AMN107, a Novel Aminopyrimidine Inhibitor of Bcr-Abl, Has *In vitro* Activity against Imatinib-Resistant Chronic Myeloid Leukemia

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Abstract Resistance to or intolerance of imatinib in patients with Philadelphia chromosome–positive chronic myelogenous leukemia (CML) has encouraged the development of more potent Bcr-Abl inhibitors. AMN107 is a novel, orally bioavailable ATP-competitive inhibitor of Bcr-Abl. The effects of AMN107 were compared with those of imatinib on imatinib-sensitive (KBM5 and KBM7) and imatinib-resistant CML cell lines (KBM5-STI571$^{R1.0}$ and KBM7-STI571$^{R1.0}$). Compared with the antiproliferative activity of imatinib, AMN107 was 43 times more potent in KBM5 (IC_{50} of 11.3 versus 480.5 nmol/L) and 60 times more potent in KBM7 (IC_{50} of 4.3 versus 259.0 nmol/L) cells. IC_{50} for AMN107 and imatinib were 2,418.3 and 6,361.4 nmol/L, respectively, in KBM5-STI571$^{R1.0}$, and 97.2 and 2,497.3 nmol/L, respectively, in KBM7-STI571$^{R1.0}$ cells. AMN107 inhibited autophosphorylation of Bcr-Abl kinase more effectively than imatinib in all cell lines. They had similar effects on cell cycle progression and apoptotic response in these cell lines. Among severe combined immunodeficient mice bearing KBM5 cells, mean survival times of groups treated with 10, 20, and 30 mg/kg/d of AMN107, starting day 20 after leukemic cell grafting and continuing for 20 days, were 144%, 159%, and 182%, respectively, compared with controls. These results strongly support investigation of the clinical efficacy of AMN107 in patients with CML.

Identification of the central role of the *BCR-ABL* gene and its product, a chimeric protein having a tyrosine kinase domain rendered permanently active through autophosphorylation of its ATP-binding site (1), has stimulated the development of drugs targeting this pathway. The most effective of these drugs developed to date, imatinib (Gleevec; refs. 2–4), is now thought to conduct much of its activity by stabilizing an inactive, non–ATP-binding tyrosine kinase conformation (5, 6). By preventing activation of the kinase through autophosphorylation, imatinib critically impacts transmission of downstream regulatory signals resulting in target-specific inhibition of cell proliferation (2–4).

Imatinib has significant activity in all three phases of Philadelphia chromosome–positive chronic myelogenous leukemia (CML)—chronic, accelerated, and blastic (7–14). However, responses to imatinib in patients with more advanced disease are generally transient (7–10). In early chronic CML, high cytogenetic and molecular response rates are obtained (11–15), particularly with higher-dose imatinib regimens (15). If patients are unable to tolerate an adequate imatinib dose, response rates are lower (15). Resistance to imatinib may manifest at the hematologic, cytogenetic, or molecular level particularly in patients with blastic phase disease (7–9) and may be evidenced by the development of more advanced CML (8, 10). There is a wealth of information documenting heterogeneity of cellular mechanisms associated with inherent or acquired resistance to imatinib *in vitro* (16–24) and *in vivo* (16–18, 25–28). Imatinib resistance in CML can be caused by several mechanisms: increased expression or mutation of the kinase, resulting in reduction or loss of imatinib effectiveness; changes in the ability of the cells to maintain an effective intracellular imatinib concentrations at the cellular level; or emergence of additional mutations decreasing the dependence of CML on Bcr-Abl (16–18). *In vitro*, increasing drug concentrations can overcome some mechanisms of imatinib resistance (19), and clinical studies have replicated this finding in patients with CML (15, 29). These data have encouraged the search for more potent Bcr-Abl inhibitors for treatment of CML (30).

AMN107 is a novel inhibitor of the protein tyrosine kinase activity of Bcr-Abl. Structural biology studies have confirmed that the molecule binds to an inactive conformation of the protein, occupying a region of space analogous to that which would be occupied by ATP in the active conformation of the enzyme (Fig. 1). In animals, AMN107 is well absorbed following oral administration, has a good pharmacokinetic profile, and is well tolerated. Therefore, AMN107 may prove to be an effective treatment for Bcr-Abl–associated diseases, including CML. An important preclinical issue in the development of AMN107 is its

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Received 12/17/04; revised 4/5/05; accepted 4/15/05.

