Published in final edited form as:

Semin Oncol. 2011 April ; 38(0 1): S3–S9. doi:10.1053/j.seminoncol.2011.01.016.

Nilotinib: a Novel, Selective Tyrosine Kinase Inhibitor

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Abstract

The development of tyrosine kinase inhibitors (TKIs) for the treatment of chronic myelogenous leukemia (CML) was based on the discovery that CML stem and progenitor cells overexpress the abnormal fusion protein kinase BCR-ABL. The prototype TKI, imatinib, selectively inhibits BCR-ABL, as well as several other kinases, including DDR, KIT, PDGFR, and CSF-1R. Although the management of CML improved dramatically with the introduction of imatinib, not all patients benefit from treatment because of resistance or intolerance. Consequently, research efforts have focused on developing more potent TKIs with the ability to circumvent imatinib resistance. Nilotinib, a second-generation oral TKI, was rationally designed based on the crystal structure of imatinib to be highly active against a wide range of imatinib-resistant BCR-ABL mutants and is approved for the treatment of newly diagnosed or imatinib-resistant or -intolerant CML, and has shown superiority over imatinib in first-line in newly diagnosed CML. Furthermore, the activity of nilotinib against KIT and PDGFR α has led to its evaluation in advanced gastrointestinal stromal tumors (GIST). The purpose of this review is to describe the development of nilotinib, providing a structural explanation for the differential activity of nilotinib and imatinib in GIST. Activity of nilotinib against KIT and PDGFR and emerging evidence of differences in cellular uptake between nilotinib and imatinib are discussed.

Keywords

Nilotinib; AMN107; fusion proteins; BCR-ABL; stem cell factor receptor; receptors; platelet-derived growth factor; gastrointestinal stromal tumors; drug resistance

INTRODUCTION

The design and development of tyrosine kinase inhibitors (TKIs) for the treatment of chronic myelogenous leukemia (CML) was driven by the knowledge that the protein kinase BCR-ABL, encoded by the *BCR-ABL* fusion oncogene, is constitutively activated in patients with

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this disease. This oncogene is present in 95% of patients with CML and is the result of a chromosomal aberration known as the Philadelphia chromosome (Ph), which arises from the accidental fusion of the *BCR* gene with the gene encoding for the intracellular non-receptor tyrosine kinase c-ABL.¹ In normal cells, the activity of ABL1 is tightly controlled; in contrast, BCR-ABL fusion proteins have constitutive catalytic activity leading to cell transformation and ultimately uncontrolled cellular proliferation and reduced apoptosis.^{2,3} Based on this premise, BCR-ABL kinase represents a logical therapeutic target for the development of drugs to treat CML.

The small-molecule prototype TKI, imatinib mesylate (Glivec[®], formerly known as STI-571, Novartis Pharma AG, Basel, Switzerland), selectively targets BCR-ABL and also the stem cell factor receptor (KIT), discoidin domain receptor (DDR), and platelet-derived growth factor receptor (PDGFR) tyrosine kinases.⁴ Imatinib was the first TKI to be licensed for the treatment of CML; however, despite its well-established efficacy in this setting,⁵ many patients fail on imatinib therapy due to the development of resistance or loss of response. Resistance to imatinib may be categorized as primary, manifesting as a lack of efficacy from the very start of therapy, or secondary (acquired resistance), defined as a lack of response in patients who initially responded to treatment.⁶ Point mutations within the kinase domain of BCR-ABL that reduce the binding affinity of imatinib to the protein are the most common cause of imatinib resistance. However, increased expression of BCR-ABL kinase through gene amplification, overexpression of the SRC family of kinases, and pharmacokinetic and pharmacodynamic factors also are understood to play a role in the development of resistance in some patients.^{6,7}

