Refining targeted therapies in chronic myeloid leukemia: development and application of nilotinib, a step beyond imatinib

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Abstract: The BCR-ABL kinase inhibitor imatinib mesylate is currently the standard therapy for patients with chronic myeloid leukemia (CML). Despite the remarkable results achieved with imatinib for the treatment of CML, the emergence of resistance to this drug has become a significant problem. Mutations within the ABL kinase domain have been identified as the main mechanism of resistance to imatinib. Other mechanisms include genomic amplification of BCR-ABL and modulation of drug efflux or influx transporters. Several strategies have been developed to overcome the problem of imatinib resistance, including dose escalation of imatinib, combination treatments, or novel targeted agents. Nilotinib is a tyrosine kinase inhibitor 30-fold more potent than imatinib, active against a wide range of mutant clones, except T315I. Phase I–II trials of nilotinib showed high activity in imatinib-resistant CML and Ph+ acute lymphoblastic leukemia. We here review the development of nilotinib and the activity of this agent in CML patients and in other forms of sensitive neoplasms.

Keywords: myeloid leukemia, nilotinib, imatinib, neoplasms

Introduction

Chronic myelogenous leukemia (CML) is a clonal disorder caused by the malignant transformation of a pluripotent hematopoietic stem cell. It is characterized by the Philadelphia chromosome (Ph), a genetic abnormality which arises from the reciprocal translocation t(9; 22) (q34; q11) (Faderl et al 1999). This translocation fuses the genes encoding BCR and ABL, resulting in expression of the constitutively active protein tyrosine kinase, BCR-ABL. The Ph chromosome is present in more than 90% of adult CML patients, in 15%–30% of adult acute lymphoblastic leukemia (ALL), and in 2% of acute myelogenous leukemia (AML) (Goldman and Melo 2003). Different molecular weight isoforms are generated, based on different breakpoints and mRNA splicing. Most CML patients have a fusion protein of 210 kDa while approximately 30% of Ph+ ALL cases and few CML cases are associated to 190 kDa BCR-ABL protein (Sattler et al 2003). CML normally progresses through three clinically recognized phases: about 90% of patients are diagnosed during the typically indolent chronic phase (CP), which is followed by an accelerated phase (AP) and a terminal blastic phase (BP). Twenty to 25% of patients progress directly from CP to BP and the time course for progression can be extremely varied. The mechanisms behind CML progression are not fully understood (Goldman and Melo 2003). There is increasing evidences that Src family kinases are involved in CML progression through induction of cytokine independence and apoptotic protection (Donato et al 2003).

The function of BCR-ABL has allowed the design and development of imatinib, a small-molecule kinase inhibitor that targets PDGFR, c-Kit, and ABL kinases

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(Deininger et al 2005). Imatinib provides an effective and durable therapy for CML: a recent 6-year follow-up of phase III International Randomized IFN versus STI571 (IRIS) study, showed that this agent induced complete hematologic remission in the majority (98%) of newly diagnosed patients in chronic phase of the disease and complete cytogenetic remission (CCR) in about 87% of patients (Druker et al 2006).

With the high rate of complete cytogenetic responders, the goal of therapy has become the achievement of molecular responses, as measured by the reduction or elimination of BCR-ABL transcript. Major molecular response (MMR) in the IRIS trial was defined as a >3 log reduction in transcript from baseline. Obtaining MMR was associated with significantly better long-term remission duration and progression-free survival (PFS). At 60-month follow-up, achievement of CCR and MMR by 12 months was associated with a PFS of 97% compared with 89% for patients with CCR but with less MMR (Druker et al 2006). Early molecular response predicted a better outcome: progression of disease correlated with failure to achieve a 1 log reduction in transcript level by 3 months and a 2 log reduction by 6 months (Druker et al 2006).

In 2006, recommendations for definitions of failure/resistance and suboptimal response to imatinib were proposed by European LeukemiaNet (Baccarani et al 2006); failure was defined as no complete hematologic response at 3 months, no cytogenetic response at 6 months, less than partial cytogenetic response at 12 months, less than complete cytogenetic response at 18 months, or loss of complete hematologic response (CHR) or CCR, or acquisition of BCR-ABL mutations at any time. Suboptimal response was defined as incomplete hematologic response at 3 months, less than partial CR at 6 months, less than complete cytogenetic response at 12 months, and less than MMR at 18 months, or acquisition of cytogenetic abnormalities in Ph+ cells, mutations of BCR-ABL or loss of MMR at any time (Baccarani et al 2006).

Despite the apparent success of imatinib therapy, the IRIS study at 5 years follow-up provides an indication of imatinib failure: 31% of patients have discontinued imatinib, with an estimated resistance (lack or loss of response) of 14% (Druker et al 2006). The estimated annual rate of treatment failure after the start of imatinib therapy was 3.3% in the first year, 7.5% in the second year, 4.8% in the third year, 1.5% in the fourth year, and 0.9% in the fifth year. The corresponding annual rates of progression to AP or BP were 1.5%, 2.8%, 1.6%, 0.9%, and 0.6%, respectively.

Resistance can be defined on the basis of its time of onset: primary resistance is a failure to achieve a significant hematologic or cytogenetic response, whereas secondary or acquired resistance is the progressive reappearance of the leukemic clone after an initial response to the drug (Baccarani et al 2006). Resistance is also defined on the basis of clinical and laboratory criteria used for detection of leukemia, which includes hematological, cytogenetic, and molecular resistance. Hematological resistance is a lack of normalization of peripheral blood counts and spleen size; cytogenetic resistance is a failure to achieve a major cytogenetic response; and molecular resistance represents the failure to achieve or loss of complete or MMR (Hochhaus and La Rosee 2004).

Several mechanisms may contribute to imatinib resistance, including increased expression of BCR-ABL kinase through gene amplification, decreased intracellular drug concentrations caused by drug efflux proteins (OCT1), clonal evolution, or over-expression of Src kinases (Lyn, Hck) involved in BCR-ABL independent activation of alternative pathways (Apperley 2007). Emergence of clones expressing mutant forms of BCR-ABL, in which amino acid substitutions in the ABL kinase domain impair imatinib binding trough disruption of critical contact point by inducing a switch from the inactive to active conformation are the most frequent cause of resistance.

