Nilotinib

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Abstract

With imatinib still being linked to the breakthrough in CML therapy and probably being the most prescribed drug, second-generation TKIs are increasingly gaining importance. Showing higher response rates while not leading to more adverse events, nilotinib has become an attractive option in the first-line treatment of chronic-phase chronic myeloid leukemia. By reaching deep and long-lasting molecular remissions, discontinuation of TKIs is becoming one of the central topics of future CML therapy. Stopping nilotinib seems safe and provides a stable remission in about half of the eligible patients, though long-term data are still missing.

Keywords

CML · TKI · Nilotinib

1 Introduction

Since the beginning of the century, treatment of chronic-phase Philadelphia-chromosome-positive chronic myeloid leukemia (CML) is largely based on tyrosine kinase inhibitors (TKIs) targeting the oncogenic origin of the disease. After the discovery of the Philadelphia chromosome more than fifty years ago, understanding of the underlying oncogenic mechanism started to grow. This reciprocal chromosomal translocation was found to form a fusion protein identified as the dysregulated BCR-ABL tyrosine kinase. After proving that this kinase leads to unregulated growth of the leukemia cells, approaches to pharmacologically counteract it arose (Nowell and Hungerford 1960; Rowley 1973; Druker et al. 1996).

The introduction of imatinib, a potent inhibitor of the BCR-ABL kinase, led to a change of pace in treating CML, showing high rates of cytogenetic and even molecular remission (Druker et al. 2006). Thus, it quickly became the new standard in CML therapy. Despite this success, some patients showed primary or secondary resistance or insufficient response to imatinib. The main mechanism was found to be additional point mutations of the kinase domain, preventing the optimal effect of imatinib on a molecular level (Gorre et al. 2001; O'Hare et al. 2007). In other cases, failure of imatinib therapy could not be further characterized (Apperley 2007). Additionally, relevant side effects could not be tolerated in some cases and could even lead to discontinuation of the targeted therapy, especially in light of a daily administration (Druker et al. 2006). Therefore, the need for second-generation BCR-ABL inhibitors followed the initial excitement after the introduction of imatinib.

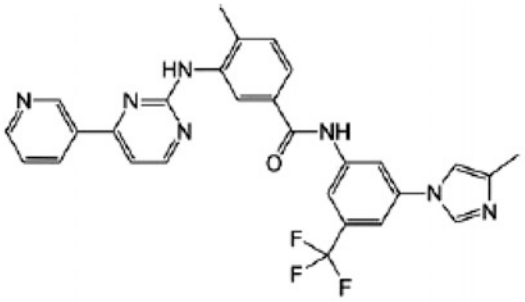
Nowadays, second-generation TKIs for the treatment of CML have been used for over ten years, one of them being nilotinib. The drug not only shows superior effectiveness in both first- and second-line CML but also leads to deep and long-lasting remissions (Kantarjian et al. 2007; Hochhaus et al. 2016a, b). Therefore, the focus increasingly shifts to tolerability in light of high rates of disease control. Furthermore, questions are raised, if and which patients are able to maintain their remission status even after discontinuation of TKI.

With growing experience in the use of nilotinib even in a first-line setting, emphasis will be put on recent clinical data instead of known preclinical findings.

2 Structure and Mechanism of Action

After clinical proof of the antileukemic effect of imatinib, research for further substances with increased activity started. The crystallographic analysis of imatinib interacting with the kinase domain of the BCR-ABL fusion protein was the basis for further development. Nilotinib, formerly known as AMN107, was designed by replacing an *N*-methylpiperazine group in the imatinib molecule (Manley et al. 2004; Weisberg et al. 2005). The molecular structure is displayed in Fig. 1. This novel molecule was found to have a 10- to 50-fold higher BCR-ABL kinase inhibition activity compared to imatinib. Besides this effect against unmutated BCR-ABL, nilotinib was also proven to show sufficient activity against most kinase domain mutations known at that time to cause imatinib resistance (Weisberg et al. 2005). The inhibiting effect is accomplished by preventing the BCR-ABL kinase from switching to an active conformation.

