. Mechanisms of resistance to imatinib and second-generation tyrosine inhibitors in chronic myeloid leukaemia. *Clin. Cancer Res.* 15, 7519–7527 (2009).
- 12 Jabbour EJ, Cortes J, Kantarjian HM. Resistance to tyrosine kinase inhibition therapy for chronic myelogenous leukemia: a clinical perspective and emerging treatment options. *Clin. Lymphoma Myeloma Leuk.* 13(5), 515–529 (2013).
- 13 Branford S, Rudzki Z, Walsh S *et al.* Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance and mutations in the ATP phosphate-binding loop (p-loop) are associated with a poor prognosis. *Blood* 102(1), 276–283 (2003).
- 14 Jabbour E, Kantarjian H, Jones D *et al.* Frequency and clinical significance of BCR-ABL mutations in patients with chronic myeloid leukaemia treated with imatinib mesylate. *Leukemia* 20(10), 1767–1773 (2006).
- 15 Soverini S, Colarossi S, Gnani A *et al.* Contribution of ABL kinase domain mutations to imatinib resistance in different subsets of Philadelphia-positive patients: by the GIMEMA working party on chronic myeloid leukemia. *Clin. Cancer Res.* 12(24), 7374–7379 (2006).
- 16 Nicolini FE, Corm S, Le QH *et al.* Mutation status and clinical outcome of 89 imatinib mesylate-resistant chronic myelogenous leukaemia patients: a retrospective analysis from the French intergroup of CML. *Leukemia* 20(6), 1061–1066 (2006).
- 17 Sherbenou DW, Wong MJ, Humayun A *et al.* Mutations of the BCR-ABL kinase domain occur in a minority of patients with stable complete cytogenetic response to imatinib. *Leukemia* 21(3), 489–493 (2007).
- 18 Khorashad JS, de Lavallade H, Apperley JF *et al.* Finding of kinase domain mutations in patients with chronic phase chronic myeloid leukaemia responding to imatinib may identify those at high risk of disease progression. *J. Clin. Oncol.* 26(29), 4806–4813 (2008).
- 19 Savona MR, Saglio G. Identifying the time to change BCR-ABL inhibitor therapy in patients with chronic myeloid leukemia. *Acta Haematol.* 130(4), 268–278 (2013).
- 20 Kantarjian H, Giles F, Wunderle L *et al.* Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. *N. Engl. J. Med.* 354(24), 2542–2551 (2006).
- 21 Talpaz M, Shah NP, Kantarjian H *et al.* Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. *N. Engl. J. Med.* 354(24), 2531–2541 (2006).
- 22 Cortes J, Jones D, O'Brien S *et al.* Results of dasatinib therapy in patients with early chronic-phase chronic myeloid leukemia. *J. Clin. Oncol.* 28(3), 398–404 (2010).
- 23 Cortes J, Jones D, O'Brien S *et al.* Nilotinib as front-line treatment for patients with chronic myeloid leukemia in early chronic phase. *J. Clin. Oncol.* 28, 392–397 (2010).
- **MDACC results of Phase II trial at 1 year.**
- 24 Rosti G, Palandri F, Castagnetti F *et al.* Nilotinib for the frontline treatment of Ph(+) chronic myeloid leukemia. *Blood* 114(24), 4933–4938 (2009).
- **GIMEMA results of Phase II trial at 1 year.**
- 25 Saglio G, Kim DW, Issaragrisil S *et al.* Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. *N. Engl. J. Med.* 362(24), 2251–2259 (2010).
- **ENESTnd follow-up at 1 year.**
- 26 Kantarjian H, Shah NP, Hochhaus A *et al.* Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. *N. Engl. J. Med.* 362(24), 2260–2270 (2010).
- **DASISION follow-up at 1 year.**
- 27 Rosti G, Castagnetti F, Gugliotta G *et al.* Excellent outcomes at 3 years with nilotinib 800 mg daily in early chronic phase, Ph+ chronic myeloid leukemia (CML): results of a phase 2 GIMEMA CML WP Clinical Trial. *Blood* 116(Abstr. 359) (2010).
- 28 Gugliotta G, Castagnetti F, Breccia M *et al.* Early CP CML, nilotinib 400mg twice daily frontline: beyond 3years, results remain excellent and stable (a GIMEMA CML Working party trial). *Blood* 118, Abstract 2756 (2011).
- **GIMEMA trial follow-up beyond 3 years.**
- 29 Quintas-Cardama A, Kantarjian HM, Luthra R *et al.* Efficacy of frontline nilotinib therapy in patients with newly diagnosed Philadelphia chromosome (Ph)-positive chronic myeloid leukemia in early chronic phase (CML-CP). *Blood* 118, Abstract 454 (2011).
- **MDACC trial results at 4 years.**
- 30 Hughes TP, le Coutre PD, Jootar S *et al.* ENESTnd 5-year follow-up: continued benefit of frontline nilotinib compared with imatinib in patients with chronic myeloid leukemia in chronic phase (CML-CP). *Haematologica* 99, Abstract S677 (2014).