The first reported clinical resistance BCR-ABL mutation was T315I, a single amino acid substitution of the threonine 315 residue to an isoleucine: this blocked imatinib binding by suppressing a drug kinase hydrogen bond and by creating a steric clash between imatinib and the bulky hydrocarbon side chain of the isoleucine residue in the mutant, while preserving the ATP-binding and kinase activity of the BCR-ABL protein. To date, more than 50 different point mutations encoding for more than 40 distinct single amino acid substitutions in the BCR-ABL kinase domain have been isolated from CML-relapsed patients, and preclinical studies have demonstrated that mutations outside the domain can also result in molecular conformations of BCR-ABL that impair imatinib binding. Mutations are more frequent in patients with acquired resistance compared with primary resistance patients, whereas in early CP patients they are rare (Hochhaus et al 2007).

Early detection of BCR-ABL mutants may identify patients who are likely to become resistant to imatinib therapy, in whom optimization of treatment such as dose escalation, drug combination, or second-generation tyrosine kinase inhibitors are requested. There is currently no

consensus on which technique should be used for mutation analysis screening, and highly sensitive detection methods can increase the detection rate of point mutations. Allele specific oligonucleotide polymerase chain reaction methods have permitted the detection of mutants prior to imatinib therapy. Mutations can also be detected in an automated manner by denaturing high-performance liquid chromatography (DHPLC), which has been described as a highly sensitive screening method, even when the site of mutation is unknown (Baccarani et al 2008). Recent work by Ernst et al (2008) proved that the occurrence of mutations during imatinib therapy is predictive of relapse and that mutations may be detectable several months before relapse; their identification could provide clinical benefit for patients and lead to early reconsideration of therapeutic strategies (Ernst et al 2008).

Higher doses of imatinib and combination therapy with other agents have been used to overcome imatinib resistance (Kantarjian et al 2003). Imatinib dose escalation is an accepted clinical strategy, and doubling the dose in patients treated with standard dose allowed hematological and cytogenetic responses in half of patients treated with this procedure. Some investigators have questioned the durability of responses after imatinib dose escalation (Marin et al 2003; Zonder et al 2003). Increasing the dose of imatinib is likely useful only in a subset of patients with imatinib cytogenetic resistance, whereas alternative treatment options are required for the other subset of resistant patients.

A series of compounds commonly referred to as second-generation tyrosine kinase inhibitors, have been developed to improve the results obtained with imatinib. In this article, we provide an overview of development and clinical results of nilotinib, a new tyrosine kinase inhibitor that received approval by US Food and Drug Administration (FDA) on October 2007, for the use in treatment of patients with CP and AP CML resistant or intolerant to imatinib.

Development and biochemical and molecular characterization of nilotinib

The development of nilotinib was based upon the crystal structure of the imatinib-ABL complex (Figure 1, Schindler et al 2000; Manley et al 2004). Based on these 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 H-bond interactions to Glu286 and Asp381 (Manley et al 2004). This approach resulted in the discovery of AMN107, or nilotinib. Nilotinib has superior potency compared with imatinib as an inhibitor of BCR-ABL in vitro and in vivo: it is 10- to 30-fold more potent, as assessed by its ability to block proliferation of BCR-ABL dependent cell lines derived from CML patients (K562, Ku-812F) and cell lines (32D or Ba/F3) transfected to express the BCR-ABL protein (Figure 2; Weisberg et al 2005). Similarly, nilotinib is 10- to 20-fold more active than imatinib in reducing BCR-ABL autophosphorylation (IC_{50} values ranging from 20–60 nM). Unlike imatinib, this drug 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 Thr315, the amido-NH and side chain carboxylate of Glu 286 and the amido C = O with the backbone-nh of the Asp 381 (Manley et al 2005). The increased potency of nilotinib against ABL is the result of a better fit with the active site in the kinase domain. Like imatinib, nilotinib binds the hydrophobic pocket referred to as the DGF (asparagines, glycine, phenylalanine)

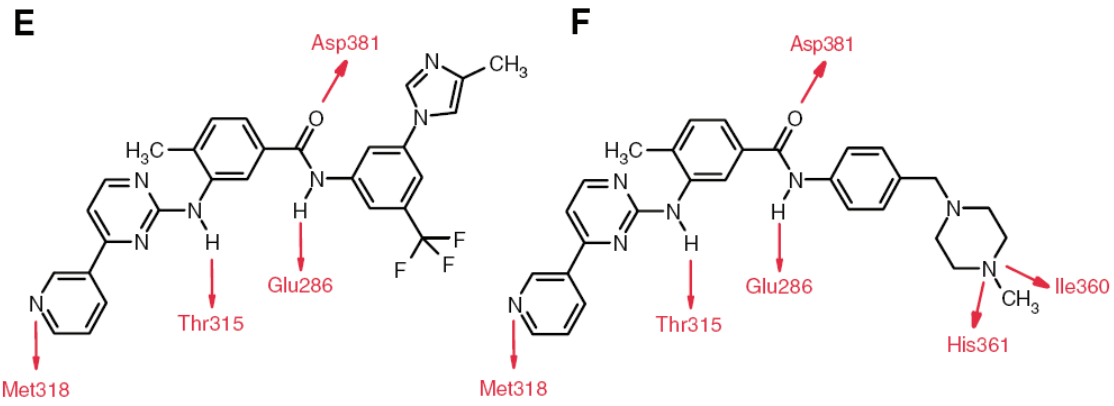

Figure 1 The molecular structures of nilotinib (B) and imatinib (C) are depicted, with their respective H-bond interactions with the ABL kinase domain (red).