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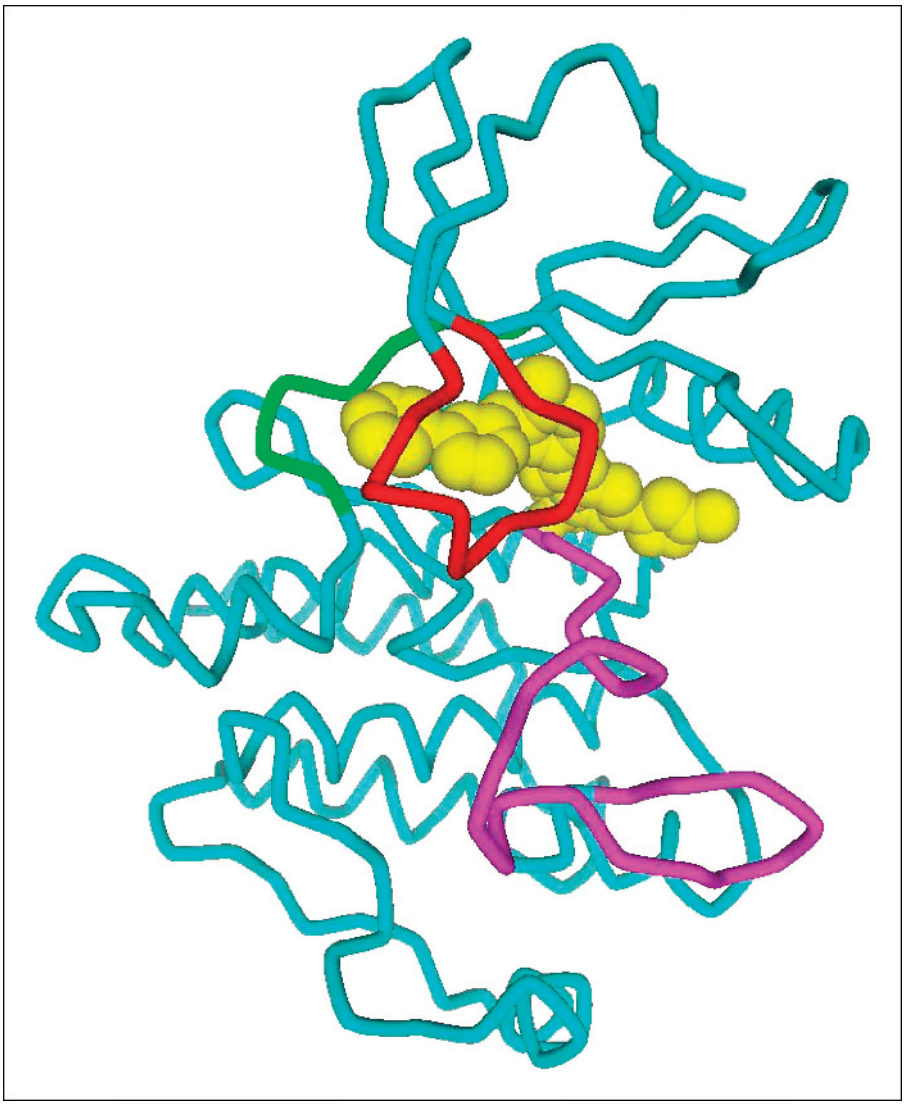

Fig. 1. Schematic diagram, based on an X-ray crystal structure, of the Abl kinase domain (*cyan*) in complex with AMN107 (*yellow*). The hinge region is shown in green, the glycine-rich loop is shown in red, and the activation loop is shown in magenta. In this structure, the activation loop adopts an inactive "DFG out" conformation similar to that observed in the imatinib-Abl complex. The picture was kindly supplied by S. Cowan-Jacob. Novartis Institutes of Biomedical Research, Basel, Switzerland.

activity relative to that of imatinib in both imatinib-sensitive and imatinib-resistant *BCR-ABL*–positive leukemic cells. We investigated the activity of AMN107, relative to imatinib, in a series of human Philadelphia chromosome–positive cell lines. These cell lines differ by degree of sensitivity to imatinib and by mechanisms of resistance to imatinib. We then assessed the efficacy of AMN107 in a severe combined immunodeficient (SCID) mouse model of CML.

