

# Nilotinib for the treatment of newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia: review of the latest clinical evidence

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Nilotinib is a second-generation tyrosine kinase inhibitor 30-fold more potent than imatinib, with high affinity and selectivity on breakpoint cluster region/V-abl Abelson murine leukemia viral oncogene homolog 1, and also active against a wide range of mutant clones. Phase II trials of nilotinib showed high activity in imatinib-resistant or -intolerant chronic myeloid leukemia patients. Recently, the results of nilotinib as frontline treatment showed high efficacy and superiority as compared with imatinib. Two independent Phase II trials (Italian Group for Adult Hematologic Diseases [GIMEMA] and MD Anderson Cancer Center [MDACC] experiences), testing nilotinib as single agent at standard dose in newly diagnosed patients, showed high rate of cytogenetic and molecular responses with few cases of disease progression. The Phase III randomized Evaluating Nilotinib Efficacy and Safety in Clinical Trials – newly diagnosed patients (ENESTnd) study results demonstrated higher cytogenetic and molecular responses after 24 months (overall major molecular response rate, 62% for nilotinib 300 mg twice daily, 59% for nilotinib 400 mg twice daily compared with 37% for imatinib; overall best complete cytogenetic response rate, 87% for nilotinib 300 mg twice daily, 85% for nilotinib 400 mg twice daily compared with 77% with imatinib), lower rate of progression compared with imatinib (0.7% for nilotinib 300 mg twice daily, 1.1% for nilotinib 400 mg twice daily and 4.2% with imatinib). This article provides substantial evidence on the efficacy and relative tolerability of nilotinib in the management of early chronic phase chronic myeloid leukemia patients.

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Chronic myeloid leukemia (CML) is a clonal disorder caused by the malignant transformation of a pluripotent stem cell. It is characterized by the Philadelphia chromosome, a genetic abnormality that arises from the reciprocal translocation t(9;22) (q34;q11) [1–3]. This translocation fuses parts of the genes encoding for breakpoint cluster region (*BCR*) and V-abl Abelson murine leukemia viral oncogene homolog 1 (*ABL1*), resulting in expression of the constitutively active protein tyrosine kinase, *BCR-ABL1*, which represents the target of different compounds [4–6]. Imatinib mesylate is a inhibitor of *ABL1* and its derivative *BCR-ABL1*, as well as other tyrosine kinases [7–10]. Imatinib provides an effective and durable therapy for CML: a 7-year follow-up of Phase III International Randomized interferon versus STI571 study, showed that this agent induces complete hematologic remission in the majority (98%) of newly diagnosed patients in chronic phase (CP) of the disease and complete cytogenetic response (CCyR) in approximately 87% of patients [11]. A

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recent 8-year follow-up showed an overall survival (OS) of 85% [12]. However, the emergence of resistance to imatinib has dampened the enthusiasm for this drug in countries where second-generation tyrosine kinase inhibitors (TKIs) are available [13]. Several mechanisms may contribute to the phenomenon, including increased expression of *BCR-ABL1* through gene amplification, decreased intracellular drug concentrations caused by drug efflux proteins (such as P-glycoprotein) 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 [14–18]. However, 40% of resistance is attributed to the emergence of clones expressing mutated forms of *BCR-ABL1* with amino acid substitutions in the *ABL1*-kinase domain that impair imatinib binding through either disruption of the critical contact point or by inducing a switch from the inactive to the active conformation; in addition, 20% of patients who take imatinib do not achieve CCyR [19–24]. All these evidences have determined the rationale for the creation of new compounds (nilotinib, dasatinib and bosutinib), which have been explored in patients resistant and/or intolerant to imatinib. Recently, safety and efficacy of second-generation TKIs in newly diagnosed patients was reported in Phase II trials with single-arm dasatinib [25] or nilotinib [26,27] or in randomized Phase III trials [28,29] that tested the efficacy of nilotinib or dasatinib versus imatinib. Currently, after the results of these trials, either dasatinib and nilotinib are approved for the treatment of newly diagnosed CML patients. The aim of the present review is to report recent clinical evidences regarding nilotinib in newly diagnosed CP-CML patients.

#### Nilotinib: structural data

Nilotinib is structurally related to imatinib but is 30-fold more potent and active against *BCR-ABL1*. Based on the imatinib complex structural data, a more potent and selective compound could be designed by incorporating alternative binding groups for the *N*-methylpiperazine group, while retaining an amide pharmacophore to keep the hydrogen-bond interactions to Glu-286 and Asp-381 [30]. As compared with imatinib, nilotinib makes only four hydrogen-bond interactions with the ABL kinase domain, involving the pyridyl-*N* and the backbone-NH of Met-318, the anilino-NH and the side-chain hydroxyl of Thr-315, the amido-NH and side-chain carboxylate of Glu-286 and the amido C=O with the backbone-NH of the Asp-381 [31]. Nilotinib is able to block proliferation of *BCR-ABL1*-dependent cell lines derived from CML patients (K562 and Ku-812F) and transfected cell lines (32D or Ba/F3) to express the *BCR-ABL1* protein. The drug effectively inhibits, with