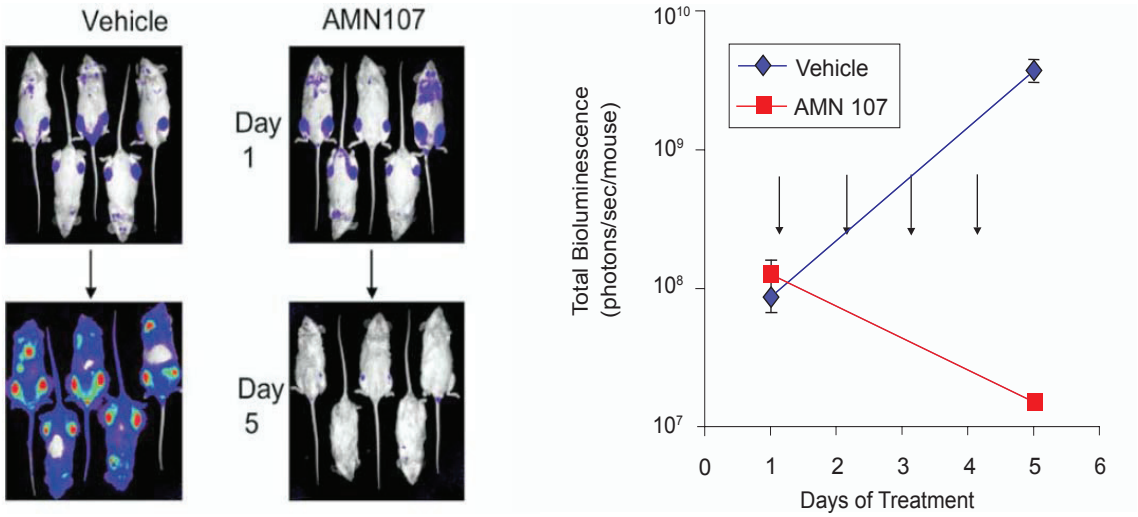

Figure 2 Activity of nilotinib against 32D.p210- and 32D-E255V-Luc+ cells in vivo. Adapted from Weisberg E, Manley PW, Breitenstein W, et al. 2005. Characterization of AMN107, a selective inhibitor of native and mutant BCR-ABL. *Cancer Cell*, 7:129–41. Copyright © 2005 with permission from Elsevier.

motif, which is displaced away from the ATP-binding site (DGF-out) upon adoption of the active conformation by the BCR-ABL kinase (Hubbard et al 1994; Liu and Gray 2006). This specific mode of binding is the basis of the selectivity of imatinib and nilotinib. Nilotinib also inhibits the tyrosine kinase activity of the PDGF and c-Kit receptors, displaying similar efficacy to imatinib, and possessing greater selectivity toward BCR-ABL. Nilotinib showed no activity against a wide panel of other protein kinases at concentrations below 3 μM, including c-Src.

Nilotinib is more potent than imatinib against BCR-ABL mutant isoforms: in vitro results

Nilotinib is >30-fold more potent than imatinib in the killing of wild-type BCR-ABL expressing cells (Manley et al 2005; Weisberg et al 2005), and 43- to 60-fold more potent than imatinib against cell lines KBM5 and KBM7 (Golemovic et al 2005). It has no activity against T315I even at concentrations up to 10 μM: as with imatinib, the lack of activity against T315I is the result of nilotinib binding closely to the T315 residue, such that loss of the hydroxyl side chain and additional methyl group of the isoleucine inhibits binding (O'Hare et al 2005). Four mutant isoforms (E255K, E255V, L248R, Y253H) were found to confer intermediate sensitivity to nilotinib when transfected into Ba/F3 cells (Weisberg et al 2005). In vitro activity was demonstrated in severe combined immune-deficient mice (NOD-SCID) subjected to sublethal doses of radiation and transplanted with murine p210 positive cells engineered to express mutations; activity was demonstrated also in bone marrow cells from normal mice transduced with normal and mutant cells (E255V): treatment with nilotinib prolonged survival and reduced the accumulation of leukemic cells in the spleen, bone marrow, lymphonodes and liver (Weisberg et al 2005). Recently, using cell-based screening, it was identified which mutants might arise under nilotinib therapy: in contrast to imatinib, resistance to nilotinib was associated with a limited spectrum of BCR-ABL kinase mutations. With the exception of T315I, all of the mutations that were identified were effectively suppressed when the nilotinib concentration was increased to 2,000 nM, which is the peak of range of plasma concentration measured in patients treated with 400 mg twice daily. Resistance mutations that may arise in condition of suboptimal nilotinib concentration include p-loop mutations, such as Y253H and E255V. Because plasma levels of up to 3,600 nM were measured in phase I and II, p-loop mutations may emerge less frequently than with imatinib therapy and it is expected that nilotinib induces responses in cases in which p-loop mutations cause resistance to imatinib mesylate (von Bubnoff et al 2006).

With a N-ethyl-N-nitrosourea (ENU)-based mutagenesis screen, imatinib, nilotinib, and dasatinib (a 325-fold more potent inhibitor active against BCR-ABL and src kinases family) were compared; 20 different mutations were recovered upon treatment with imatinib, while only 10 and 9 were recovered after exposure to nilotinib or dasatinib, respectively. The mutations recovered after exposure to intermediate concentrations of nilotinib included T315I,

Y253H, and E255V, whereas at concentrations close to the maximal achievable plasma levels, only T315I was isolated (Bradeen et al 2006). In 2007, Ray et al conducted a saturation mutagenesis screening for nilotinib-resistant BCR-ABL subclones, using a method previously described by Azam et al and Cools et al for imatinib against BCR-ABL and PKC412 against FLT3 (Ray et al 2007; Azam et al 2003; Cools et al 2004). With the exception of T315I, all mutations detected in this study were inhibited by nilotinib plasma level concentrations: this suggested that the clinical resistance to nilotinib might be predominantly associated with the emergence of T315I. The results of the mutagenesis screen also indicated that the mutations of the p-loop, C-helix, SH2 contact, and the A-loop might occur less frequently or would be manageable with moderate dose escalation of nilotinib (Ray et al 2007).