Materials and Methods

Cell lines. Human cell lines used in this study included the Philadelphia chromosome–positive KBM5 (31) and KBM7 (32) cell lines, and the imatinib-resistant counterparts to these cell lines (19), KBM5-STI571$^{R1.0}$ and KBM7-STI571$^{R1.0}$. KBM5 cells contain multiple copies of the Philadelphia chromosome, express p210 Bcr-Abl protein, but lack normal c-Abl (33). KBM5-STI571$^{R1.0}$ cells have a T315I mutation in the ATP-binding site of Bcr-Abl (34). KBM7 cells have a near-haploid karyotype and express p210 Bcr-Abl. In KBM7-STI571$^{R1.0}$ cells, prominent features associated with resistance to imatinib are amplification of the *BCR-ABL* fusion gene and a corresponding increase in the Bcr-Abl protein (19). KBM5 and KBM7 cells differ in their response to imatinib: In KBM5 cells, imatinib does not elicit an apoptotic response but does cause G_0-G_1 cell cycle arrest; in KBM7 cells, imatinib causes increased apoptosis with no significant alterations in cell cycle progression (19).

The two imatinib-resistant cell lines were grown in the presence of 1 μmol/L concentration of imatinib at a proliferation rate similar to the proliferation rate of untreated parental cells. Acute myelomonocytic leukemic cell lines HL60 and U937 were used as *BCR-ABL*–negative controls. All cell lines were maintained in Iscove's modified Dulbecco's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin-streptomycin solution (Gemini Bio-Products, Woodland, CA).

Drugs. Imatinib and AMN107 monohydrochloride were provided by (Novartis, East Hanover, NJ). Stock dilutions were prepared in DMSO and stored as 10 mmol/L solutions at −20°C. Only freshly thawed aliquots were used in experiments.

Proliferation inhibition assay. The 3-(4,5-dimethylthiazol-2-yl)-5-(carboxymethoxyphenyl)-2(4-sulophenyl)-2*H*-tetrazolium (MTS) assay (CellTiter 96 Aqueous One Solution Reagent, Promega Corporation, Madison, WI) was used to measure the effect of imatinib and AMN107 on proliferation of human leukemic cells *in vitro*. The assay was done according to the manufacturer's recommendations. Cells were seeded in triplicate in 96-well microtiter plates (Falcon, Franklin Lakes, NJ), incubated in the presence of different drug concentrations for 72 hours and the proliferation was measured as a percentage of the proliferation of untreated cells. Each experiment was done at least thrice. The drug

concentration resulting in 50% inhibition of cell proliferation (IC_{50}) was determined. The resistance index was calculated by dividing the IC_{50} for resistant cells by the IC_{50} for parental cells.

Detection of caspase-3 activity. The fluorogenic substrate PhiPhiLux G1D2 (Oncoimmunin, Gaithersburg, MD) was used to monitor caspase-3 activity by means of flow cytometry. Following treatment with imatinib or AMN107, cells were washed in Ca^{2+}-free PBS, resuspended in 25 μL of substrate solution, and incubated for 1 hour in a humidified chamber at 37°C in the dark. After incubation, cells were washed and resuspended in Ca^{2+}-free PBS. Propidium iodide was added to permit identification and exclusion of necrotic cells during analysis. Prepared cell samples were run on a flow cytometer (FACScan, Becton Dickinson, Franklin Lakes, NJ), and the resulting data were analyzed using the program CellQuest (Becton Dickinson).