Nilotinib (Tasigna[®], formerly known as AMN107, Novartis Pharma AG, Basel, Switzerland) is a new oral TKI, rationally designed to overcome imatinib resistance in CML.⁸ In Phase I and II studies, nilotinib achieved good tolerability and durable responses in adult patients with Ph+ CML resistant or intolerant to at least one prior therapy, including imatinib.^{9,10} This subsequently resulted in the approval of nilotinib for the treatment of patients with newly diagnosed CML or imatinib-resistant/-intolerant patients with chronic or accelerated-phase CML.^{11–13} In addition to inhibiting BCR-ABL, nilotinib, similar to imatinib, also has potent activity against the DDR, KIT, PDGFR, and colony stimulating factor receptor-1 (CSF-1R) tyrosine kinases.^{8,14,15} Mutations in the genes encoding for these tyrosine kinases have been shown to play a key role in the pathogenesis of certain malignancies, including gastrointestinal stromal tumors (GIST), subtypes of melanoma, and pigmented villonodular synovitis (PVNS).^{16–18} In recent years, the introduction of TKI therapy, in the form of imatinib, has significantly improved the outcome of patients with GIST.¹⁹ Further progress continues to be made in the treatment of this malignancy, with the ongoing clinical evaluation of nilotinib and other TKIs in patients with advanced GIST in several different treatment settings.

CHEMICAL STRUCTURE OF NILOTINIB

A phenylamino-pyrimidine derivative, nilotinib was rationally designed based on the crystal structure of imatinib and an understanding of the molecular mechanism of imatinib activity in relation to inhibition of BCR-ABL kinase (Figure 1).²⁰ Imatinib has a high affinity for

ABL kinase. Like others, this kinase incorporates a highly conserved bi-lobed structure with an ATP binding domain situated in a deep cleft between the N- and C- terminal lobes. Adjacent to this is the centrally located activation loop that incorporates a conserved Asp-Phe-Gly (DFG) sequence and controls catalytic activity by switching between different states in a phosphorylation-dependent manner.³ Autophosphorylation of the activation loop is essential for BCR-ABL activity, as without it the activation loop is not properly positioned for catalysis and ATP binding is not feasible.²¹ Imatinib achieves BCR-ABL kinase inhibition by binding to the inactive, unphosphorylated conformation of the kinase (DFG-out), thereby reducing the availability of the catalytically active phosphorylated conformation (DFG-in), necessary for ATP binding. This blocks signal transduction, ultimately resulting in inhibition of proliferation and loss of viability and proliferation.^{3,21,22} As there is much greater structural heterogeneity between the inactive conformations of protein kinases in comparison to the highly conserved active conformations, drugs like imatinib have much greater target selectivity than those agents that bind to active conformations. Examination of spatial changes in the ABL kinase domain that allow imatinib to bind led researchers to hypothesize that the selectivity and potency of imatinib for BCR-ABL kinase could be improved upon by filling the imatinib binding site more efficiently by taking advantage of its lipophilicity. Based on this principle, nilotinib was designed to maintain binding to the inactive conformation of the ABL kinase domain, while incorporating alternative binding groups to the *N*-methylpiperazine moiety and preserving an amide pharmacophore to retain H-bond interactions with Glu286 and Asp381.²⁰ Substitution of the *N*-methylpiperazine moiety present in imatinib by a phenyl group bearing trifluoromethyl and imidazole substituents in the nilotinib structure greatly contributes to the potency of nilotinib by reducing the requirement for hydrogen bonding with nilotinib (4 H-bond interactions compared with 6 H-bonds for imatinib). As described later in this review, these structural modifications provide nilotinib with greater affinity and inhibitory activity compared with imatinib against wild-type BCR-ABL kinase, while preserving activity against KIT and PDGFR kinases.^{8,20} In addition, the structural differences between nilotinib and imatinib are reflected in minimal cross-intolerance between the two drugs with respect to non-hematologic toxicities in patients with CML.^{14,23} Among patients with imatinib-resistant CML, nilotinib has not been associated with the toxic effects commonly seen with imatinib treatment, such as fluid retention, edema, and weight gain.⁹

NILOTINIB: MECHANISM OF ACTION IN GIST

Because of its chemical structure, nilotinib has demonstrated anticancer activity in CML through its ability to selectively inhibit autophosphorylation of BCR-ABL. Small changes in the mode of binding to ABL enable nilotinib to inhibit cellular proliferation of imatinib-sensitive and -resistant CML cell lines more potently than imatinib.^{8,20,24,25} Additional preclinical work indicated that the anticancer activity of nilotinib in CML is antiproliferative rather than proapoptotic.²⁶