a potency 10- to 20-fold more compared with imatinib, the autophosphorylation of *BCR-ABL1* on Tyr-177, an important binding site for the Grb2 adapter protein. Tyr-177 is involved in CML pathogenesis through regulation of several pathways, including the activation of phosphatidylinositol-3 kinase and Ras/Erk [32]. Similar to imatinib, nilotinib binds inactive conformation of ABL, but with subtle alterations in its structure that allow a better topographical fit. With similar efficacy of imatinib, nilotinib also inhibits the tyrosine kinase activity of the PDGF and c-Kit receptors. Nilotinib has shown no activity against a wide panel of other protein kinases at concentrations below 3  $\mu$ M, including c-Src. Unlike imatinib, nilotinib is not a substrate for efflux transporter P-glycoprotein pump ABCB1 and intake transporter hOCT-1 [33]. Even at concentrations up to 10  $\mu$ M, it has no activity against T315I; as for imatinib, the lack of activity against T315I is the result of nilotinib binding closely to the T315 residue, implying that loss of the hydroxyl side chain and additional methyl group of the isoleucine inhibits binding [34]. E255K, E255V, L248R and Y253H mutant clones transfected into the Ba/F3 cells were found to confer intermediate sensitivity to nilotinib [35,36]. Weisberg *et al.* reported on *in vitro* activity against different situations: nilotinib is able to reduce accumulation of leukemic cells in spleen, bone marrow, liver and lymph nodes of immunodeficient mice transplanted with p210 mutated positive cells and in bone marrow cells with E255K mutant clone [34].

#### Resistance to nilotinib

Bradeen *et al.* showed that in a *N*-ethyl-*N*-nitrosourea-based mutagenesis screening, which compared imatinib, nilotinib and dasatinib, under nilotinib therapy only ten mutations were responsive, including T315I, Y253H, E255V, but at concentrations close to the maximal levels, only T315I was isolated [37]. Another report by Ray *et al.* in a saturation mutagenesis screening for nilotinib, showed that only T315I mutation was associated to clinical resistance to this drug [38]. Hughes *et al.* reported baseline mutation data in 281 out of 321 patients treated in Phase II trial: 55% was the frequency of mutations at baseline in imatinib-resistant patients [39]. A total of 35 different mutations affecting 27 amino acid residues were identified; 23% of patients had mutations that, *in vitro*, were sensitive to nilotinib ( $IC_{50} < 150$  nM), whereas 14% of patients had mutations less sensitive to the drug ( $IC_{50} > 150$  nM). Another 15% of patients had mutations with unknown *in vitro* sensitivity. The results showed that among patients with baseline mutations with high ( $IC_{50} < 150$  nM) or unknown sensitivity to the drug, efficacy was similar compared with patients without baseline mutations. Response rates in patients with mutations with an  $IC_{50} > 150$  nM were less favorable. In particular,

none of the patients with E255V, Y253H and F359 mutations achieved CCyR. Dose escalation to 600 mg twice daily (b.i.d.) did not improve response rates in patients with less sensitive mutations. These type of mutations were also associated most frequently to progression of disease. Emergence of new mutations during nilotinib therapy was registered in 53 patients: most common mutations reported were E255K/V, T315I, F359C/V, G250E and Y253H. G250E mutation had an  $IC_{50}$  of 145 nM close to the  $IC_{50}$  used to define less sensitive mutations to nilotinib; however, although this is one of the most common mutations that emerges with nilotinib, usually it is not reported associate to progression [40]. Two reports discussed on the possible synergistic association of nilotinib and imatinib: Weisberg *et al.* showed cooperation between the two drugs *in vitro* in a panel of *BCR-ABL1* imatinib-sensitive and imatinib-resistant expressing cells [41]. In particular, the drug association was able to overcome resistance due to E255V, E255K, F317L, M351T and F486S, but not to Y253H or T315I in Ba/F3 cells carrying multiple point mutations. White *et al.*, using assay measuring intracellular uptake and retention (IUR) with  $^{14}C$ -labeled imatinib and nilotinib, proved the effect of adding unlabeled nilotinib to the  $^{14}C$ -labeled imatinib IUR and unlabeled imatinib to  $^{14}C$ -labeled nilotinib IUR, with a significant increase in the IUR of 1  $\mu M$   $^{14}C$ -labeled nilotinib when either 1 or 2  $\mu M$  imatinib was added [42]. Other experiments conducted by White *et al.* assessing the effect of temperature (37 vs 4°C) on nilotinib uptake and retention in an ABCB1-expressing cell line, suggested that nilotinib is transported by ABCB1 and that the inhibition of ABCB1-mediated efflux might be the cause for the increased IUR for nilotinib that is observed when both drugs are combined. Brendel *et al.* [43] and others [44] reported that nilotinib is an high-affinity substrate of ABCG2 multidrug transporter: Hegedus showed *in vitro* that nilotinib stimulated both ABCB1 and ABCG2 ATPase activities, but 500 nM of the drug was needed to reach the maximum stimulation of ABCB1 whereas stimulation of ABCG2 was reached with 25 nM [44]. Also other groups described the role of transporters: Davies *et al.* studied nilotinib interactions with ABCB1, ABCC1 and ABCG2 in primitive CD34<sup>+</sup> cells and in cell lines [45]. They reported that nilotinib is neither dependent on active import by Hct1 nor by effluxed transporters. Indeed, they reported that the drug may be an inhibitor of ABCB1 and ABCG2 [45].