Cell cycle analysis by flow cytometry. To investigate the impact of drug exposure on cell cycle distribution and apoptotic response, cells were exposed to imatinib or AMN107 for 24, 48, or 72 hours. Equipotent concentrations of the drugs, ranging between IC_{60} and IC_{90}, were chosen on the basis of the MTS-determined cell sensitivities. After drug treatment, cells were collected, washed in Ca^{2+}-free PBS, and fixed overnight in 70% ethanol at −20°C. Cells were then washed twice in cold PBS, resuspended in hypotonic propidium iodide solution [25 μg of propidium iodide per milliliter, 0.1% Triton X-100, 30 mg of polyethylene glycol per milliliter, and 3,600 units of RNase per milliliter, dissolved in 4 mmol/L sodium citrate buffer (pH 7.8); Sigma], and incubated for at least 1 hour at 4°C in the dark. Cellular DNA contents were determined by flow cytometry (FACScan). Cell cycle distribution was analyzed using ModFit LT software (Becton Dickinson). Cells with hypodiploid DNA content were considered apoptotic.

Immunoprecipitation of BCR-ABL. Following treatment of cells with AMN107 or imatinib for 3 hours, Bcr-Abl was immunoprecipitated as previously reported (4, 19). Aliquots of 20×10^6 cells per cell line were treated with different concentrations of imatinib or AMN107 for 3 hours. Cells were then collected, washed thrice with cold PBS, resuspended in 250 μL of lysis buffer, and incubated on ice for 1 hour. Cell lysate was then centrifuged at 14,000 rpm at 4°C for 40 minutes. Supernatant was removed and mixed with 25 μL of anti–Bcr-Abl P6D monoclonal antibody (kindly provided by Dr. R.B. Arlinghaus, University of Texas M. D. Anderson Cancer Center, Houston, TX) for 1 hour on ice. Fifty microliters of protein-A/G agarose slurry (sc-2003, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were added; the solution was incubated overnight at 4°C with constant rotation. The antibody-protein complex was subjected to Western blotting.

Western blotting. The immunoprecipitated complex was eluted from the agarose with 2× loading buffer and run on a 9.5% SDS-PAGE gel. Western blotting was done overnight at 4°C after incubation of membranes with mouse antiphosphotyrosine (py99, Santa Cruz Biotechnology) diluted in 2.5% bovine serum albumin and 2.5% nonfat milk (dilution 1:8,000). The active band for phosphorylated Bcr-Abl was detected using conjugated HOURP-sheep anti-mouse antibody (NA931V, Amersham). Detection was done by enhanced