Synergistic effects between imatinib and nilotinib

Based on previously a described interaction between imatinib and nilotinib in mouse expressing p210 positive cells, Weisberg et al showed positive cooperative effects of combination of nilotinib with imatinib in a panel of imatinib-sensitive and imatinib-resistant BCR-ABL expressing cells in vitro: the result was a synergistic/additive effect against Ba/F3 cells carrying multiple point mutations, such as E255V, E255K, F317L, M351T, and F486S, but not against Y253H or T315I (Weisberg et al 2007). Recent studies of White et al (2007) using ^{14}C-labeled imatinib and nilotinib in an assay measuring intracellular uptake and retention (IUR), assessed the effect of adding unlabeled nilotinib to the ^{14}C-labeled imatinib IUR and, conversely, unlabeled imatinib to ^{14}C-labeled nilotinib IUR. While there was variation in the degree of response between patients, the data demonstrated a significant increase in the IUR of 1 μM ^{14}C-labeled nilotinib when either 1 or 2μM imatinib was added. Other experiments by White et al assessing the effect of temperature (37 °C vs 4 °C) on nilotinib uptake and retention in an ABCB1-expressing cell line suggested that nilotinib is transported by ABCB1. Therefore, imatinib inhibition of ABCB1-mediated efflux may be the cause for the increased IUR for nilotinib that is observed when both drugs are combined (White et al 2007).

Application of nilotinib: phase I studies in CML

In a phase I dose-escalation study, nilotinib was given to 119 patients with imatinib-resistant CML or acute lymphoblastic leukemia (17 patients in CP, 56 in AP, 24 in myeloid BP, 22 in lymphoid BP or Ph+ ALL). Daily doses ranged from 50 to 1,200 mg once daily or 400 mg and 600 mg twice daily, and were assigned in 9 dose cohorts of patients, to evaluate the safety and tolerability of the drug (Table 1). Dose escalation was permitted for patients with inadequate response and no toxic effects. The maximum tolerated dose was established at 600 mg twice daily. The treatment was established for a median duration of 120 days (range 1–385). Complete blood count was obtained weekly for the first 8 weeks and then every other week. Patients were evaluated for cytogenetic analysis at baseline and if they had response. Safety assessment included evaluation of side effects, hematologic and cardiac assessment, electrocardiography, and physical examination. Pharmacokinetic study revealed that the median time to peak nilotinib concentrations was 3 hours, the half-life was 15 hours, and the steady state level was achieved by day 8. With a daily dose, the peak concentrations and the area under the concentration-time curve increased among patients receiving a daily dose of 400 mg and reached a plateau among patients receiving more than 400 mg. Four hundred mg and 600 mg were administered twice daily and tested in 32 and 18 patients, respectively. The mean serum trough level at the steady-state level was 1.0 μM at 400 mg daily, 1.7 μM at 400 mg twice daily, and 2.3 μM at 600 mg twice daily, all of these concentrations exceeding the IC50 of cellular phosphorylation of 32 of 33 BCR-ABL kinase mutants (19–709 nM). No differences were observed between patients with or without BCR-ABL mutations, except for 2 patients with T315I mutation who exhibited marked resistance to nilotinib. The most frequent grade 3–4 toxicities were hematologic, with thrombocytopenia occurring in 21% and neutropenia in 14% of patients. The non-hematologic profile was similar to that

Table 1 Phase I and II results

	CML phase	No of patients	Response rate (%)		
			CHR	Major cytogenetic response	Complete cytogenetic response
Phase I	CP	17	92	35	35
	AP	56	51	27	14
	My BP	24	8	21	4
	Ly BP	22	0	11	11
Phase II	CP	387	77	57	41
	AP	129	26	31	19
	My BP	105	11	38	29
	Ly BP	31	13	48	32

Abbreviations: AP, accelerated phase; BP, blastic phase; CP, chronic phase; CHR, complete hematologic response; CML, chronic myeloid leukaemia.

of imatinib and included peripheral oedema, weight gain, and cutaneous rash. Grade 3–4 unconjugated bilirubinemia was reported in 14% of patients receiving 600 mg twice daily: this increase was not accompanied by an increase in levels of amino-transferase or evidence of increased hemolysis. Grade 3–4 elevations of lipase occurred in 5% of patients and grade 3 elevation of amylase level was reported in 1 patient. From more than 2,200 electrocardiograms performed in a cohort of 119 patients, the only abnormality associated with the drug was the corrected QT interval by Fridericia's formula, which was increased from 5 to 15 msec. Furthermore, pericardial effusion and atrial fibrillation were described in 1 patient. The evaluation of the responses showed that among 17 CP patients, 9 (53%) had a cytogenetic response, including 6 patients with CCR and 3 patients with minimal response. CHR occurred in 92% of CP patients. Among AP patients, 72% of those with hematologic disease obtained a CHR: a total of 55% of patients obtained a cytogenetic response, which was complete in 8 patients, partial in 7, and minor in 5. Among patients with myeloid BP, 42% achieved a CHR and 29% a cytogenetic response (1 patient CCR); of the 9 patients with lymphoid BP or Ph+ ALL, 33% achieved a CHR and 22% a cytogenetic response (1 patient CCR). In patients with AP or BP who received nilotinib 400 mg or 600 mg twice daily, a significant decrease in phosphorylation of AKT, CRKL, STAT1, and STAT5 was observed on day 15 of therapy compared with baseline (Kantarjian et al 2006).

Jørgensen et al had reported on the efficacy of nilotinib on CD34+ CML cells: in this subset, imatinib and nilotinib were equipotent for the inhibition of BCR-ABL activity, and produced equivalent effect but incomplete reduction in CRKL phosphorylation at 5 μM. As for imatinib, the most primitive cells persisted and accumulated over 72 hours with nilotinib, and remained caspase-3 negative. The combination of nilotinib and imatinib produced further accumulation of this cell population, suggesting additive antiproliferative effects (Jørgensen et al 2007). Recently, it was demonstrated that nilotinib inhibited mitogen-activated protein kinase (MPAK), AKT and STAT5 phosphorylation in CML CD34+ cells in the absence of growth factors, but did not suppress AKT and STAT5 activity and resulted in increased MAPK activity in the presence of growth factors. For this reason nilotinib did not show increased efficacy in reducing non-dividing CML progenitors compared with imatinib (Konig et al 2008).