Although nilotinib was not rationally designed specifically to treat GIST, its *in vitro* activity against kinases other than BCR-ABL, particularly KIT- and PDGFR α (both of which are known to be oncogenic in GIST) led to its evaluation in this setting. Specific oncogenic mutations within the KIT or PDGFR α receptor tyrosine kinase lead to constitutive

phosphorylation and activation of the receptor resulting in uncontrolled cellular proliferation and the subsequent development of several human malignancies, including GIST. Four different regions of KIT are known to be mutated in GIST, and of these the juxtamembrane portion coded for by exon 11 is most often affected (~67% of GIST), followed by mutations in exon 9 (~10%), exon 13 (~1–2%), and exon 17 (~1%).²⁷ Across all known mutation types, approximately 80% of GIST harbor mutations in *KIT*, while 5–8% have mutations in *PDGFRA* and 12–15% are wild-type.²⁸

Activity against KIT and mutant cell lines

In vitro studies have shown nilotinib to be a potent inhibitor of several *KIT* mutant cell lines (Table 1). Nilotinib was as effective as imatinib at inhibiting the proliferation of exon 11 *KIT*^{V560del} and exon 13 *KIT*^{K642E} mutant cells²⁰ and also potently inhibited the proliferation of exon 11 *KIT*^{V560G} mutant cells *in vitro* (IC₅₀ 108 nM).²⁹ Activity against xenografts harboring *KIT*^{V560G} mutant-tumors has also been demonstrated *in vivo*.³⁰ Against murine Ba/F3 cells expressing wild-type KIT, nilotinib also was a more potent inhibitor of cellular proliferation compared with other TKIs, achieving an IC₅₀ of 35 nM compared with 245, 316, 910, and 3132 nM for the TKIs sunitinib, dasatinib, sorafenib, and imatinib, respectively.³¹

In addition to its activity against cell lines expressing single *KIT* mutations, nilotinib also has demonstrated *in vitro* activity against double mutations involving *KIT* exons 13 or 17, which confer resistance to imatinib. Approximately half of all patients with GIST who experience an initial response to imatinib therapy will develop imatinib resistance as a result of secondary mutations in the KIT kinase domain. These most commonly occur in exon 17, although single amino acid mutations in exons 13 and 14 also have been reported.³² Nilotinib was more effective than dasatinib and sorafenib in inhibiting the growth of imatinib-resistant cells carrying *KIT* double mutants involving exon 11 and exons 13 (*KIT*^{V560del/V654A}) or 17 (*KIT*^{V559D/D820Y}) mutations. For *KIT*^{V560del/V654A} mutations, an IC₅₀ value of 192 nM was reported for nilotinib compared with 585 and 1074 nmol/L for dasatinib and sorafenib, respectively. Corresponding IC₅₀ values for the *KIT*^{V559D/D820Y} mutation were 297, 432, and 944 nM, respectively.³³ In contrast to imatinib, nilotinib also inhibited the proliferation of FDC-P1 cells expressing *KIT*^{V560G/V564A} mutations to a similar extent to wild-type *KIT* in autophosphorylation and cell proliferation assays, suggesting that nilotinib may be less susceptible to resistance arising from the V654A mutation that is frequently associated with imatinib resistance.^{29,34} By modifying the structure of the kinase domain, this missense mutation is thought to confer resistance by interfering with interactions between imatinib and the receptor.

Activity against PDGFRα and PDGFRβ mutant cell lines

In studies of other receptor tyrosine kinases implicated in the oncogenesis of GIST, nilotinib achieved potent and selective inhibition of PDGFRα and PDGFRβ. As is the case with imatinib, nilotinib potently inhibited the autophosphorylation of A31 cells transformed by *PDGFRA* and *PDGFRB* double mutations (Table 1).²⁰ Nilotinib also showed *in vitro* and *ex vivo* activity similar to imatinib against FIP1L1-PDGFRα,³⁵ a fusion protein associated with the myeloproliferative syndrome hypereosinophilia (HES),³⁶ as well as the D842V- and

V561-*PDGFRA*-Ba/F3 mutant cell lines.³⁷ Against EOL-1 cell lines with constitutive activation of FIP1L1-*PDGFRα*, nilotinib was as effective as imatinib at inhibiting proliferation (IC₅₀ 0.54 and 0.20 nM, respectively) and inducing apoptosis. Nilotinib and imatinib also were equipotent as inhibitors of *PDGFRα* phosphorylation in EOL-1 cells and primary cells from patients with HES.³⁵ Furthermore, in Ba/F3 cells transformed by *FIP1L1-PDGFRα*, nilotinib inhibited cell proliferation (IC₅₀ <25 nM) and phosphorylation of this fusion kinase and the downstream signaling target STAT5.³⁸ Nilotinib also showed *in vitro* activity against Ba/F3 cells expressing FIP1L1-*PDGFRα*/T674I, a mutation that renders these cells resistant to imatinib.³⁹ The possibility that the development of resistance to imatinib in patients with HES may parallel that in CML suggests that nilotinib may have a potential role in the treatment of CML and other myeloproliferative disorders. Additional antitumor effects have been observed against *PDGFRB* mutations in that nilotinib prolonged survival of mice with *TEL-PDGFRB* mutations in an *in vivo* model.³⁸