Of note in this context, nilotinib was also shown to have an inhibitory effect on KIT, PDGFR, DDR1, and NQO2 (Rix et al. 2007).



Chemical name:	4-Methyl-3-((4-(3-pyridinyl)-2-pyrimidinyl)amino) -N-(5-(4-methyl-1H-imidazol-1-yl)-3- (trifluoromethyl)phenyl)benzamide
Synonym:	AMN107
Molecular weight:	529.52
Molecular formula:	$C_{28}H_{22}F_3N_7O$

Fig. 1 Molecular structure and chemical characteristics of nilotinib. Adopted from O'Hare et al. (2005)

Table 1 Comparison of IC_{50} values of imatinib and nilotinib in wild-type and mutated BCR-ABL

Mutation of BCR-ABL	IC_{50} (nM) imatinib	IC_{50} (nM) nilotinib
Wild type	260–678	<10–25
M244V	1600–3100	38–39
G250E	1350 to >20,000	48–219
Y253F Y253H	6400–8953 6400–17,700	182–725 450–1300
E255K	3174–12,100	118–566
E255V	6111–8953	430–725
F311L	480–1300	23
T315I	6400 to >20,000	697 to >10,000
V379I	1000–1630	51
F359V	1400–1825	91–175

Baccarani et al. (2013), Bradeen et al. (2006), von Bubnoff et al. (2006), Gorre et al. (2001), Hochhaus et al. (2013), O'Hare et al. (2005), Ray et al. (2007), Redaelli et al. (2012), Soverini et al. (2006), Weisberg et al. (2006)

3 Preclinical Data

As noted above, nilotinib was found to be more potent in inhibiting BCR-ABL as imatinib. These findings could be confirmed by studies both on murine and human cell lines in vitro. Additionally, nilotinib led to fewer rates of BCR-ABL autophosphorylation in exposed cells compared to imatinib (Golemovic et al. 2005; Weisberg et al. 2005). When tested in a mouse model with an induced CML, nilotinib was able to significantly prolong survival and reduce the overall burden of tumor cells in imatinib-resistant clones (Weisberg et al. 2005). Thus, most mutations of the kinase domain could be overcome, important exceptions being, for example, T315I, T315V, and L248R. IC_{50} values of some mutations are shown in Table 1. Maximum plasma concentration of nilotinib was 2329 ± 1233 nM.

4 Clinical Data

4.1 Nilotinib Phase I Trial

After demonstration of a superior effect in both unmutated and mutated BCR-ABL-positive CML cells in vitro, a Phase I trial in CML patients resistant to imatinib was conducted. In this dose escalation study, patients of all stages of disease (chronic, accelerated, and blastic phase), who developed resistance to prior imatinib therapy, were randomly assigned to receive 100–1200 mg of nilotinib once per day or 400–600 mg twice daily. A steady-state level of the drug in blood

serum was reached after eight days of intake. At this level, the exposure to the drug was higher when administered at 400 mg twice daily than at 800 mg once per day, thus making two doses per day favorable. Furthermore, peak concentrations and the area under the curve were found to rise in increasing dosages up to 400 mg and then steadied. The half-life of nilotinib was about 15 h.

Toxicity was found to be reasonable up to doses of 600 mg twice per day.

In this highly heterogenous study population, a notable response to therapy could be noted. Of 33 patients in blastic phase at the beginning of treatment, 13 developed a complete hematological response (CHR) with 9 patients showing cytogenetic response. In the accelerated phase cohort, 33 out of 46 patients achieved CHR and 22 showed any cytogenetic response. Of patients in chronic phase, 11 out of 12 with active disease at baseline achieved CHR, whereas 9 out of the total 17 showed cytogenetic response (Kantarjian et al. 2006).

