AMN107: Tightening the grip of imatinib

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The Abl inhibitor imatinib is a highly effective therapy for patients with chronic myeloid leukemia. Relapses are relatively uncommon in newly diagnosed patients, but are the rule in patients with more advanced disease. Mutations in the BCR-ABL gene are the most common cause of relapse. Working from the imatinib chemical structure, a higher-affinity family member, AMN107, was designed. AMN107 is approximately 20-fold more potent than imatinib, and this translates into improved inhibitory activity against most of the common BCR-ABL mutations. The implications of these results, and the potential role this and other novel ABL inhibitors may have in treating patients with CML, are discussed.

Targeted molecular therapy with the selective Bcr-Abl tyrosine kinase inhibitor imatinib (STI571, Gleevec) has revolutionized the treatment of chronic myeloid leukemia (CML) (Druker et al., 1996, 2001). The clinical efficacy of imatinib can be attributed to several factors: the critical role of the Bcr-Abl kinase in the pathogenesis of CML, exceptional target specificity of imatinib along with excellent pharmacokinetics, and a high level of tolerability. Conspicuously absent from this list is "extremely high potency"—imatinib is a rather average inhibitor from the standpoint of binding affinity (IC_{50}: 300 nM versus wild-type Bcr-Abl). Despite the clinical success of imatinib therapy, an important question is raised: would an inhibitor combining the attributes of imatinib with a substantially higher affinity for Bcr-Abl be more effective as a therapeutic agent for CML? The identification and characterization of just such an inhibitor, AMN107, are presented in this issue of *Cancer Cell* by Weisberg et al. (2005). Their data suggest that AMN107 may also find application in the treatment of imatinib-resistant CML.

AMN107 (Figure 1) was developed using a rational drug design strategy in which imatinib served as the lead compound. The crystal structure of the Abl kinase domain in complex with imatinib reveals that few changes to the inhibitor structure are likely to be tolerated (Nagar et al., 2002; Schindler et al., 2000). The drug fits into the canonical ATP binding site lining the groove between the N and C lobes and penetrates into the central region of the kinase. Thus, the close match between the core of the inhibitor and its binding site prohibits alterations within this region of imatinib. However, the methylpiperazinyl group of imatinib lies along a partially hydrophobic, surface-exposed pocket of the Abl kinase, and is more amenable to modification. Methodical replacement of this ring system led to the discovery of AMN107, a close relative of imatinib with more than 20-fold improved affinity for wild-type Bcr-Abl. As a testament to the Bcr-Abl-specific design requirements built into AMN107, the improvement in binding affinity for Bcr-Abl is not carried through to Kit or PDGFR. Although these kinases are inhibited by AMN107, their affinities for AMN107 are very similar to those of imatinib. The relative ranking of affinities with respect to imatinib is PDGFR > Kit > Bcr-Abl, while the order for AMN107 is Bcr-Abl > PDGFR > Kit. Thus, AMN107 is a more potent and more specific inhibitor of the Abl kinase than imatinib.

Weisberg et al. initially established that AMN107 blocks proliferation of cell lines expressing wild-type Bcr-Abl with ~20-fold greater potency than imatinib. Results from a murine CML model demonstrate that AMN107 prolongs survival of nude mice injected with 32D cells expressing wild-type Bcr-Abl and of mice transplanted with bone marrow infected with retrovirus expressing Bcr-Abl. In all cases, treatment with AMN107 resulted in a significant reduction in the accumulation of leukemic cells in the spleen, bone marrow, liver, and lymph nodes.

Although a more potent Bcr-Abl kinase inhibitor might be better than imatinib for treating newly diagnosed patients with chronic phase CML, the real push in the Bcr-Abl inhibitor field has been to develop alternate inhibitors to treat imatinib-resistant CML. Currently, >95% of newly diagnosed patients with chronic phase disease achieve durable responses on imatinib therapy. Relapses are observed in only a small percentage of these patients, but are the rule for patients with advanced disease. The leading cause of acquired resistance to imatinib is reactivation of Bcr-Abl kinase activity due to mutations in its kinase domain that decrease its sensitivity to imatinib by 3- to >100-fold (reviewed in Deininger et al., 2004). To date, at least 30 different mutations within the Bcr-Abl kinase domain have been identified in patient samples (reviewed in Hochhaus and La Rosée, 2004), and in vitro experiments confirm that these mutations reduce imatinib sensitivity to a variable degree.