CELLULAR UPTAKE OF NILOTINIB AND IMATINIB IN GIST CELL LINES

Cellular uptake mechanisms of imatinib and nilotinib appear to be different.⁴⁰ Imatinib is transported intracellularly via an active transporter mechanism involving the organic cation transporter-1 (OCT-1) influx protein.⁴⁰ Inhibition of OCT-1 results in significantly reduced intracellular uptake and retention (IUR) of imatinib at all concentrations ($P < 0.001$) but does not significantly affect the IUR of nilotinib ($P > 0.05$). This difference (OCT-1 transport) may contribute to the interpatient variability in drug blood levels observed with imatinib but not nilotinib. Moreover, because the majority of substrates transported by OCT proteins are cations, imatinib uptake also may be influenced by the degree of protonation of the basic N-methylpiperazine group at physiological pH, a group that is absent in nilotinib. The lack of identified active transport mechanisms for nilotinib intracellular concentration suggests that nilotinib transport is mostly passive. Because imatinib and nilotinib display similar potency *in vitro*, a more uniform cellular uptake via passive transport may result in an advantage for nilotinib over imatinib in diseases driven by KIT and *PDGFR*.⁴¹

Analysis of the cellular uptake of nilotinib and imatinib in GIST cell lines is suggestive of increased cellular uptake or decreased cellular efflux of nilotinib compared with imatinib. In an investigation involving two human GIST patient-derived cell lines, both of which expressed constitutively activated KIT, uptake of nilotinib was significantly higher than that of imatinib. Compared with imatinib, uptake of nilotinib was 7-fold higher in the imatinib-sensitive cell line GIST882 and 10-fold higher in the imatinib-resistant cell line GIST GDG1. Furthermore, combining the two compounds had no influence on the uptake of either agent in GIST882 cells, and uptake of imatinib was not influenced by co-administration with nilotinib in GIST GDG1 cells. However, the uptake of nilotinib was significantly increased when combined with imatinib 1 μM in GIST GDG1 cells (Figure 2).⁴² A more recent investigation also reported an increase in the IUR of nilotinib following co-administration with imatinib. Assays to measure IUR in five newly diagnosed patients with CML and two cell lines revealed an increase in the IUR of ¹⁴C-labelled nilotinib (1 and 2 μM) following administration of unlabelled imatinib; however, when the converse was performed (administration of ¹⁴C-labelled imatinib and unlabelled nilotinib), the IUR of imatinib was decreased in most patients.⁴³ These findings have led to the suggestion that the increase in

nilotinib intracellular uptake mediated by imatinib may be responsible for the synergy observed between the two drugs in *BCR-ABL* leukemic cell lines.⁴⁴

Based on the differential cellular uptake of nilotinib and imatinib, it has been suggested that nilotinib may be less susceptible to cellular transport-driven imatinib resistance.⁴² Available evidence indicates that multi-drug-resistant (MDR) P-glycoprotein (PGP) functions as a transporter of imatinib in transformed cell lines and that MDR1 expression acts to confer drug resistance.^{40,45–47} One of the consequences of transport-driven imatinib resistance in GIST cells and the resulting decrease in intracellular concentrations of imatinib may be a reduced likelihood of cellular apoptosis, leading to the generation of imatinib-resistant subclones. Decreased intracellular concentrations of imatinib also may potentiate the selection of cells harboring mutant copies of the *BCR-ABL* gene, making the tumor cell resistant to the compound.⁴⁰ However, nilotinib does not appear to be a substrate of imatinib transport pumps, suggesting a stability in intracellular drug levels that may benefit patients with GIST, particularly those in whom imatinib resistance has developed.⁴²