Tanaka et al. could show that the intestinal absorption of nilotinib is altered depending on the kind of food intake. For example, it could be shown that the area under the curve of nilotinib was increased up to 50% after a meal with high content of fat (Tanaka et al. 2009). These findings were consistent with previous data of healthy volunteers (Kagan et al. 2005; Tanaka et al. 2009). Thus, intake of nilotinib is recommended at least two hours after the last meal; afterwards, the patient should be fasting for another hour.

4.2 Nilotinib Second- and Third-Line Therapy

After successful use of nilotinib in patients resistant to imatinib, the only approved TKI at that time, controlled studies were initiated for further investigations of the drugs effects.

In 2007, Kantarjian et al. first published a study of 280 patients with Philadelphia-positive CML in chronic phase resistant or intolerant to imatinib, treated with nilotinib 400 mg twice daily in a single arm. A first analysis after six months of treatment revealed a rate of 31% of patients with a complete cytogenetic remission (CCyR). Almost half of the study population (48%) achieved at least a major cytogenetic remission (MCyR), defined as <35% Philadelphia-positive cells. With the exception of the T315I mutation, a majority of mutational and non-mutational mechanisms of imatinib resistance were overcome (Kantarjian et al. 2007).

The four-year update of the same Phase II trial showed that 31% of patients were still under the study drug at 48 months. Furthermore, the median administered daily dose of nilotinib was found to be 789 mg, which comes close to the optimal dose of 800 mg split up in 400 mg twice daily according to the study protocol. Thus, nilotinib was found to be safe and tolerable. After four years, 59% of patients had reached a MCyR with 45% being in CCyR. Interestingly, rates of MCyR were identical after 24 and 48 months, indicating that an early response is associated with better outcome (Kantarjian et al. 2011a, b; Giles et al. 2013). This fact was supported by an analysis, showing that deep molecular remissions after 3 and 6 months

were associated with better overall survival and progression-free survival. Nilotinib had to be discontinued in the first 48 months mainly because of disease progression (30%), but it has to be noted that only 3% of patients progressed to accelerated or blastic phase of CML. Another 21% of patients had to stop nilotinib because of adverse events.

Parallel to nilotinib, another second-generation TKI, dasatinib, was developed. Giles et al. investigated the effectiveness of nilotinib after failure of imatinib and dasatinib. A majority of patients (67%) previously treated with dasatinib had to discontinue this drug due to intolerance instead of resistance. Interestingly, 79% of patients switching from dasatinib had not reached MCyR before. After switching to nilotinib, 43% reached MCyR, while 79% reached CHR (Giles et al. 2010).

Further studies have been conducted, investigating the efficacy of nilotinib in the second or further line of therapy for patients in accelerated or blastic phase. After 24 months of therapy, patients in accelerated phase showed any hematologic response in 55% of cases with 31% achieving CHR and 32% achieving MCyR (le Coutre et al. 2008; le Coutre et al. 2012). After the same period of time, patients in myeloid blastic phase and lymphoid blastic phase achieved a major hematologic response in 60 and 59% and MCyR in 38 and 52%, respectively (Giles et al. 2008).

Thus, nilotinib was proven to be effective in all stages of CML after failure of prior TKI therapy. Usual dosages applied were 400 mg of nilotinib twice per day, while today's standard is 300 mg twice daily.

Recently, the ENESTfreedom extension trial could show that switching to a higher dosed nilotinib regimen (400 mg twice daily) leads to sufficient response rates in patients with non-optimal disease control under treatment with imatinib 400 mg once daily or nilotinib 300 mg twice daily. After change of treatment, 32% of patients pre-treated with imatinib and 39% pre-treated with regular doses of nilotinib reached major molecular response (MMR). However, estimated progression-free survival and overall survival were worse in the group switching from imatinib. Toxicity rates were not significantly higher compared to the standard dose nilotinib group (Hughes et al. 2014a, b). Thus, dose escalation of nilotinib is an option for patients not eligible for another second-generation or third-generation TKI.