Phase II studies

Results from a phase I dose escalation study indicated that nilotinib produces significant hematologic and cytogenetic responses in all phases of CML (Table 1). The primary endpoints of a phase II study was to determinate the rate of major cytogentic response in patients with CP after resistance or intolerance to imatinib. A first draft from an interim analysis on the first 280 consecutively enrolled CP-CML patients was published in 2007 (Kantarjian et al 2007a). Based on pharmacokinetic study of phase I, nilotinib was administered at 400 mg twice daily and could be escalated to 600 mg twice daily if patients had not obtained an hematologic response at 3 months, a major cytogenetic response at 12 months, or if they showed loss of hematologic or cytogenetic response or disease progression at any time. A total of 318 patients were enrolled and treated at 63 different institutions. The results referred to the initial cohort of 280 patients with at least 6 months of follow up: 194 (69%) patients were imatinib resistant and 86 (31%) patients were imatinib intolerant. Nilotinib was interrupted at a very low rate (median days 18) and the median intensity of nilotinib was 797 mg/day, very close to the intended daily dose of 800 mg/day. Sixty-five percent of patients remained on study, with the main causes of discontinuation being adverse events (15%) and disease progression (11%). Complete hematologic response was achieved in 68% of patients with resistance and 90% of patients with intolerance to imatinib, with a total response of 74%. Rates of major cytogenetic responses were 48% in patients with imatinib intolerance and 47% in patients with imatinib resistance. Complete cytogenetic response was reached in 30% of patients with imatinib resistance and in 47% of patients with imatinib intolerance (overall 31%); in addition 2% of patients entered the study while in CCR and maintained the response and 1% had missing baseline assessment but CCR was detected during the study. Partial cytogenetic response was achieved in 16% of patients overall (19% in imatinib-resistant and 12% in imatinib-intolerant); in addition 1% of patients entered the study while in PCR and maintained this response. Rate of patients that persisted in MCR was 96% (only 5 out of 134 patients discontinued therapy due to progression or death) and estimated 1-year overall survival was 95% (a total of 10 patients died: 2 during treatment and 8 during the long term follow up). Mutational study was available for 182 patients and 28 different mutations were detected: MCR was achieved in 42% of mutated patients (versus 51% of non-mutated) and CCR was achieved in 23% of mutated patients (versus 35% of non-mutated). CHR and MCR were observed across all BCR-ABL genotypes, with the exception of the T315I mutation, identified in 4 patients, and the E255V and E274K mutations, identified in 1 patient each. The rates of responses appeared to be affected

by preclinical IC50 to nilotinib: patients with mutations with IC50 less than 100 nM reached MCR at a rate of 53% versus 15% of patients with mutations with IC50 ranging from 201 to 800 nM. Patients with IC50 more than 10,000 nM did not achieve cytogenetic response. The patients with less sensitive mutations (IC > 200 nM) showed complete in vitro suppression with increased exposure to nilotinib. It is possible that, as suggested by Kantarjian et al (2007a), longer treatment duration may result in further improvement in clinical responses in patients with less sensitive mutations.

The safety profile was similar to that observed in the phase I study. The majority of serum chemistry abnormalities ranged from mild to moderate and included elevations of AST/ALT in 4% of patients and elevations of bilirubin and lipase, occurred in 9% and 14% of patients, respectively. The etiology of hyperbilirubinemia was explored in 62 patients by examining the polymorphisms in the uridine diphosphate glucurosyltransferase 1A1 (UGT1A1) gene: the results indicated that the repeat of TA, which predisposes to Gilbert syndrome, predicted for susceptibility to side effect under nilotinib. Comparison of non-hematologic adverse events occurred in patients with imatinib-intolerance subsequently treated with nilotinib, showed the presence of a same effect in only 2 out of the 86 observed patients, thus indicating the lack of cross-intolerance between the two drugs. Preclinical studies showed that nilotinib could potentially prolong the QT interval: an incidence of 1% of QTc intervals exceeding 500 milliseconds according to Fridericia correction was reported. Pleural effusions were observed in only 1% of patients on nilotinib therapy (Kantarjian et al 2007a).

The same phase II multicenter international single arm study also examined the efficacy and safety of nilotinib in patients with accelerated phase resistant or intolerant to imatinib. AP was defined by one or more of the following features: >15% but <30% blasts in the bone marrow or in blood, >30% blasts plus promyelocytes in blood or bone marrow, peripheral blood basophils >20%, and/or thrombocytopenia $<100 \times 10^9$/L unrelated to therapy. All patients were initially treated with nilotinib 400 mg twice daily and dose escalation to 600 mg twice daily was permitted for failure to obtain return to CP by 1 month, loss of an achieved hematologic or cytogenetic response, or disease progression. Primary endpoint was the rate of hematologic responses, defined as CHR, bone marrow response, or return to CP. Secondary endpoints included time to hematologic response, duration of hematologic response (HR), MCR, time to and duration of MCR, and overall survival. A total of 119 patients from 36 different institutions were enrolled: the majority had received previous therapy other than imatinib and 81% of all patients were imatinib resistant, with 49% previously treated with at least 800 mg. Among the 119 patients an overall HR rate of 47% was observed in a median of 1 month of treatment. Of these patients who achieved HR, 70% remained in response at 12 months. In a median of 2 months of treatment, 35 patients (29%) achieved a MCR and 19 patients (16%) a CCR. Median duration of MCR was 15.4 months. Thirty-five patients with additional chromosome abnormalities (ACA) at baseline were included in the study: 21 of them responded (60%), similarly to patients without ACA. The estimated 1-year overall survival was 79%. Mutational screening was available for 51 patients and 17 different BCR-ABL mutations were identified. HR was obtained in 48% of mutated patients compared with 45% of non-mutated, and MCR was achieved in 21% of mutated versus 36% of non-mutated patients. HR and MCR were observed across a variety of BCR-ABL mutant genotypes. The T315I mutation was the only one imatinib resistant among 32 tested mutations. The most commonly reported non-hematologic events possibly related to nilotinib were rash (22%), pruritus (20%), constipation (11%), nausea, headache, and fatigue (10%). The most common hematologic adverse events of grade 3–4 were thrombocytopenia (35%) and neutropenia (21%). The serum biochemistry abnormalities observed were mild to moderate, and grade 3–4 elevations in AST and ALT were recorded in 1% and 2% of patients, respectively. Grade 3–4 elevations in bilirubin occurred in 9% and lipase in 18% of patients. No patients experienced a prolongation in QTc interval. Estimated 1-year overall survival was 79% (Le Coutre et al 2008).