### Results of Phase II studies in CP patients resistant or intolerant to imatinib

Phase II trials (summarized in Table 1) confirmed the efficacy of the drug in three cohorts of patients in CP, accelerate phase (AP) and blast crisis (BC), respectively.

A total of 321 CP patients with resistance or intolerance to imatinib were treated with nilotinib 400 mg b.i.d. [46]. A total of 72% of these patients had received prior therapy with imatinib, at more than 600 mg/day. Resistance (primary or acquired) was defined as either treatment with imatinib >600 mg/day with disease progression, no hematological response in bone marrow after 4 weeks, or presence of any of the following mutations: L248, G250, Q252, Y253, E255, T315, F317, H396, in patients receiving <600 mg/day. Intolerance was defined as for patients without major cytogenetic response (MCyR) who discontinued for: persistent grade 3/4 imatinib-related adverse events, despite optimal supportive care, or persistent grade 2 imatinib-related adverse events, persisting for more than 1 month despite optimal supportive care or recurring more than three-times with imatinib dose reduction. Median age of enrolled patients was 58 years (range 21–85), with a median duration of CML of 58 months. A total of 70% of patients were considered as resistant and 30% as intolerant. Median duration of nilotinib therapy was 18.4 months (range <1–36) and median dose intensity was 789 mg, similar to the planned dose of 800 mg/day. Dose reductions and discontinuations were 25 and 56%, respectively. At a minimum follow-up of 24 months, 94% of patients reached a complete hematologic response in a median time of 1 month; overall, 59% of patients achieved a MCyR (56% of resistant and 66% of intolerant patients), in a median time of 1.4 months. Of these, 44% reached a CCyR (41% of resistant and 51% of intolerant patients). Major molecular response (MMR; ratio *BCR-ABL1/ABL1* <0.1 International Scale [IS]) was obtained in 28% of patients. At 24 months, progression-free survival (PFS) was 64% and OS was 87%; 78% of patients who achieved MCyR, maintained this response [46]. Mutational screening was performed in 281 patients and 114 of these (41%) had a baseline mutation, with higher incidence in imatinib-resistant as compared with imatinib-intolerant patients (55 vs 10%). A total of 23% of patients had a mutation sensitive to nilotinib ( $IC_{50}$  <150 nM), including p-loop, a-loop and other regions; mutations with  $IC_{50}$  >150 nM occurred in 14% of resistant patients and affected only three amino-acid residues (Y253H, E255K/V and F359C/V); 15% of patients had a mutation with unknown  $IC_{50}$ . After 12 months of therapy, cytogenetic responses of mutated patients (both with  $IC_{50}$  <150 nM and with unknown  $IC_{50}$ ) were comparable to those of nonmutated patients (MCyR 49 vs 60%, respectively). MCyR in mutated patients with  $IC_{50}$  >150 nM was less favorable (19%). Rate of disease progression was higher in mutated patients compared with nonmutated patients (46 vs 26%); in patients with  $IC_{50}$  >150 nM

Table 1. Results of Phase II and III trials in newly diagnosed chronic myeloid leukemia patients.

	CML phase	Patients (n)	Response rate (%)			Ref.
			CCyR	MMR	CMR	
MDACC <sup>a</sup>	Early CP and AP	49	98	74	24 <sup>a</sup>	[26]
GIMEMA <sup>a</sup>	Early CP	73	81	71	27 <sup>a</sup>	[45]
ENESTnd <sup>b</sup>	Early CP	Nilotinib 300 mg b.i.d. (282)	87	62	44 <sup>b</sup>	[46]
		Nilotinib 400 mg b.i.d. (281)	85	59	36 <sup>b</sup>	
		Imatinib 400 mg (283)	77	37	20 <sup>b</sup>	

<sup>a</sup>In best response at 12 months follow-up. CMR was considered as one leukemic cell in 10<sup>5</sup>. Nilotinib was used at 400 mg b.i.d.  
<sup>b</sup>In intention-to-treat analysis at 30 months follow-up. CMR was defined as negative transcript at RQ-PCR and nested PCR. Nilotinib was used at 400 mg b.i.d.  
<sup>c</sup>In intention-to-treat analysis on the whole population on core treatment at 24 months follow-up. CMR was defined as 4-log reduction.  
AP: Accelerate phase; b.i.d.: Twice daily; CCyR: Complete cytogenetic response; CML: Chronic myeloid leukemia; CMR: Complete molecular response; CP: Chronic phase; ENESTnd: Evaluating Nilotinib Efficacy and Safety in Clinical Trials – newly diagnosed patients; GIMEMA: Italian Group for Adult Hematologic Diseases; MDACC: MD Anderson Cancer Center; MMR: Major molecular response.