On the other hand, Cortes et al. could show that in a case of an insufficient response to first-line imatinib, switching the TKI to nilotinib at a dose of 400 mg twice a day might lead to better rates of remission than a dose escalation of imatinib. Among the 191 patients enrolled, who had not reached a complete cytogenetic remission (CCyR) yet, 50% of the nilotinib group reached this endpoint after six months. Of patients escalated to 600 mg imatinib once per day in the other arm, only 42% reached CCyR at the same point of time (Cortes et al. 2016). Statistically, these findings were not significant, but they raise interest in further studies in this field covering a longer study interval.

In summary, nilotinib stays a potent option for second or further line therapy of CML, enabling decent rates of remission after failure of prior TKI therapy.

4.3 Nilotinib First-Line Therapy

The first and most relevant study of frontline nilotinib usage was ENESTnd. In this randomized open-label multicentre Phase III trial, nilotinib was tested against the standard therapy with imatinib in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Patients in the control arm received 400 mg of imatinib once per day, whereas patients in the study arms received 300 mg or 400 mg of nilotinib twice daily, respectively.

The first data were published by Saglio et al. in 2010 after a 12-month treatment period. The primary endpoint was defined as the rate of major molecular remission (MMR), equaling $\leq 0.1\%$ BCR-ABL according to the International Scale. Rates of MMR were 44% for the group treated with 300 mg nilotinib twice a day, 42% at 400 mg nilotinib twice daily, and 22% in the imatinib group. These findings were highly significant in favor of nilotinib. Reinforcing these facts, rates of complete cytogenetic remission were significantly higher in the nilotinib groups (80% in the 300 mg arm and 78% in the 400 mg arm) compared to patients treated with imatinib (65%). Furthermore, time to progression of the disease was notably longer under treatment with nilotinib ($p = 0.01$ in the nilotinib 300 mg group, $p = 0.004$ in the 400 mg group), while toxicity rates were comparable between imatinib and nilotinib groups (Saglio et al. 2010).

These findings led to the approval of nilotinib as first-line therapy in the USA and the European Union at the end of 2010 and the beginning of 2011, respectively.

In the 2016 update of the ENESTnd trial, both 300 mg and 400 mg of nilotinib administered twice daily were found to lead to rates of deep molecular remission ($MR^{4,5}$, see below) in more than 50% of patients (54 and 52%, respectively) after a study period of five years. In contrast, 31% of patients treated with imatinib reached the same milestone. Since toxicity was significantly higher in the group receiving 400 mg twice a day, the dosage of 300 mg twice daily should be considered standard in first-line therapy, especially in light of the excellent results (Hochhaus et al. 2016a, b). Table 2 shows further details retrieved from the 5-year update.

This work confirmed that nilotinib leads to faster responses, even on the molecular level. Previously, Jain et al. were already able to show that early responses are an individual prognostic factor, as earlier responses to therapy are associated with better outcome, e.g., regarding progression or overall survival (Jain et al. 2013). These findings were confirmed by Hughes et al., who could prove another advantage of nilotinib versus imatinib: Whereas a high Sokal risk score was associated with lower rates of early molecular response (here defined as $\leq 10\%$ BCR-ABL at 3 or 6 months), the same was not true in the nilotinib group.

In the ENEST1st study, a remarkable number of 1089 patients were treated in a single arm with nilotinib 300 mg twice per day in the first-line setting. For the first time, molecular remission was not only set as a primary endpoint in a trial of this size, but the cutoff was set to $\leq 0.01\%$ BCR-ABL according to the International Scale. This milestone was reached by 38.4% of all patients after a treatment interval of 18 months. This endpoint was especially important as it leads a way to a possible discontinuation of the drug after successful primary therapy (Hochhaus et al. 2016a, b).