Results of nilotinib treatment in patients with Ph+ CML in blast crisis (CML-BC) resistant to or intolerant of imatinib were presented at ASH 2007 (Giles et al 2007). The primary endpoint was a confirmed HR. Nilotinib was given at the dose of 400 mg twice daily with the possible dose escalation to 600 mg twice daily for inadequate response. Data were presented for 135 patients with blast crisis (BC) (103 myeloid and 29 lymphoid). Hematologic response was recorded in 39% of myeloid BC patients and in 38% of lymphoid BC patients, with 5% of marrow response being observed in myeloid BC and in 3% in lymphoid BC. The most common grade 3–4 hematologic abnormalities were neutropenia (67%), thrombocytopenia (62%), and anemia (42%). The most common grade 3–4 non-hematologic side effects were: pneumonia (11%), pyrexia (7%), nausea (4%), diarrhea (4%), and asthenia (4%). Nilotinib was administered at a dose of 400 mg twice daily also in patients with CML-CP, CML-AP, and CML-BP who

previously received and either failed or were intolerant to both imatinib and dasatinib (Giles et al 2007). A total of 67 patients were reported with CML-CP (27), CML-AP (15), and CML-BP (25 total; 15 myeloid, 8 lymphoid). A total of 22 (33%) patients with dasatinib failure remained on nilotinib and 45 (67%) discontinued. Of 17 patients with CML-CP who did not have a CHR at baseline, 11 (65%) achieved a CHR at 4-month follow up. Of all 22 CML-CP patients, 7 (32%) had a MCR (3 complete, 4 partial). Of 13 patients with CML-AP, 3 (23%) showed no evidence of leukemia and 3 (23%) had a return to chronic phase after 4 months of nilotinib therapy. Of 20 patients with BP, 3 (15%) achieved CHR, 1 (5%) had return to CP, and 6 (30%) had disease progression. The most common grade 3/4 hematologic adverse events reported were neutropenia (51%), thrombocytopenia (44%), and anemia (21%). The most frequent grade 3–4 non-hematologic adverse events were pyrexia (8%), anorexia and headache (3%), diarrhoea, asthenia, constipation, fatigue, and myalgia (2% each) (Giles et al 2007).

Recently, the MD Anderson group described the outcome of 420 patients after imatinib failure, with 374 of them being resistant and 46 intolerant to the drug. Prognosis in this subset of patients is usually reported to be poor, but there are no data on survival after subsequent treatments with stem cell transplant or new TKI. In the cohort of 88 patients with CP, median survival was not reached in this study and the estimated 3-year survival rate was 72%. Prognosis was better after receiving dasatinib or nilotinib versus allogeneic stem cell transplantation (SCT) versus other therapies for patients in CP, but not for patients in more advanced phase of disease (Kantarjian et al 2007b).

The activity of nilotinib is currently being evaluated in the frontline setting of CP patients. Preliminary results were presented at the 2007 ASH meeting (Cortes et al 2007): 32 patients with early CP received 400 mg twice daily; Sokal risk was low in 21 patients, intermediate in 6, and high in 3. The rates of CCR at 3, 6, and 12 months were 95%, 100%, and 100%, respectively, and compared favorably with those observed in historical control patients treated with imatinib at 400 and 800 mg. MMR was achieved by 3 patients at 3 months, by 7 patients at 6 months, and by 5 patients at 12 months. Grade 3–4 neutropenia and thrombocytopenia were observed in the rate of 7% and 3%, respectively. Other grade 3–4 adverse events included elevation of lipase, bilirubin, and back pain. Twelve patients had treatment interruptions due to pain and lipase elevation. Only 3 patients discontinued therapy: 2 switched to imatinib and 1 performed SCT (Cortes et al 2007).

A biomarker study using the established pCRKL assay in imatinib-resistant CML and Ph+ ALL treated with nilotinib was recently published (La Rosee et al 2008). With this study a minimun dose (200 mg) required for effective BCR-ABL inhibition in imatinib-resistant/intolerant leukemia was established; the preclinical profile of nilotinib as active drug against mutant BCR-ABL was largely confirmed. Differences were noted in the PCRKL/CRKL blood baseline ratio between CML and Ph+ ALL, with values being lower in the latter subset of patients. The limitation of this assay is for advanced disease, where effective CRKL inhibition could reflect differential compartmentalization of the leukemic clone, when comparing CML and Ph+ ALL. Reactivation of BCR-ABL shortly after starting nilotinib treatment was seen in Ph+ ALL patients with progressive disease with p-loop mutations (Y253H, E255K) or T315I. This tool was suggested as a possible surrogate to establish effective dosing of nilotinib and to characterize resistance mechanisms (La Rosee et al 2008).

Activity of nilotinib against KIT and PDGFR malignancies

Nilotinib selectively inhibits Bcr-Abl, Kit, and PDGFR tyrosine kinases, but does not significantly affect any of the kinases required for IL-3 signaling, such as JAK2, or a broad variety of other receptor tyrosine kinases or tyrosine kinase oncogenes (Weisberg et al 2005). These data also suggested that nilotinib can be a reasonable alternative therapy for the treatment of malignancies driven by mutated forms of Kit and PDGFR kinases, such as GIST (gastrointestinal stromal tumors) and hypereosinophilic syndromes (HES). Verstovsek et al demonstrated that nilotinib is effective in HMC-1(560) mast cells carrying wild-type codon 816 c-kit, and is potent as imatinib in inhibiting cellular proliferation and in inducing apoptosis. By contrast, in HMC-1(560,816) cells bearing a c-kit mutation in codon 816, neither drug exerted a significant effect. Nilotinib was also as effective as imatinib in inhibiting phosphorylation of c-kit in HMC-1(560) cells. Nilotinib had little effect on survival of bone marrow mast cells with 816 c-kit mutation obtained from patients with systemic mastocytosis (Verstovsek et al 2006a). Guo et al investigated on the efficacy of second-line tyrosine kinase inhibitors, such as sorafenib, dasatinib, and nilotinib, against the commonly observed imatinib-resistant Kit mutations [(Kit(V654A), Kit(T670I), Kit(D820Y), and Kit(N822K)] expressed in the Ba/F3 cellular system: they found that nilotinib inhibited the growth of imatinib-resistant cells carrying Kit(V560del/V564A) and Kit (V599D/D820Y) more efficaciously than dasatinib and sorafenib (Guo et al 2007). However, nilotinib lacked activity against the mutant Kit (T670I) or double Kit (WK557-8del/T670I), resembling the lack of activity by imatinib, dasatinib, and nilotinib against