the rate was 69%. New mutations were observed in 48 patients and were more frequent in subjects who were mutated at baseline. Several mechanisms of resistance to nilotinib were hypothesized, because the majority of patients who progressed did not have newly detectable mutations [39]. Mahon *et al.* generated nilotinib-resistant cell lines (AR230, LAMA84 and K562) and investigated the possible mechanisms of resistance to nilotinib: BCR–ABL overexpression and MDR1 overexpression were found in LAMA84 cells. The authors found that upregulated expression of Lyn kinase signaling may play an important role in *in vitro* nilotinib resistance. Moreover, *in vivo* data showed that only two out of seven patients resistant to nilotinib had an increase of Lyn mRNA expression; in three tested patients no increase in mRNA was found and all patients showed resistance mediated to the onset of mutations [47]. In a Phase II trial, hematological grade 3/4 toxicity included 31% neutropenia, 30% thrombocytopenia and 10% anemia (Table 2). Grade 3/4 nonhematological events were very infrequent, with only 2% of patients experiencing skin rash, headache and diarrhea; more frequent were laboratory abnormalities with 16% grade 3/4 elevated lipases, hypophosphatemia (15%), hyperglycemia (12%) and increased total bilirubin (7%). Only eight patients experienced electrocardiographic QT interval according to Fridericia correction (QTcF) prolongation greater than 60 ms from baseline [42]. In elderly patients treated in the Phase II trial the safety profile was similar to that of the younger patients: biochemical abnormalities were transient and generally clinically asymptomatic, with elevated lipases in 23% and increased bilirubin in 3% of patients. Hematological grade 3/4 events included

neutropenia in 30%, thrombocytopenia in 36% and anemia in 15% of patients. Incidence of pleural/pericardial effusions and bleeding events of grade 3/4 was only 1%; the incidence of cardiac events (myocardial infarction 4%, congestive heart failure 1%, QTcF prolongation 2%) did not increase as compared with the incidence reported in younger patients [48].

GIMEMA experience in newly diagnosed CML patients with standard dose nilotinib

A multicenter Phase II trial was conducted by the Italian GIMEMA CML Working Party (ClinicalTrials.gov; NCT00481052) [101] with nilotinib standard dose 400 mg b.i.d. as a single arm.

Primary end point was the achievements of CCyR at 1 year: 73 patients were enrolled (median age 51 years) and the last median follow-up presented at the last 2010 American Society of Hematology (ASH) meeting was 30 months [49]. Sokal risk at baseline identified 45% of patients as low, 41% as intermediate and 14% as high risk. The cumulative CCyR rate at 12 months was 100%. CCyR rate at each milestone was: 78% at 3 months, 96% at 6, 12 and 18 months, 92% at 24 months, 81% at 30 months. The cumulative rate of MMR was 96%, while the rates of MMR at 3, 6, 12, 18, 24 and 30 months were 52, 66, 85, 81, 82 and 71%, respectively. The cumulative rate of complete molecular response (CMR; tested at least once) was 70%, while the rates of CMR (in intention-to-treat analysis) at 12, 24 and 30 months were 12, 27 and 27%, respectively. None of the patients who achieved a MMR progressed to 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. At the last 24–30 months follow-up, a mean daily dose of 750–800 mg was maintained in 64% of patients. Adverse events were mostly grade 1/2 and were manageable with appropriate dose adaptations. Two patients (3%) showed a prolongation of the QTcF above 450 ms (none >500 ms). Four patients permanently discontinued the drug: three patients discontinued after 9, 15 and 27 months on treatment for recurrent episodes of amylase and/or lipase increase (no pancreatitis) and one patient discontinued after 25 months due to atrial fibrillation, unrelated to the study drug. Three of these patients are currently on imatinib second-line and one is on dasatinib third-line. At 30 months the OS, PFS

and failure-free survival are 99% and event-free survival (EFS) is 91%. The long-term follow-up showed that the rate of failures was very low during the first 3 years of treatment and that responses remained stable [49].

### MDACC experience in newly diagnosed CML patients

MDACC conducted an experience in untreated CP-CML patients (or with <1 month of imatinib therapy) and in a cohort of patients with previously untreated CML in AP: the primary end point was the achievement of MMR at 12 months [26]. The results at 12 months in 61 patients who received nilotinib at the standard dose of 400 mg b.i.d. were published: median age of the group was 46 years and 12 patients (20%) were pretreated with imatinib and four (6%) had clonal evolution at baseline. Overall, 98% of patients obtained complete hematologic response in a median time of 3 weeks. The incidence of CCyR was 98% at a median time of 3 months, with responses being durable. A total of 38 of 51 evaluable patients achieved MMR at 12 months, evaluated as best response: 12 patients (24%) reached CMR. Hematological grade 3/4 adverse events were represented by anemia in 5%, neutropenia 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 100% and EFS was 89% at 24 months. In total, 43 patients experienced a temporary drug interruption and six patients discontinued therapy due to toxicity (four patients), progression to BC (one patient) and refusal (one patient). Median dose intensity was similar to the planned dose. A comparison with historical imatinib data demonstrated that nilotinib induced more rapid and higher responses, even if compared with high-dose imatinib data [26].

### ENESTnd study follow-up at 24 months

The ENESTnd trial is a Phase III, international, randomized study that has demonstrated the superior efficacy of nilotinib over imatinib. A recent update with 24-month follow-up was presented at the last 2010 ASH meeting [50]. A total of 846 CP patients were randomized to nilotinib 300 mg b.i.d. (n = 282), nilotinib 400 mg b.i.d. (n = 281) and imatinib 400 mg/day (n = 283). The rationale for 300 mg b.i.d. was that similar AUC and  $C_{min}$  between 300 mg b.i.d. and 400 mg b.i.d. was found and that a low dose of the drug may reduce the risk of QTcF prolongation. Patients were randomized according to Sokal score. Primary end point was the achievement of MMR ( $\leq 0.1\%$  BCR-ABL<sup>IS</sup>) rate at 12 months, whereas key secondary end points were the duration of MMR, time to MMR and CCyR, progression to AP/BC