Table 2 Five-year outcome of patients treated with nilotinib or imatinib in first line

Parameter	Imatinib 1 × 400 mg/day (%)	Nilotinib 2 × 300 mg/day (%)	Nilotinib 2 × 400 mg/day (%)
MMR	60.4	77.0	77.2
MR^4	41.7	65.6	63.0
$MR^{4,5}$	31.4	53.5	52.3
Progression of CML	7.4	3.5	2.1
New mutations of BCR-ABL	7.8	4.3	3.9
New T315I Mutation	1.4	0.7	1.1
Grade 3/4 AE	58.9	60.6	71.5
Cardiovascular events	2.1	7.5	13.4
Deaths	7.7	6.4	3.6

Adopted from Hochhaus et al. (2016a, b)

Concluding, nilotinib has not only reached the status of a first-line option for patients with newly diagnosed CML in chronic phase. It has even proven a higher efficacy in terms of response rates as well as time to response compared to the established therapy with imatinib.

4.4 Nilotinib Discontinuation

As mentioned above, nilotinib is able to achieve faster and deeper remissions than imatinib in the majority of patients. With growing experience and a large number of patients in deep molecular remissions, aspirations of stopping the medication came up. Initially, this idea was realized for patients treated with imatinib, showing continuous remission rates between 39 and 51.9% after discontinuation of the drug (Mahon et al. 2010; Mori et al. 2015).

The ENESTfreedom study was the first to determine the outcome of controlled discontinuation in patients in deep molecular remission treated with nilotinib (Hochhaus et al. 2017). The patient population had to be treated with nilotinib for at least two years and was required to be in deep molecular remission ($MR^{4,5}$, see below) for at least one year. After discontinuing nilotinib, 51.6% of these patients were found to stay in major molecular remission during the first 48 weeks. Relapsing patients were retreated with nilotinib with 98.8% reaching at least MMR again. The most common adverse event during the study was musculoskeletal pain, which had been described after imatinib discontinuation before (Mahon et al. 2010; Mori et al. 2015; Hochhaus et al. 2017).

The rates of treatment-free remission, defined as maintaining at least MMR, are similar to the findings with imatinib mentioned above. However, since more patients are able to achieve remission levels necessary for discontinuation when

Table 3 Sustained molecular remission after discontinuation of TKI therapy

Trial	Drug	TF interval	MMR rate[a] (%)	MMR rate after reinitiation[b] (%)	Reference
EURO-SKI	Ima	18 months	53	–	Mahon et al. (2016)
STIM	Ima	12 months	41	62	Mahon et al. (2010)
ISAV	Ima	36 months	51.9	100	Mori et al. (2015)
ENESTfreedom	Nil	48 weeks	51.6	99	Hochhaus et al. (2017)
ENESTop	Nil	48 weeks	57.9	98	Hughes et al. (2016)
STOP 2G-TKI	Nil	48 months	61.4	–	Rea et al. (2016)

Ima Imatinib; *Nil* Nilotinib; TF interval: (median) treatment-free follow-up (i.e., time after TKI discontinuation)
[a]Percentage of patients with sustained MMR (or better) after TKI discontinuation
[b]Percentage of patients regaining MMR after TKI re-exposition following molecular relapse

treated with nilotinib, in total numbers more patients are getting the chance of discontinuation and treatment-free remission.

Table 3 shows success rates of different trials testing the discontinuation of imatinib and nilotinib after achieving deep molecular remission. Noteworthy are the very high numbers of patients, who were able to re-gain MMR or better after restart of TKI therapy following relapse. Therefore, discontinuation seems safe even considering that nearly half of patients are relapsing.

The ongoing ENESTpath trial is the first to have the objective of determining optimal conditions for a possible stop of therapy after reaching a deep molecular remission. A first analysis showed that 30.5% of patients with prior imatinib therapy and non-optimal molecular response were able to achieve $MR^{4,5}$ after 12 months (Rea et al. 2015). Thus, switching to nilotinib might be favorable for an intended TKI discontinuation. On the other hand, this study is making nilotinib the only TKI available at publishing date with a noted possibility of discontinuation at start of therapy.