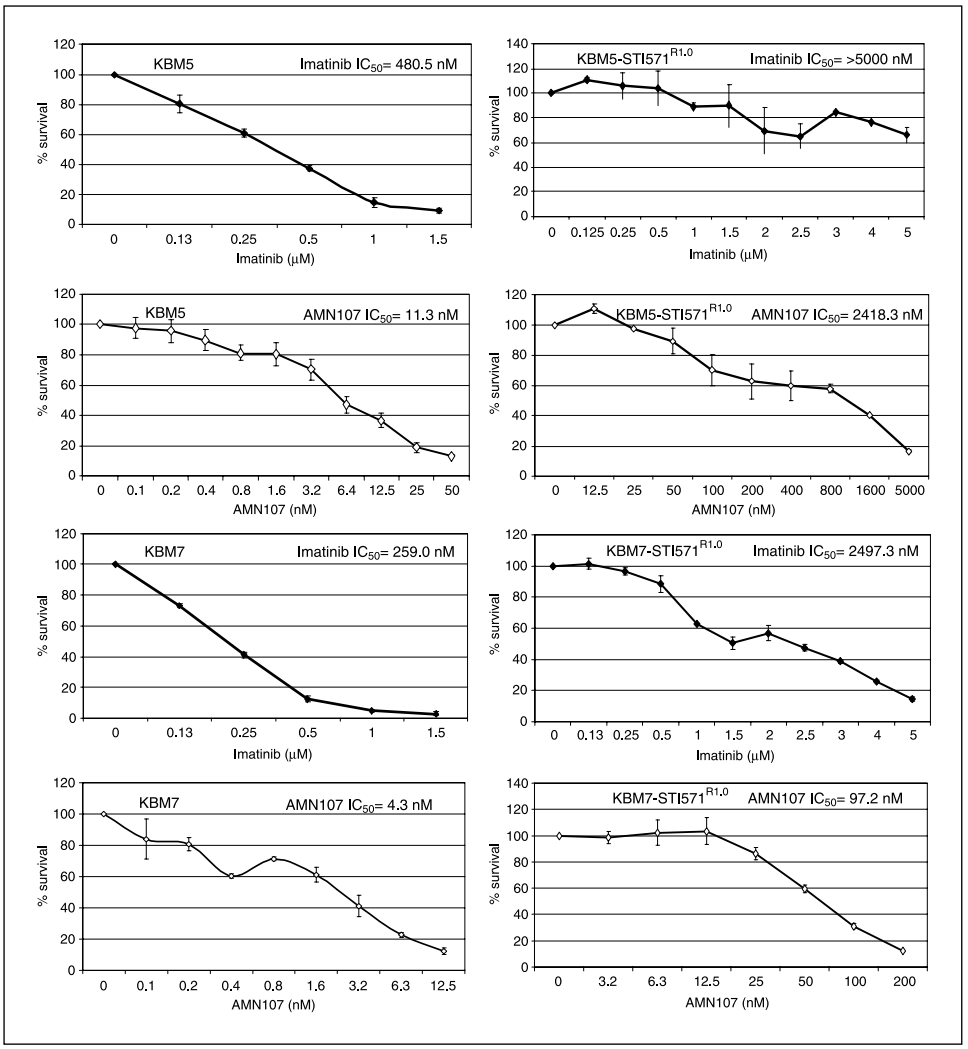

Fig. 2. Effect of increasing concentrations of imatinib and AMN107 on imatinib-sensitive (KBM5, KBM7) and imatinib-resistant (KBM5-STI571$^{R1.0}$, KBM7-STI571$^{R1.0}$) cell lines *in vitro*. *Points,* means of three MTS assays.

chemiluminescence as specified by the manufacturer (ECL, Amersham, Piscataway, NJ).

Stripped membranes were reprobed with mouse anti Bcr-Abl antibody (8E9; dilution 1:4,000) overnight at 4°C. The active band for Bcr-Abl was detected with HOURP-sheep anti-mouse antibody.

In vivo *experiments.* To investigate the efficacy of AMN107 *in vivo*, the SCID mouse model of clinically advanced blastic phase CML was selected (31). The experiments were done according to a research protocol approved by the Animal Care and Use Committee at M. D. Anderson Cancer Center. After acclimatization and 1 day after whole-body irradiation (250 cGy), SCID mice (5-6 weeks old, female, Taconic, Germantown, NY) were injected i.p. with 2.4×10^7 KBM5 cells (31) and randomly assigned to treatment groups. Starting 20 days after KBM5 cell inoculation, at a time when most mice were visibly ill and some had evident tumor masses, mice were treated with AMN107 at doses of 10, 20, or 30 mg/kg body weight i.p. daily for 20 days. AMN107 was dissolved in DL-lactic acid (L-6661, Sigma) and then diluted 80-fold with 5% glucose water (G8644, Sigma) containing 1% pluronic F68 solution (v/v; P5556, Sigma) to 0.14 mol/L, the final molarity of DL-lactic acid. Mice in the control group received vehicle only. Animals displaying signs of pain and suffering were euthanized by CO asphyxiation. Survival was measured to the time of spontaneous death or CO asphyxiation.

We expressed the median survival time of treated animals as a percentage of the median survival time of control animals. By National Cancer Institute criteria, if the mean survival time of treated animals exceeds 125% of that of control animals, the treatment has significant antitumor activity.

Results

Effect of imatinib on cell proliferation. The proliferation rates, viability, and saturation density of the resistant sublines were similar to those of the corresponding parental cell lines cultured concomitantly without imatinib. The IC_{50} values for imatinib in KBM5 and KBM7 cells were 0.48 and 0.24 μmol/L, respectively. The IC_{50} values for imatinib in KBM5-STI571$^{R1.0}$ and KBM7-STI571$^{R1.0}$ cells were 6.40 and 3.30 μmol/L, respectively. The calculated resistance indexes of 13.3 for KBM5-STI571$^{R1.0}$ cells and 13.8 for KBM7-STI571$^{R1.0}$ confirmed comparable degrees of imatinib resistance (Fig. 2).