- **ENESTnd follow-up at 5 years.**
- 31 Giles FJ, Baccarani M, Brummendorf T *et al.* Deep molecular responses in patients with newly diagnosed chronic myeloid leukemia receiving nilotinib as assessed

- within the EUTOS laboratory network in the ENEST1st study. *Blood*, 122, Abstract 4030 (2013).
- 32 Giles FJ, O'Dwyer M, Swords R. Class effects of tyrosine kinase inhibitors in the treatment of chronic myeloid leukemia. *Leukemia* 23, 1698–1707 (2009).
- 33 Larson RA, Hochhaus A, Saglio G *et al.* Cardiac safety profile of imatinib and nilotinib in patients (pts) with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP): results from ENESTnd. *Blood* 116, Abstract 2291 (2010).
- **Cardiotoxicity reported within ENESTnd trial.**
- 34 Rea D, Mirault T, Gaultier JF *et al.* Prospective analysis of plasma cholesterol and triglycerides in patients with chronic phase-chronic myeloid leukemia during treatment with the 2nd generation tyrosine kinase inhibitor nilotinib. *Blood* 122, Abstract 4042 (2013).
- 35 Hiwase DK, Yeung DT, Carne L *et al.* Hypercholesterolemia in imatinib intolerant/resistant CML-CP patients treated with nilotinib: a retrospective analysis. *Blood* 122, Abstract 1503 (2013).
- 36 Rea D, Mirault T, Cluzeau T *et al.* Early onset hypercholesterolemia induced by the 2-nd generation tyrosine kinase inhibitor nilotinib in patients with chronic phase-chronic myeloid leukemia. *Haematologica* 99(7), 1197–1203 (2014).
- 37 Aichberger KJ, Herndlhofer S, Scherthaner GH, Schillinger M, Mitterbauer-Hohendanner G, Sillaber C, Valent P. Progressive peripheral arterial occlusive disease and other vascular events during nilotinib therapy in CML. *Am. J. Hematol.* 86(7), 533–539 (2011).
- 38 le Coutre P, Rea D, Abruzzese E, Dombret H, Trawinska MM, Herndlhofer S, Dorken B, Valent P. Severe peripheral arterial disease during nilotinib therapy. *J. Natl Cancer Inst.* 103(17), 1347–1348 (2011).
- 39 Giles F, Mauro MJ, Hong F *et al.* Rate of peripheral arterial occlusive disease in patients with chronic myeloid leukemia in the chronic phase treated with imatinib, nilotinib, or non-tyrosine kinase therapy: a retrospective cohort analysis. *Leukemia* 27(6), 1310–1315 (2013).
- 40 Kim TD, Rea D, Schwarz M *et al.* Peripheral artery occlusive disease in chronic phase chronic myeloid leukemia patients treated with nilotinib or imatinib. *Leukemia* 27(6), 1316–1321 (2013).
- 41 Hochhaus A, Saglio G, Larson RA *et al.* Nilotinib lowers the incidence of BCR-ABL mutations and improves the molecular response kinetics compared with imatinib in patients (pts) with newly diagnosed chronic myeloid leukemia (CML). *Blood* 116, Abstract 3431 (2010).
- 42 Mahon FX, Rea D, Guilhot J *et al.* Intergroupe Français des Leucémies Myéloïdes Chroniques. Discontinuation of imatinib in patients with chronic myeloid leukemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. *Lancet Oncol.* 11(11), 1029–1035 (2010).
- 43 Ross DM, Branford S, Seymour JF *et al.* Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study. *Blood* 122(4), 515–522 (2013).
- 44 Rousselot P, Charbonnier A, Cony-Makhoul P *et al.* Loss of major molecular response is accurate for restarting imatinib after imatinib discontinuation in CP-CML patients with long lasting CMR: importance of fluctuating values of MRD and interferon. *Haematologica* 97(Suppl.1) 77 (2012)
- 45 Mahon FX, Nicolini FE, Noel MP *et al.* Preliminary report of the STIM2 study: a multicentre stop imatinib trial for chronic phase chronic myeloid leukemia de novo patients on imatinib. *Blood* 122, Abstract 654 (2013).
- 46 Saussele S, Richter J, Guilhot J *et al.* First interim analysis of a PAN-European stop trial using standardized molecular criteria: results of the EURO-SKI trial. *Haematologica* 99, Abstract LB2440 (2014).
- 47 Rea D, Rousselot P, Guilhot F *et al.* Discontinuation of second generation (2G) tyrosine kinase inhibitors (TKI) in chronic phase (CP)-chronic myeloid leukemia (CML) patients with stable undetectable BCR-ABL transcripts. *Blood* 120, Abstract 916 (2012).
- 48 Conroy RM, Pyorala K, Fitzgerald AP *et al.* Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur. Heart J.* 24(11), 987–1003 (2003).
- 49 Defina M, Ippoliti M, Gozzetti A *et al.* Evaluation of residual CD34(+) Ph(+) progenitor cells in chronic myeloid leukemia patients who have complete cytogenetic response during first-line nilotinib therapy. *Cancer* 118(21), 5265–5269 (2012).