4.5 Resistance to Nilotinib

Resistance to tyrosine kinase inhibitors in the treatment of CML is largely based on additional mutations of the kinase domain, leading to ineffective binding of the drug. These mechanisms were first identified in the context of imatinib resistance (Shah et al. 2002). In most cases, these mutations are not present at the time of diagnosis but develop over the course of treatment (Soverini et al. 2006; Ernst et al. 2011). In total, nilotinib was found to develop fewer kinase domain (KD) mutations than imatinib (Hochhaus et al. 2013). Furthermore, mutations were mainly identified in the p-loop of KD, contrasting the findings of imatinib (Bradeen

et al. 2006; Ray et al. 2007). This can partly explain the efficacy of switching TKIs after developing resistance (Giles et al. 2013).

In a sub-analysis of the ENESTnd trial, the incidence and character of developing mutations under TKI therapy was investigated (Hochhaus et al. 2013). Shortly summarized, additional mutations emerged more seldom under treatment with nilotinib than imatinib; furthermore, progression to advanced stages of CML was not as frequent. The rate of new mutations when treated with nilotinib was 4.9% during the first three years. The most common mutations developing under nilotinib therapy were Y253H, E255K/V, and F359C/V. The European Leukemia Net recently released guidelines including an overview of the most frequent kinase domain mutations and their resistance to individual TKIs. Interestingly, mutations often acquired under imatinib treatment are usually sensitive to nilotinib, whereas the aforementioned frequent mutations developing under nilotinib are mostly resistant to imatinib as well (Baccarani et al. 2013).

An exception stays the crucial T315I mutation, which shows resistance to both first- and second-generation TKIs, emphasizing the status of ponatinib and research for further generation drugs. By changing amino acids at the binding site of nilotinib (as well as imatinib and dasatinib), this exact binding is hindered, leading to resistance to the drug (Gorre et al. 2001).

Shortly, nilotinib therapy leads to fewer mutations of BCR-ABL conferring drug resistance compared to imatinib. Furthermore, most mutations acquired under treatment with imatinib can be overcome by further treatment with nilotinib. One of the most significant resistances still is the T315I mutation.

5 Toxicity

Despite being closely related to the first-generation drug imatinib, nilotinib shows quite a distinct profile in terms of toxicity (Kantarjian et al. 2006).

Already in early studies it was shown, that treatment discontinuation because of higher grade (grade 3/4) adverse events (AEs) was noticeably low with nilotinib. Further investigation even noted lower rates of the mentioned events as in a comparable study population treated with imatinib, thus making nilotinib an overall well-tolerated drug (Giles et al. 2012, 2013; Larson et al. 2012; le Coutre et al. 2012).

As typical in nearly all antileukemic drugs, hematological toxicity was most common. Grade 3 or 4 anemia could be noted in 3.9% of cases with neutropenia and thrombocytopenia showing rates of 11.8 and 10.4% of the same grades, respectively (Larson et al. 2012). Dose reductions or interruptions of the drug were common, whereas discontinuation was rare.

Among the non-hematological AEs, rash and fluid retentions were most common with both of these rarely occurring in higher grades. Even more important, fluid retention rates were significantly lower than with imatinib with the same

percentage of clinical relevant effusions (1.8%). Other clinical side effects included pancreatitis, hepatotoxicity, and significant bleeding.

Interestingly, Kim et al. could show high rates of thyroid dysfunction in patients treated with nilotinib. Both hypo- and hyperthyroidism were common. The mechanism still is unclear, and discontinuation of nilotinib was very rare in the study population. The same effect was found under treatment with imatinib and dasatinib. Thyroid dysfunction was noted in 25% of patients treated with imatinib, 55% of patients treated with nilotinib, and 70% of patients under dasatinib treatment, respectively (Kim et al. 2010).