Effect of AMN107 on cell proliferation and potency of AMN107 compared with that of imatinib. AMN107 was a more potent inhibitor of proliferation than imatinib in both KBM5 and KBM7 cells. The mean IC_{50} values for imatinib and AMN107 were 480.5 and 11.3 nmol/L, respectively, for KBM5 cells and 259.0 and 4.3 nmol/L, respectively, for KBM7 cells. Thus, AMN107 was 43 times as potent as imatinib in KBM5 cells and 60 times as potent as imatinib in KBM7 cells (Fig. 2). AMN107 was also a more potent proliferation inhibitor than imatinib in KBM5-STI571$^{R1.0}$ and KBM7-STI571$^{R1.0}$ cells. In KBM5-STI571$^{R1.0}$ cells, characterized by the presence of a T315I mutation (34), the mean IC_{50} values for imatinib and AMN107 were 6,361 and 2,418 nmol/L, respectively, indicating that AMN107 was thrice as potent as imatinib (Fig. 2). In KBM7-STI571$^{R1.0}$ cells, which have amplification of the *BCR-ABL* fusion gene and a corresponding increase in Bcr-Abl protein (19), mean IC_{50} values for imatinib and AMN107 were 2,497 and 97 nmol/L, respectively, indicating that AMN107 was 26 times as potent as imatinib (Fig. 2). The differences in the relative potency of AMN107 in these two imatinib-resistant cell lines suggested that different mechanisms of resistance to imatinib might determine the activity of AMN107. Sensitivity to AMN107 was Bcr-Abl selective. Philadelphia chromosome–negative acute myelomonocytic leukemia (U937, HL-60) cell lines lacked sensitivity to AMN107 or imatinib at concentrations of up to 10 μmol/L (data not shown).

Cell cycle analysis and caspase-3 activity. Exposure of KBM5 cells to AMN107 or imatinib resulted in an accumulation of cells in the G_0-G_1 phase of the cell cycle (Table 1). Similar but less pronounced perturbation of cell cycle progression was observed in KBM5-STI571$^{R1.0}$ cells exposed to imatinib or AMN107 (Table 1). AMN107 was more effective than imatinib in inducing accumulation of cells in G_0-G_1. In KBM7 and in KBM7-STI571$^{R1.0}$ cells, the effect of AMN107 or imatinib on cell cycle distribution was much less pronounced. Overall, at equipotent drug doses, the effect of AMN107 on cell cycle progression was similar to that of imatinib and was comparable in imatinib-sensitive and imatinib-resistant cells.

In KBM7 cells, intermediate to high concentrations of imatinib induced a time-dependent activation of caspase-3 and an increase in apoptosis. In KBM5 cells, these effects were not seen except at the longest (72 hours) imatinib exposure

Table 1. Percentage of cells in G_0-G_1 phase of cell cycle (A) and percentage of apoptotic cells (B) in sensitive and resistant sublines after treatment with AMN107 and imatinib at equipotent doses

Exposure time (h)	KBM5 (~IC_{90})			KBM5-STI571R1.0 (~IC_{60})			KBM7 (~IC_{90})			KBM7-STI571R1.0 (~IC_{80})		
	0	Imatinib (1,000 nmol/L)	AMN107 (25 nmol/L)	0	Imatinib (8,000 nmol/L)	AMN107 (3,000 nmol/L)	0	Imatinib (1,000 nmol/L)	AMN107 (25 nmol/L)	0	Imatinib (4,000 nmol/L)	AMN107 (160 nmol/L)
A. Percentage of cells in G_0-G_1												
24	32.0	73.9	75.7	25.9	43.0	63.9	30.3	25.7	32.6	34.9	54.8	59.7
48	32.1	87.4	88.4	27.5	41.0	55.7	29.0	35.5	41.5	29.5	48.0	49.5
72	24.5	76.3	83.3	29.6	52.0	60.8	26.5	36.8	36.6	34.3	45.1	48.9
B. Percentage of apoptotic cells												
24	4.8	5.3	5.6	9.0	11.4	17.2	11.5	24.8	24.7	28.7	37.9	36.6
48	3.6	8.3	7.9	8.3	13.7	29.7	9.2	44.7	40.6	28.8	49.0	61.1
72	3.8	14.7	17.6	10.3	15.1	30.0	15.0	59.4	53.5	25.9	53.8	67.4

NOTE: Propidium iodide staining and flow cytometry were used to determine cell cycle contents. PhiPhiLux G1D2 kit was used to detect caspase-3 activity by flow cytometry.