the homologous T315I mutation (Guo et al 2007). Nilotinib was potent as imatinib in inducing apoptosis and inhibiting proliferation of EOL-1 cells, with IC(50) values of 0.54 nM. In addition, both imatinib and nilotinib inhibited the phosphorylation of PDGFR-alpha tyrosine kinase with equivalent efficacy (Verstovsek et al 2006b). Stover et al proved that in vitro, nilotinib inhibited proliferation of Ba/F3 cells transformed by both TEL-PDGFRbeta and FIP1L1-PDGFRalpha with IC50 values of less than 25 nM and inhibited phosphorylation of the fusion kinases and their downstream signaling targets. The imatinib mesylate-resistant mutant TEL-PDGFRbeta T681I was sensitive to nilotinib, whereas the analogous mutation in FIP1L1-PDGFRalpha, T674I, was resistant (Stover et al 2005). In contrast to these results is the experience of von Bubnoff, who proved nilotinib capable of suppressing the growth of Ba/F3 cells transfected with FIP1LI-PDGFRa harbouring the T674I mutation with an IC50 of 376 nM. This drug also induced apoptosis in T674I positive cells. The authors explained the conflicting findings by lower concentrations of nilotinib used in the experiments of Stover et al (2005) (von Bubnoff et al 2006).

Closing remarks

The current first-line therapy for newly diagnosed CML patients is imatinib. However, imatinib resistance represents a significant clinical problem that may not be overcome by an increase in dose. Novel inhibitors may work to some extent for imatinib-resistant patients. Nilotinib has been shown to be active in clinical phase II trials: data supported preclinical findings demonstrating the high activity of this drug against BCR-ABL positive cells after failure of imatinib therapy owing to resistance or intolerance. The rational design of nilotinib was based on the scaffold of imatinib, resulting in a compound with increased affinity and high potency against BCR-ABL mutants, except for T315I. Nilotinib has been approved by the FDA for the treatment of patients in CP or AP CML after failure with imatinib therapy. Studies on nilotinib as a first-line therapy in early CP are in process and preliminary results suggest that this drug compares favorably with imatinib. Updates of these investigations are needed to confirm the remarkable activity of nilotinib.

Disclosures

Neither author has any conflicts of interest to disclose.

References

Apperley JF. 2007. Mechanisms of resistance to imatinib in chronic myeloid leukaemia (part I and II). *Lancet Oncol*, 8:1018–29.

Azam M, Raz T, Nardi V, et al. 2003. A screen to identify drug resistance variants to target directed anti-cancer agents. *Biol Proced Online*, 5:204–10.

Baccarani M, Saglio G, Goldman J, et al 2006; European Leukemia Net. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European Leukemia Net. *Blood*, 108:1809–20.

Baccarani M, Pane F, Saglio G. 2008. Monitoring treatment of chronic myeloid leukemia. *Haematologica*, 93:161–9.

Bradeen HA, Eide CA, O'Hare T, et al. 2006. Comparison of imatinib mesylate, dasatinib and nilotinib in a N-Ethyl-N-nitrosourea (ENU)-based mutagenesis screen: high efficacy of drug combinations. *Blood*, 108:2332–8.

Cools J, Mentens N, Furet P, et al. 2004. Prediction of resistance to small molecule FLT3 inhibitors: implications for molecularly target therapy of acute leukemia. *Cancer Res*, 64:6385–9.

Cortes J, O'Brien S, Jabbour E, et al. 2007. Efficacy of nilotinib in patients with newly diagnosed, previously untreated Philadelphia chromosome positive chronic myelogenous leukemia in early chronic phase [abstract]. *Blood*, 110:abstract 29.

Donato NJ, Wu JY, Stapley J, et al. 2003. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukaemia cells selected for resistance to STI571. *Blood*, 101:690–8.

Deininger M, Buchdunger E, Druker BJ. 2005. The development of imatinib as a therapeutic agent for chronic myeloid leukaemia. *Blood*, 105:2640–53.

Druker BJ, Guilhot F, O'Brien S, et al 2006; IRIS investigators. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med*, 355:2408–17.

Ernst T, Erben P, Muller MC, et al. 2008. Dynamics of BCR-ABL mutated clones prior to hematologic or cytogenetic resistance to imatinib. *Haematologica*, 93:186–92.

Faderl S, Talpaz M, Estrov Z, et al. 1999. Chronic myelogenous leukaemia: biology and therapy. *Ann Intern Med*, 131:207–19.

Giles F, Larson RA, Kantarjian H, et al. 2007. Nilotinib in patients with Philadelphia chromosome-positive chronic myelogenous leukaemia in blast crisis who are resistant or intolerant to imatinib [abstract]. *Blood*, 110:abstract 1025.

Goldman JM, Melo JV. 2003. Chronic myeloid leukaemia-advances in biology and new approaches to treatment. *N Engl J Med*, 349:1451–64.

Golemovic M, Verstovsek S, Giles F, et al. 2005. AMN107, a novel aminopyrimidine inhibitor of BCR-ABL, has in vitro activity against imatinib-resistant chronic myeloid leukaemia. *Clin Cancer Res*, 11:4941–7.

Guo T, Agaram NP, Wong GC, et al. 2007. Sorafenib inhibits the imatinib-resistant KITT670I gatekeeper mutation in gastrointestinal stromal tumour. *Clin Cancer Res*, 13:4874–81.