Preclinical analyses hinted at a prolongation of the QTc interval by nilotinib; thus, electrocardiographic controls had to be conducted during all studies. Recent findings state no higher rate of relevant QTc prolongation under nilotinib than under imatinib with absolute numbers being considerably low (Larson et al. 2013). Nevertheless as the induction of cardiac arrhythmias could lead to severe complications up to cardiac death, the recent NCCN guidelines demand further precaution. Blood levels of potassium and magnesium should be taken care of and elevated to normal if necessary. Furthermore, the combination of additional drugs prolonging the QTc interval should be avoided (see below). During treatment, ECG should be performed regularly, in case of a prolonged QTc interval nilotinib should be either reduced in dosage or discontinued (Radich et al. 2017).

An increasing attention was paid to cardiovascular diseases and events in recent years. These were not obvious in the first years of nilotinib usage but emerged as distinct risks of the drug. Aischberger et al. and le Coutre et al. first described the increased risk of developing peripheral artery occlusive disease (PAOD) under treatment with nilotinib (Aichberger et al. 2011; le Coutre et al. 2011). These findings were later confirmed by analyses of the ENESTnd study, revealing a newly diagnosed PAOD in 1.4% and 1.8% of patients treated with nilotinib 300 mg twice daily and 400 mg twice daily. In contrast, no patient in the imatinib arm developed PAOD during the study course (Larson et al. 2013). Even prospective analyses were able to show significantly higher rates of PAOD and early stages of peripheral circulation disorders revealed by ankle–brachial index (ABI) (Kim et al. 2013). Most cases were found to occur in the first 48 months of nilotinib therapy. As a pathogenetic correlate, elevated levels of glucose and LDL were found in patients with nilotinib, pointing at a general role in atherosclerosis. Supporting these findings, other atherosclerotic-driven events such as ischemic heart attack or stroke were also more common in patients treated with nilotinib (Quintás-Cardama et al. 2012). The 2016 update of the ENESTnd trial revealed a rate of 4.7% of grade 3/4 cardiovascular events in patients treated with nilotinib 300 mg twice daily and 8.7% of patients treated with nilotinib 400 mg twice daily. In contrast, these events occured only in 1.8% of patients in the imatinib arm (Hochhaus et al. 2016a, b).

Giles et al. could recently show that age has a relevant effect on the cardiovascular toxicity of nilotinib (Giles et al. 2017). Details are shown in Table 4.

Steegmann et al. suggest regular assessment of cardiovascular risk profiles in their ELN toxicity recommendations. When treating with nilotinib, laboratory tests and the ABI should be performed every six to twelve months. In patients with a

Table 4 Rates of cardiovascular events in different age groups treated with nilotinib

Cardiovascular events	18–39 years (%)	40–59 years (%)	60–74 years (%)	≥ 75 years (%)
Total	0.8	5.3	10	13.5
Ischemic heart disease	0.4	2.8	5.7	9.6
PAOD	0.4	1.8	3.0	1.9
Ischemic cerebrovascular event	0	0.8	1.3	1.9

PAOD peripheral artery occlusive disease
Adopted from Giles et al. (2017)

high cardiovascular risk, nilotinib initiation is not recommended. In case of newly diagnosed PAOD, the drug should be discontinued (Steegmann et al. 2016).

In summary, nilotinib stays a favorable option in both first- and second-line treatment of CML with less overall toxicity compared to imatinib. Nevertheless, the distinct toxicity profile needs to be considered. Prolongation of the QTc interval and cardiovascular events might be rare but are of great risk concerning morbidity and mortality.

6 Drug Interactions

Two factors should be taken into account when thinking about the interaction of nilotinib with other drugs: On the one hand, the majority of patients of patients still requires a lifelong CML therapy, and on the other hand, most patients are diagnosed in the middle to elderly age. Thus, prescription of other medication and polypharmacy are common in CML patients.

As described above, nilotinib is known to prolong the QTc interval. Therefore, physicians need to take care of concomitant intake of other drugs with the same side effect. Known substances are amiodarone, digoxin or several opioids such as methadone (Radich et al. 2017).