NILOTINIB MECHANISM OF ACTION IN PVNS

In addition to the *BCR-ABL*, *KIT*, and *PDGFR* kinases, nilotinib has also shown *in vitro* activity against *CSF1R*, albeit weaker than that of imatinib, which is strongly expressed in PVNS.^{14,18} This benign but painful neoplastic condition, also known as tenosynovial giant cell tumor (TGCT), affects the synovium in young and middle-aged adults with the potential for severe joint morbidity and results from a specific alteration that affects the *CSF1* gene.^{18,48} Inhibition of *CSF-1R* with imatinib has been reported to produce tumor remission in patients with PVNS, suggesting that targeting this pathway can provide additional treatment options beyond surgery for this tumor type.^{49–51} Nilotinib is being investigated for this indication.

CONCLUSIONS

The unique binding characteristics of nilotinib—combined with the limitations of other available TKIs—have led to the investigation of nilotinib in GIST. Although the prototype TKI imatinib transformed the treatment of patients with GIST, the increasing incidence of acquired resistance to imatinib and, subsequently to sunitinib, in patients with this malignancy suggests that other therapeutic agents are needed.^{32,34} Sunitinib is commonly used as a second-line TKI following imatinib failure, but it typically provides only ~6 months of additional time to tumor progression and ~6 months of additional progression-free survival.⁵²

Among the multiple mechanisms believed to contribute to imatinib resistance in GIST, secondary mutations in the *KIT* kinase domain, most commonly exon 17 and to a lesser extent exons 13 and 14, are known to make a significant contribution.³² Notably, nilotinib has demonstrated potent antiproliferative activity against cell lines harboring such *KIT* mutations, including *KIT* double mutants involving exon 11 and exons 13 or 17,³³ and also against cells harboring the missense V654A mutation that is frequently associated with imatinib resistance.^{29,34} These findings, together with emerging evidence suggesting a

reduced susceptibility of nilotinib to cellular transport-driven imatinib resistance, highlight the potential activity of nilotinib against *KIT* mutations responsible for clinical resistance in certain patients with GIST. Recently reported data with nilotinib in patients with GIST who had progressed on available approved methods of treating the disease show that it provides significant clinical benefit in this patient population.^{53,54} Moreover, the structural differences between nilotinib and imatinib suggest potential differences in safety as evidenced by the minimal cross-intolerance between the two drugs. Based on this premise, nilotinib was evaluated in the third-line setting, with assessment of these data indicating that it may have some clinical benefit in patients that have progressed on imatinib and sunitinib (see article 3 of this series). Other trials are under way that are designed to leverage nilotinib's unique biochemical properties compared with other TKIs in the treatment of patients in the first-line setting (ENEST g1) and as an alternative to dose escalation for patients progressing on imatinib 400 mg/d (LANGIST). These trials are described in the third article in this series. Taken together, nilotinib is a novel TKI that has potent activity against GIST cells and has been shown to provide clinical benefit in GIST patients who have limited treatment options.

Acknowledgments

Financial support for medical editorial assistance was provided by Novartis Pharmaceuticals. We thank Angelo Russo for his medical editorial assistance with this manuscript.

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Figure 1.

Molecular structures of nilotinib and imatinib²⁰

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Figure 2.

Intracellular uptake of nilotinib and imatinib in GIST882 cells and imatinib-resistant GIST GDG1 cells⁴²

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Table 1

Activity of nilotinib and imatinib against kinases involved in the pathogenesis of GIST. IC₅₀ values are presented as means.

<i>KIT</i> mutation (cell lines)	Nilotinib IC ₅₀ , nM	Imatinib IC ₅₀ , nM	Reference
Exon 11 <i>KIT</i> ^{V560del} (Ba/F3)	26	27	20
Exon 13 <i>KIT</i> ^{K462E} (GIST882)	160	120	20
Exon 11 <i>KIT</i> ^{V560G} (HMC-1 ⁵⁶⁰)	108	74	55
Exon 11/exon 13 <i>KIT</i> ^{V560del/V654A} (Ba/F3)	192	3927	33
Exon 11/exon 17 <i>KIT</i> ^{V559D/D820Y} (Ba/F3)	297	3202	33
<i>PDGFRA</i> + <i>PDGFRB</i> (A31)	71 ^a	74 ^a	20
<i>PDGFRB</i> (Tel Ba/F3)	57	39	20

^aData shown represent kinase autophosphorylation.