Hochhaus A, La Rosee P. 2004. Imatinib therapy in chronic myelogenous leukaemia: strategies to avoid and overcome resistance. *Leukemia*, 18:1321–31.

Hochhaus A, Erben P, Ernst T, et al. 2007. Resistance to targeted therapy in chronic myelogenous leukemia. *Semin Hematol*, 44:S15–S24.

Hubbard SR, Wei L, Ellis L, et al. 1994. Crystal structure of the tyrosine kinase domain of the human insulin receptor. *Nature*, 372:746–54.

Jørgensen HG, Allan EK, Jordanides NE, et al. 2007. Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+ CML cells. *Blood*, 109:4016–9.

Kantarjian HM, Talpaz M, O'Brien S, et al. 2003. Dose escalation of imatinib mesylate can overcome resistance to standard-dose therapy in patients with chronic myelogenous leukemia. *Blood*, 101:473–75.

Kantarjian H, Giles F, Wunderle L, et al. 2006. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. *N Engl J Med*, 354:2542–51.

Kantarjian H, Giles F, Gattermann N, et al. 2007a. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. *Blood*, 110:3540–6.

Kantarjian H, O'Brien S, Talpaz M, et al. 2007b. Outcome of patients with Philadelphia chromosome positive chronic myelogenous leukaemia post-imatinib mesylate failure. *Cancer*, 109:1556–60.

Konig H, Holtz M, Modi H, et al. 2008. Enhanced BCR-ABL kinase inhibition does not result in increased inhibition of downstream signalling pathways or increased growth suppression in CML progenitors. *Leukemia*, 22:748–55.

La Rosee' P, Holm-Eriksen S, Konig H, et al. 2008. Phospho-CRKL monitoring for the assessment of BCR-ABL activity in imatinib-resistant chronic myeloid leukemia or Ph+ acute lymphoblastic leukemia patients treated with nilotinib. *Haematologica*, 93:765–9.

Le Coutre P, Hochhaus A, Heim D, et al. 2006. A phase II study of nilotinib, a novel tyrosine kinase inhibitor administered to patients with hypereosinophilic syndrome (HES) [abstract]. *Blood*, 108:abstract 4912.

Le Coutre P, Ottmann OG, Giles F, et al. 2008. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is active in patients with imatinib-resistant or -intolerant accelerated-phase chronic myelogenous leukemia. *Blood*, 111:1834–9.

Liu Y, Gray NS. 2006. Rational design of inhibitors that bind to inactive kinase conformations. *Nat Chem Biol*, 2:358–364.

Manley PW, Cowan-Jacob SW, Fendrich G, et al. 2004. Urea derivatives of STI571 as inhibitors of BCR-ABL and PDGFR kinases. *Bioorg Med Chem Lett*, 14:5793–7.

Manley PW, Cowan-Jacob SW, Fendrich G, et al. 2005. Molecular interactions between the highly selective pan-BCR-ABL inhibitor, AMN107, and the tyrosine kinase domain of Abl. *Blood*, 106:940a.

Marin D, Goldman JM, Olavarria E, Apperley JF. 2003. Transient benefit only from increasing the imatinib dose in CML patients who do not achieve complete cytogenetic remissions on conventional doses. *Blood*, 102:2702–3.

O'Hare T, Walters DK, Stoffregen EP, et al. 2005. In vitro activity of BCR-ABL inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res*, 65:4500–5.

Ray A, Cowan-Jacob SW, Manley PW, et al. 2007. Identification of Bcr/Abl point mutations conferring resistance to the Abl kinase inhibitor AMN107 by a random mutagenesis study. *Blood*, 109:5011–5.

Sattler M, Griffin JD. 2003. Molecular mechanisms of transformation by the BCR-ABL oncogene. *Semin Hematol*, 40:4–10.

Schindler T, Bommann W, Pellicena P, et al. 2000. Structural mechanism for STI571 inhibition of abelson tyrosine kinase. *Science*, 289:1938–42.

Stover EH, Chen J, Lee BH, et al. 2005. The small molecule tyrosine kinase inhibitor AMN107 inhibits TEL-PDGFRbeta and FIP1L1-PDGFRalpha in vitro and in vivo. *Blood*, 106:3206–13.

Verstovsek S, Akin C, Manshouri T, et al. 2006a. Effects of AMN107, a novel aminopyrimidine tyrosine kinase inhibitor, on human mast cells bearing wild-type or mutated codon 816 c-kit. *Leuk Res*, 30:1365–70.

Verstovsek S, Giles F, Quintas-Cardama A, et al. 2006b Activity of AMN107, a novel aminopyrimidine tyrosine kinase inhibitor, against human FIP1L1-PDGFRalpha-expressing cells. *Leuk Res*, 30:1499–505.

Von Bubnoff N, Manley PW, Mestan J, et al. 2006. BCR-ABL resistance screening predicts a limited spectrum of point mutations to be associated with clinical resistance to the Abl kinase inhibitor nilotinib . *Blood*, 108:1328–33.

Von Bubnoff N, Gorantla SP, Thone S, et al. 2006. The FIP1L1-PDGFRA T674I mutation can be inhibited by the tyrosine kinase inhibitor AMN107. *Blood*, 107:4970–2.

Weisberg E, Manley PW, Breitenstein W, et al. 2005. Characterization of AMN107, a selective inhibitor of native and mutant BCR-ABL. *Cancer Cell*, 7:129–41.

Weisberg E, Catley L, Wright RD, et al. 2007. Beneficial effects of combining imatinib and nilotinib in preclinical models of BCR-ABL+ leukemias. *Blood*, 109:2112–20.

White DL, Saunders VA, Quinn SR, et al. 2007. Imatinib increases the intracellular concentrations of nilotinib, which may explain the observed synergy between these drugs. *Blood*, 109:3609–10.

Zonder JA, Pemberton P, Brandt H, et al. 2003. The effect of dose increase of imatinib mesylate in patients with chronic or accelerated phase chronic myelogenous leukemia with inadequate hematologic or cytogenetic response to initial treatment. *Clin Cancer Res*, 9:2092–7.