Nilotinib was found to be metabolized via the cytochrome P450 system (CYP), precisely CYP3A4. Thus, induction or inhibition of this metabolization pathway has a significant effect on the patients' exposure to nilotinib. For example, it could be shown that ketoconazole and even grapefruit juice, known inhibitors of CYP3A4, are able to increase the exposure to nilotinib (Tanaka et al. 2011; Yin et al. 2010). Following the same principle, induction of CYP3A4, e.g., by rifampicin, leads to faster metabolization of nilotinib. As infections are a common complication in CML patients, these interactions need to be taken into account before starting an antimicrobial therapy.

Furthermore, Haouala et al. could show that nilotinib is a possible inhibitor of other cytochrome enzymes, namely CYP2C8, CYP2C9, CYP2D6, CYP3A4. Other inhibited systems include UGT1A1 and P-glycoprotein. Possible drug interactions

should therefore be considered when combining a broad variety of common substances, for example, vitamin K-antagonists (Haouala et al. 2011).

7 Biomarkers

Among the earliest markers for therapy response in CML was hematologic response in regard to peripheral blood count. After the introduction of the far more potent TKIs, precise markers were of need. These were identified as cytogenetic remission and molecular remission. The former describes the percentage of Philadelphia chromosome-positive cells in bone marrow tissue or the total absence, respectively (complete cytogenetic remission, CCyR). The latter refers to the percentage of BCR-ABL detected in a specimen on a standardized metering system, the International Scale (IS). An important milestone is the molecular remission^{4,5} ($MR^{4,5}$) defined as $\leq 0.0032\%$ IS. Defining this cutoff is especially important for a possible discontinuation of any CML-specific drug and increasingly displacing the older marker major molecular remission (MMR, more precise: $MR^3 \leq 0.1\%$ BCR-ABL) (Baccarani et al. 2009; Jain et al. 2013; Hochhaus et al. 2017).

The European Leukemia Net nowadays defines optimal response to any TKI in a first-line setting as achieving partial remissions in terms of cytogenetic and molecular marker with $\leq 35\%$ Philadelphia-positive cells and/or $\leq 10\%$ BCR-ABL three months after therapy initiation. At six months, either a CCyR or a BCR-ABL count <1% should be noted with the 12-month mark requiring a MR^3 defined as BCR-ABL $\leq 0.1\%$. The latter stays the minimum goal for optimal response to treatment at any time. If the first-line drug has to be discontinued for adverse events, the same numbers apply to the second-line treatment (Baccarani et al. 2013).

As technical progress goes on, even deeper molecular remissions can be distinguished, thus quantifying the BCR-ABL-count is becoming the most important biomarker for assessing treatment response in CML patients. Being able to perform this test on peripheral blood makes it even easier in contrast to the classic chromosome banding analysis performed on bone marrow tissue.

8 Summary and Perspective

Summarizing, nilotinib is a potent second-generation TKI for the treatment of chronic myeloid leukemia with growing importance over the last decade. Currently, it is not only approved as a first-line treatment for newly diagnosed CML in CP, it is even superior to the established therapy with imatinib in regard to rates of deep cytogenetic and molecular remission. Additionally, rates of disease progression are notably lower under treatment with nilotinib. However, the drug shows quite a different profile in terms of side effects, especially cardiovascular diseases and

events are still among the most severe ones. Therefore, cardiovascular risk factors need to be monitored regularly during nilotinib treatment. A history of cardiovascular events or a high cardiovascular risk profile still are contraindications for an initiation of the drug, even more in light of equivalent TKIs available.

With growing experience with potent second-generation TKIs, more and more patients reach long-term deep remissions, thus raising claims of therapy discontinuation. Recent studies have proven the latter to be a safe possibility, with about half of the eligible patients staying in the mentioned remission without therapy. Further studies will have to determine the long-term outcome of these cases.

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