**Table 2. Outcomes reported in newly diagnosed chronic myeloid leukemia patient trials.**

	Patients (n)	Outcome (%)			Ref.
		OS	PFS	EFS	
MDACC (at 12 months)	49	100	100	90	[26]
GIMEMA (at 30 months)	73	97	97	91	[45]
ENESTnd (at 24 months)	Nilotinib 300 mg b.i.d. (282)	97.4	98	NR	[46]
	Nilotinib 400 mg b.i.d. (281)	97.8	97.7	NR	
	Imatinib 400 mg (283)	96.3	95.2	NR	

b.i.d.: Twice daily; EFS: Event-free survival; ENESTnd: Evaluating Nilotinib Efficacy and Safety in Clinical Trials – newly diagnosed patients; GIMEMA: Italian Group for Adult Hematologic Diseases; MDACC: MD Anderson Cancer Center; NR: Not reported; OS: Overall survival; PFS: Progression-free survival.

(with and without clonal evolution), EFS, PFS and OS. 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 24 months, 26% of patients in the nilotinib 300 mg b.i.d. arm, 22% in the nilotinib 400 mg b.i.d. arm and 33% of patients treated with imatinib discontinued the treatment; disease progression rate calculated for patients on study drug was 0.7% (p = 0.006), 1.1% (p = 0.003) and 4.2%, respectively, whereas patients identified as suboptimal response/treatment failure were 9, 2 and 13, in the three arms, respectively. A recent intention-to-treat analysis including patients who discontinued the treatment, showed a progression rate of 3.3, 1.8 and 6.4%, respectively. The rate of progression on treatment was also significantly lower for nilotinib compared with imatinib when clonal evolution was included as a criterion for progression: it was 0.7% for nilotinib 300 mg b.i.d. (p = 0.003), 1.8% for nilotinib 400 mg b.i.d. (p = 0.008) and 6% for imatinib. Discontinuation rate due to adverse events was 6, 10 and 9%, respectively. Median dose intensity was 594 mg for nilotinib 300 mg b.i.d., 776 mg for nilotinib 400 mg b.i.d. and 400 mg for imatinib. As per protocol, dose escalation of nilotinib was not allowed in case of suboptimal/failure response, whereas it was allowed for imatinib to 800 mg/day. At a median follow-up of 24 months, the overall best MMR rate was superior for nilotinib 300 mg b.i.d. (62%, p < 0.0001) and nilotinib 400 mg b.i.d. (59%, p < 0.0001) compared with imatinib (37%). Cumulative incidence of MMR by 24 months was 71% for nilotinib 300 mg b.i.d., 67% for nilotinib 400 mg b.i.d. and 44% for imatinib 400 mg once daily. Superior rates of MMR were observed in both nilotinib arms compared with the imatinib arm across all Sokal risk groups. The overall best rate of BCR-ABL<sup>IS</sup>  $\leq 0.0032\%$  (equivalent to

Table 3. Grade 3/4 hematological toxicity reported in nilotinib trials in newly diagnosed chronic myeloid leukemia patients.

	Patients (n)	Anemia (%)	Neutropenia (%)	Thrombocytopenia (%)	Ref.
MDACC	49	5	11	11	[26]
GIMEMA	73	2	4	3	[45]
ENESTnd	Nilotinib 300 mg b.i.d. (282)	4	12	10	[46]
	Nilotinib 400 mg b.i.d. (281)	4	11	12	
	Imatinib 400 mg (283)	5	21	9	

b.i.d.: Twice daily; ENESTnd: Evaluating Nilotinib Efficacy and Safety in Clinical Trials – newly diagnosed patients; GIMEMA: Italian Group for Adult Hematologic Diseases; MDACC: MD Anderson Cancer Center.

4.5 log reduction) was superior for nilotinib 300 mg b.i.d. (26%,  $p < 0.0001$ ) and nilotinib 400 mg b.i.d. (21%,  $p < 0.0001$ ) compared with imatinib (10%). The overall best CCyR rate was superior for nilotinib 300 mg b.i.d. (87%,  $p < 0.001$ ) and nilotinib 400 mg b.i.d. (85%,  $p = 0.017$ ) compared with imatinib (77%). The superior efficacy of nilotinib was further demonstrated using the 2006 European LeukemiaNet 12-month milestone [51] in which fewer patients had suboptimal response or treatment failure on nilotinib 300 mg b.i.d. (4 and 3%) and nilotinib 400 mg b.i.d. (4 and 3%) compared with imatinib (13 and 11%). The 2006 European LeukemiaNet 18-month milestone application showed that fewer patients had suboptimal response or treatment failure on nilotinib 300 mg b.i.d. (24 and 4%) and nilotinib 400 mg b.i.d. (30 and 4%) compared with imatinib (45 and 16%). Estimated 24-month rate of PFS was 98% for nilotinib 300 mg b.i.d., 97.7% for nilotinib 400 mg b.i.d. and 95.2% for imatinib. There were fewer CML-related deaths on nilotinib 300 mg b.i.d. ( $n = 5$ ), and 400 mg b.i.d. ( $n = 3$ ) versus imatinib ( $n = 10$ ). Estimated OS rate (including data from follow-up after discontinuation) at 24 months was higher for nilotinib 300 mg b.i.d. (97.4%,  $p = 0.64$ ) and nilotinib 400 mg b.i.d. (97.8%,  $p = 0.21$ ) compared with imatinib (96.3%). Both drugs were well tolerated. Grade 3/4 myelosuppression registered for anemia was 4, 4 and 5%, respectively. Grade 3/4 neutropenia was 12, 11 and 21%, respectively and thrombocytopenia was 10, 12 and 9%, respectively (Table 3). Discontinuations due to adverse events or laboratory abnormalities were lower for nilotinib 300 mg b.i.d. (7% lipase elevations, 4% alanine transaminase (ALT) and total bilirubin elevations, 6% glucose elevation) compared with nilotinib 400 mg b.i.d. (8% lipase, 9% ALT, 8% total bilirubin and 5% glucose elevation) and imatinib (3% lipase and ALT, <1% total bilirubin and 0% glucose elevation) (Table 4). With longer follow up there was minimal change in the occurrence of nonhematological grade 3/4 adverse events registered, the most common being nausea, vomiting