The crystal structure of Abl in complex with imatinib revealed the structural basis by which kinase domain mutations confer imatinib resistance (Nagar et al., 2002; Schindler et al., 2000). Imatinib binds to a unique, inactive conformation of the Abl kinase domain in which the activation loop is in a closed position. Mutations that confer resistance to imatinib either affect residues directly involved in drug binding, impair the ability of the kinase domain to undergo the extensive conformational changes required for imatinib binding, or favor the active conformation of the kinase to which imatinib is unable to bind.

As reported by Weisberg et al., AMN107 also binds to the inactive conformation of the Abl kinase domain. Given the structural similarities between imatinib and AMN107, and because most imatinib-resistant mutations occur in regions of the kinase domain that contact the conserved portions of these two inhibitors, it would be predicted that the differences in sensitivity to imatinib of wild-type versus mutant Bcr-Abl would be preserved in AMN107. As shown, this is exactly the case, with most of the mutants being approximately 20-fold more sensitive to AMN107 than to imatinib (Figure 1). One exception to this is T315I, which is completely insensitive to AMN107, presumably

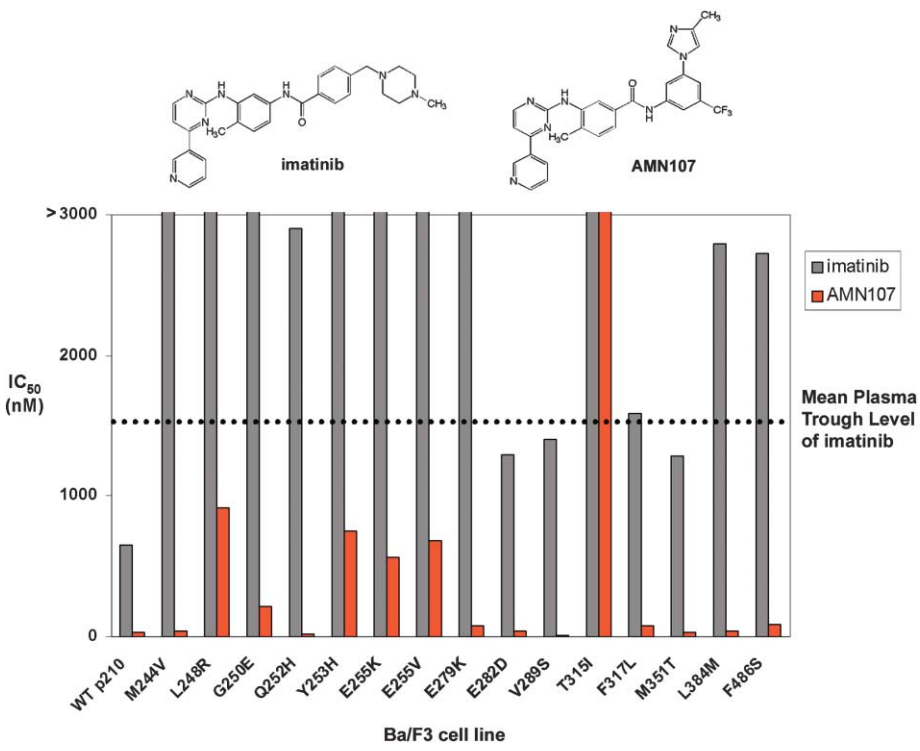

Figure 1. Comparison of imatinib and AMN107 IC_{50} values for blocking proliferation of Ba/F3 cells expressing wild-type Bcr-Abl or kinase domain mutated Bcr-Abl

Solid gray bars indicate imatinib IC_{50} values, and solid red bars indicate AMN107 IC_{50} values. Dotted black line indicates the mean trough plasma level of imatinib reported in patients 24 hr following treatment with a once-daily dose of 400 mg. The figure is based on data presented in Weisberg et al. (2005).

because this is a residue that makes a direct contact to AMN107. The other exception is M351T, which is more sensitive to AMN107 than would be predicted. This residue makes close contact with imatinib, but not with AMN107, due to the differences in chemical structure between AMN107 and imatinib. Therefore, M351T affects the binding of imatinib, but has little effect on the binding of AMN107. Although several common mutations, such as Y253F, F311L, M359V, and H396P/R were not analyzed, it seems likely that similar results would be obtained with these mutants. Regardless, the findings with the mutants challenge the notion that imatinib is conformationally or sterically excluded from the mutated Abl kinase domain, and suggest that a drug with an improved grip may have even more clinical utility than anticipated.

A comparison of IC_{50} values of imatinib and AMN107 for blocking proliferation of Ba/F3 cells expressing wild-type Bcr-Abl or imatinib-resistant mutants is illustrated in Figure 1 (based on data presented in Weisberg et al., 2005). As seen in this figure, if AMN107 drug levels match or even approach those of imatinib (the mean plasma trough concentration of imatinib at a dose of 400 mg is $\sim$1.5 μM [Druker et al., 2001; Peng et al., 2004]; dotted line in Figure 1), AMN107 would be predicted to have excellent clinical potential as a therapeutic agent for imatinib-refractory CML. At present, the safety, tolerability, and pharmacokinetic profile of AMN107, which ultimately will determine its clinical utility, remain to be established.

Another drug that has attracted attention with respect to imatinib-resistant CML is the dual Src/Abl inhibitor BMS-354825 (Deininger and Druker, 2004; Shah et al., 2004). This inhibitor, which exhibits approximately 300-fold greater affinity than imatinib for the Abl kinase, binds the kinase domain irrespective of the conformation of the activation loop. In addition, BMS-354825 makes fewer contacts with Abl than does imatinib or AMN107, and places less stringent conformational requirements on the kinase. In contrast to imatinib and AMN107, BMS-354825 is also an inhibitor of Src kinases. BMS-354825 effectively inhibited all imatinib-resistant Bcr-Abl mutants in vitro, except T315I, and prolonged the survival of mice injected with wild-type Bcr-Abl or cells expressing M351T mutant, which is marginally resistant to imatinib.

Both AMN107 and BMS-354825 fail to inhibit the T315I mutant of Bcr-Abl. Given the location of this residue in the "gatekeeper" region of the ATP binding site, this mutant may prove difficult to inhibit with ATP mimetics. In contrast, Bcr-Abl inhibitors that block the substrate binding site rather than the ATP binding site might be capable of inhibiting this mutant. A recent report profiling a non-ATP-competitive inhibitor of Bcr-Abl, ON012380, demonstrates the potential utility of this approach (Gumireddy et al., 2005). Interestingly, ON012380 inhibits wild-type and all imatinib-resistant kinase domain mutations tested, including T315I, with an IC_{50} of 10 nM or less.

The key finding from the study of imatinib resistance is that Bcr-Abl signaling remains central to disease pathogenesis, implying that selective inhibition of wild-type and imatinib-resistant Bcr-Abl signaling is still the therapeutic strategy of choice. Thus, the discovery of potent, structurally, and mechanistically distinct inhibitors of imatinib-resistant Bcr-Abl mutants represents a major advance. What is the best way to utilize these new Bcr-Abl inhibitors? Preliminary data from the phase I trials of AMN107 and BMS-354825 as single agents are encouraging (Giles et al., 2004; Sawyers et al., 2004). ON012380 has not yet entered clinical trials. Depending on the outcome of clinical trials of these agents, one potentially powerful approach would be to use these drugs in combination. The rationale for such an approach is that an inhibitor cocktail may target the widest range of resistant clones and thereby prohibit or delay the onset of acquired drug resistance. In this regard, we have preliminary data showing that the combination of imatinib and BMS-354825 have additive antiproliferative activities. However, combinations would have to be evaluated for toxicity in clinical trials.

A second potential role for these more potent Bcr-Abl inhibitors would be to target residual leukemia that persists in patients in whom imatinib induces durable remissions but fails to eradicate the disease. With imatinib, the majority of patients achieve a 3-log reduction in leukemic burden, but few achieve undetectable levels of Bcr-Abl transcripts. Although the precise mechanisms underlying disease persistence remain to be elucidated, it is conceivable that more complete suppression of Bcr-

Abl kinase with a high potency inhibitor or a combination of inhibitors may be able to target the cell population responsible for disease persistence.

AMN107, BMS-354825, and ON012380 have many more hurdles to clear before taking a place alongside imatinib, or even someday replacing it as a frontline CML therapeutic agent. However, the findings of Weisberg et al. (2005), Shah et al. (2004), and Gumireddy et al. (2005) are extremely encouraging and represent good news for patients with CML. Perhaps most remarkable is how quickly an understanding of the mechanism of imatinib resistance has translated into new treatments, demonstrating the speed at which drug development can occur.

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