and rash with nilotinib [50]. A prospective analysis was conducted to determine the effects of nilotinib in newly diagnosed CML patients with pre-existing Type 2 diabetes [52]. All grade hyperglycemia occurred in 38% of patients treated with nilotinib 300 mg b.i.d., 42% of patients treated with nilotinib 400 mg b.i.d. and 22% of patients treated with imatinib. No patients enrolled discontinued the treatment due to hyperglycemia or any other diabetic serious adverse event (diabetic ketoacidosis, hyperosmolar events and/or hospitalization).

A total of 57 diabetic patients were enrolled in the ENESTnd trial (23, 18 and 16 patients in the nilotinib 300 mg b.i.d., nilotinib 400 mg b.i.d. and imatinib 400 mg arm, respectively) with median age being higher compared with that of the whole patient population (60 vs 47 years). In total, 68% of patients were on diabetic medications (18% insulin); changes in metabolic parameters were minimal in any arm and response rates were similar to those of overall population (MMR rate 69.6, 55.6 and 25%; CCyR rate 69.6, 77.8 and 68.8%, respectively). No diabetic patients progressed to advanced phase of disease after 18-month of follow-up. Eight patients in the nilotinib arm 300 mg b.i.d., five patients in the nilotinib arm 400 mg b.i.d. and six patients in the imatinib arm, discontinued the therapy: of these, three, five and four patients, respectively, discontinued due to an adverse event or laboratory abnormality unrelated to diabetes. One diabetic patient on nilotinib 400 mg b.i.d. experienced an ischemic heart event. Hyperglycemia during nilotinib treatment was mild and manageable also in the diabetic subset of patients and did not represent an obstacle [52].

Cardiac safety profile of ENESTnd trial

Nilotinib has the potential to prolong QTc interval, a feature shared by other TKIs, and the product information for this drug contains a box warning for this effect. Few patients have been reported to have significant prolongation of the QTc interval in previous studies, and changes >500 ms were detected at low rate [53]. The cardiac safety data of the ENESTnd trial were recently reported [54]: patients were excluded from participation in the trial if they had known uncontrolled or medically significant cardiac disease, left ventricular ejection fraction <45% or QTcF interval >450 ms. Prospective assessment of QTcF was performed throughout the study via electrocardiogram and echocardiogram. QTcF increases of >30 ms from baseline occurred in 26% of patients in both nilotinib arms and in 18%

of patients in imatinib arm, whereas increases >60 ms were uncommon and occurred in less than 1% in all the arms. The highest mean values of changes in QTcF interval occurred between 3 and 6 months of therapy: 10.4, 12.4 and 7.9 ms in nilotinib 300 mg b.i.d., nilotinib 400 mg b.i.d. and imatinib arm, respectively. Nilotinib  $C_{min}$  correlates with modest QTcF changes that were not clinically relevant and mostly occurred by the first 3 months of therapy. There were no episodes of torsade de pointes or sudden death or episodes of QTcF interval prolongation >500 ms in none of the three arms. No patients discontinued therapy due to QT prolongation. A total of 11 patients experienced an ischemic heart disease event after a median treatment duration of 18 months: three patients on nilotinib 300 mg b.i.d., six patients on nilotinib 400 mg b.i.d. and two patients on imatinib arm; of these, ten patients had a pre-existing cardiac condition or predisposing risk factors and only one patient discontinued due to ischemic heart disease. Two patients experienced a myocardial infarction, whereas seven patients experienced an event consistent with left ventricular dysfunction: four patients had a reduction of left ventricular ejection fraction and three patients experienced a cardiac/congestive failure. Again, five out of the seven patients who experienced left ventricular dysfunction had pre-existing conditions and none discontinued treatment. These results showed that, with a median follow-up of 18 months, there was no increased incidence of cardiac toxic effects with nilotinib first-line [54].

#### Kinetic of molecular response & BCR–ABL mutation status in ENESTnd trial

The primary end point of the study, MMR at 12 months, was expressed according to the IS and defined as a BCR–ABL transcript level of 0.1% or less in peripheral blood on RQ-PCR assay. BCR–ABL RQ-PCR testing was conducted at baseline, at month 1, 2, 3 and then every 3 months; mutational testing of BCR–ABL was performed by long-range PCR amplification of BCR–ABL and direct sequencing at baseline and at occurrence of: fivefold increase in PCR levels, failure to achieve MMR at 12 months, loss of MMR and end of treatment [55]. A rapid decline of BCR–ABL ratio was observed in the nilotinib arms compared with imatinib, with median BCR–ABL levels for patients on nilotinib at 6 months similar to those obtained on imatinib at 18 months (median BCR–ABL: 0.19% for both nilotinib arms and 0.17% for imatinib). In addition, median time to reach MMR was

shorter with nilotinib compared with imatinib (6 and 8 months with nilotinib 300 mg b.i.d. and 400 mg b.i.d., respectively, and 10 months with imatinib). Seven patients on nilotinib 300 mg b.i.d., six patients on nilotinib 400 mg b.i.d. and seven patients in the imatinib arm lost MMR: two patients on nilotinib 400 mg b.i.d. progressed to advanced phase of disease; three patients in the imatinib arm (M244V, T315I, H396R/M351T) and two patients on nilotinib 400 mg b.i.d. (E255V and Y253H/T315I) developed mutations. Five out of seven patients on nilotinib 300 mg b.i.d. and four/six patients on nilotinib 400 mg b.i.d. regained MMR on the same assigned therapy. Mutational screening revealed 13 patients on nilotinib 300 mg b.i.d., ten patients on nilotinib 400 mg b.i.d. and 23 patients on imatinib, with newly detectable mutations during treatment. Mutations detected in the nilotinib arms were poorly sensitive to the drug: Y253H, E255K, F359V, E459K and T315I. Overall, the T315I mutation emerged in five patients on nilotinib and three on imatinib. Less than 35% of patients who developed mutations progressed to advanced phase of disease: one patient on nilotinib 300 mg b.i.d. (with E459K mutation), two patients on nilotinib 400 mg b.i.d. (with Y253H/T315I and E255V mutations) and seven patients on imatinib (with M244V, Y253H, Y253H/F359I, M351T and F359I, and two patients with E459K). 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 [55].

#### All-Ireland Cooperative Oncology Research Group CML study

From December 2008, the All-Ireland Cooperative Oncology Research Group has been conducting an open-label, single-stage, multicenter, Phase II study to evaluate safety and efficacy of nilotinib 300 mg b.i.d. in newly diagnosed CP-CML patients. Primary end point was the achievement of CCyR at 6 months, whereas secondary end points were the kinetics of molecular response evaluated at baseline and after 3 months

**Table 4. Grade 3/4 laboratory abnormalities reported in nilotinib trials in newly diagnosed chronic myeloid leukemia patients.**

	Patients (n)	Bilirubin (%)	Lipase (%)	Hyperglycemia (%)	Ref.
MDACC	49	7	4	4	[26]
GIMEMA	73	16	4	3	[45]
ENESTnd	Nilotinib 300 mg b.i.d. (282)	4	7	6	[46]
	Nilotinib 400 mg b.i.d. (281)	8	8	5	
	Imatinib 400 mg (283)	<1	3	0	

b.i.d.: Twice daily; ENESTnd: Evaluating Nilotinib Efficacy and Safety in Clinical Trials – newly diagnosed patients; GIMEMA: Italian Group for Adult Hematologic Diseases; MDACC: MD Anderson Cancer Center.

from start of treatment with standard RQ-PCR and PCR 'GeneXpert' system. A total of 37 patients were enrolled: median age 53 years (range 20–77), 28% high Sokal risk. At the ASH 2010 meeting, a median follow-up of 11 months was presented: CCyR rate was 62% at 3 months and 94% at 6 months. None of the patients progressed to advanced phase of disease and three patients went off study for persistent thrombocytopenia (one patient), persistent liver function tests (one patient) and unrelated death (one patient). Three out of 25 evaluable patients underwent dose escalation of nilotinib to 400 mg b.i.d. for suboptimal response, whereas median dose intensity was maintained to 600 mg in 82% of patients. As for toxicity, only 5% of patients experienced grade 3/4 thrombocytopenia and 19% of patients experienced lipase elevation. A good correlation was evidenced between RQ-PCR and GeneXpert system: at 3 months, the median BCR–ABL/ABL ratio was 0.45% as calculated by GeneXpert and 0.67% as evaluated by RQ-PCR, and at 6 months, BCR–ABL/ABL ratios were 0.06 and 0.01%, respectively, with the two methods. The authors stated that an individual tendency was noted for GeneXpert to underestimate BCR–ABL/ABL ratio and overestimate MMR incidence. Also the results of this trial provided evidence that nilotinib 300 mg b.i.d. is safe and effective in newly diagnosed CP-CML [56].

### Future perspective

The latest clinical evidence from Phase II and III trials using nilotinib frontline has suggested that CCyR and MMR rates remain significantly higher as compared with imatinib and that CMR rate continues to increase over time, suggesting that this drug could become the standard of care in frontline. Now, nilotinib has been approved by the US FDA as first-line treatment for newly diagnosed CML patients at the dose of 300 mg b.i.d. Three important points emerge from the 24-month follow-up of ENESTnd trial: a low incidence of progressions is confirmed as well as a low incidence of grade 3/4 hematological and nonhematological adverse events compared with imatinib. Moreover, nilotinib at either dosages of 300 and 400 mg b.i.d. reduces the incidence of suboptimal and failure patients. The ENEST1st study, aimed to explore, as primary end point, the achievement of CMR at 18 months with nilotinib 300 mg b.i.d. as single arm is ongoing. Interesting substudies are also planned inside this trial for characterization of stem cells, of mutated sub-clones and other genomic aspects. The 8-year follow-up of International Randomized interferon versus STI571 (IRIS) study with imatinib frontline emphasized some critical issues: the need to achieve a rapid cytogenetic response, because it is the response associated to long-term OS; the early achievement of

MMR, because it is associated to higher EFS and PFS; the prompt identification of suboptimal response and resistant disease, because it remains a priority in frontline treatment with standard imatinib and should drive to a quick change to a second-line therapeutic strategy. Thus, it remains to be stated which patients will continue to have benefits from imatinib therapy or whether it is the time to treat with second-generation TKIs all the newly diagnosed patients. International efforts are warranted to design therapeutic strategies to achieve the best response in all subgroups of patients, irrespective from initial stratification; probably, the evaluation of age and comorbidities at diagnosis might lead patients to be treated with one drug or another, even if we must keep in mind to maintain the possibility of a switch to a more potent drug in case of resistance. A possible individualized choice should also be offered considering some critical biological features at diagnosis, such as the type of transcript (p190 or p210), the OCT1 level and MDR1 polymorphisms: in the case of high OCT1 expression and favorable polymorphisms, imatinib should still be considered. Again, we should choose according to clinical features and stratification to Sokal and Hasford score: data of Phase III trials comparing dasatinib and nilotinib to imatinib, showed that low-risk patients treated with imatinib did not progress during treatment. No comparisons can be made between the two randomized Phase III trials comparing imatinib and second-generation TKIs: it remains unsolved for the moment which patients would benefit from treatment with dasatinib instead of nilotinib. In addition, the Dasatinib versus Imatinib Study in Treatment-Naïve CML (DASISION) trial proved the superior efficacy of dasatinib 100 mg/day versus imatinib, in terms of confirmed CCyR, MMR rate and a low rate, although not significant, of progression of disease. Long-term follow-up will provide us the real significance of a more faster CCyR reached with second-generation TKIs, dasatinib or nilotinib and the long-term safety profile of both. The issue of costs will be also considered: the costs of second-generation TKIs frontline will influence our choice when imatinib becomes generic. No data concerning compliance to nilotinib or dasatinib, used as second-line after imatinib resistance nor when used as frontline were provided, prospective trials aimed to resolve this issue will allow us to identify particular subset of patients that could benefit from one drug instead of another. Another important point emerged with ENESTnd trial is the elevated number of patients that achieved CMR at last follow-up of 24 months, compared with imatinib (26 vs 10%). One of the end points of the next Phase III trial is the standardization of CMR definition: we need to identify and improve the correct measurement of molecular response to clearly define patients who reach the status of 'cure'

(no detectable disease for long time) and the category of patients who still have detectable disease at low level but without disease recurrence. The identification of the first category of patients will allow the scientific community to test in prospective trials the possibility to definitively discontinue the drug. Recently, Mahon and colleagues reported that imatinib can be safely discontinued in patients with a CMR of at least 2 years duration: in 100 patients enrolled, the 12 months probability of persistent CMR was 41% with prompt response to imatinib after relapse [57]. The results of this study confirmed that is still uncertain the property of imatinib to completely eradicate CML stem cells [58]. Characterization of stem cell compartments in CML identified multiple mechanisms of drug resistance, including kinetic quiescence *per se*, increased expression of BCR-ABL and altered

expression of membrane drug transporters [59,60]. This finding underscores the need for treatment specifically targeting the leukemia-initiating population. Again, in the future we probably could decide how treat and discontinue the treatment according to genomic profile studies of patients at diagnosis.

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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 V-abl Abelson murine leukemia viral oncogene homolog 1.
- Nilotinib 400 mg b.i.d. in imatinib-resistant and/or -intolerant chronic myeloid leukemia (CML) patients was able to induced 44% of complete cytogenetic response with 24-month overall survival of 87%.
- Mutations less sensitive to nilotinib (IC<sub>50</sub> >150 nM: Y253H, F359C/V, E255K) had low response rates and high progression rate.
- In June 2010, the US FDA approved nilotinib as a treatment for newly diagnosed CML patients in chronic phase at the dose of 300 mg b.i.d.
- Results of ENESTnd trial showed the superiority of nilotinib (either at 400 mg or 300 mg b.i.d.) compared with imatinib, in terms of complete cytogenetic response and major molecular response achievement at 12 months.
- Follow-up of the study at 24 months, confirmed that nilotinib protects against progression of disease to blastic phase and prolongs overall survival, but more longer follow-up is needed to confirm these results.
- Nilotinib treatment is associated to low rate of myelosuppression.
- With a median follow-up of 18 months, there was no increased incidence of cardiac effects with nilotinib first line.
- Incidence of new mutations was lower with nilotinib compared with imatinib.
- Preliminary results of All-Ireland Cooperative Oncology Research Group trial confirmed that nilotinib 300 mg b.i.d. is safe and effective in newly diagnosed CML patients.
- A new Phase III trial with nilotinib 300 mg b.i.d. (ENEST1st) is ongoing with achievement of CMR at 18 months, as primary end point.

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