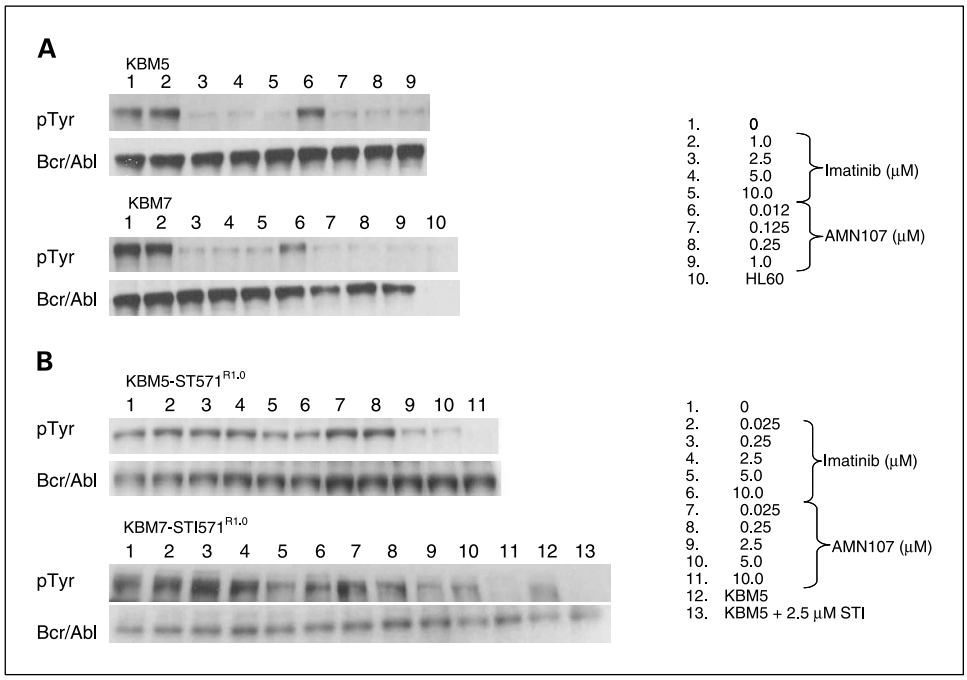

Fig. 3. Bcr-Abl expression and phosphorylation after imatinib or AMN107 exposure in imatinib-sensitive (*A*) and imatinib-resistant (*B*) leukemic cell lines. Cells (2×10^7) were exposed to different concentrations of imatinib or AMN107 for 3 hours, lysed, and whole cell lysates immunoprecipitated with Bcr-Abl antibody. Western blot analysis using antiphosphotyrosine was done on immunoprecipitated complex. After stripping, the membranes were reprobed with antibody to Bcr-Abl to assess the total amount of Bcr-Abl protein. HL60 cells were used as a Bcr-Abl – negative control. *STI*, imatinib.

(Table 1). A slight time-dependent increase in the apoptotic response was noted in KBM5-STI571$^{R1.0}$ cells exposed to AMN107 but not in KBM5-STI571$^{R1.0}$ cells exposed to imatinib. These findings indicate that AMN107 is either more potent than imatinib in inducing apoptosis by inhibiting mutated Bcr-Abl or that AMN107 and imatinib affect different pathways in these imatinib-resistant cells. In KBM7 and KBM7-STI571$^{R1.0}$ cells, imatinib and AMN107 induced similar degrees of apoptotic response, although the percentage of apoptotic cells seemed to be higher in cells treated with AMN107 for >24 hours (Table 1). Thus, at equipotent concentrations, imatinib and AMN107 induced a similar degree of apoptotic response in all but KBM5-STI571$^{R1.0}$ cells (Table 1).

Bcr-Abl expression and phosphorylation in sensitive and resistant cells. In KBM5 and KBM7 cells, a short exposure to imatinib completely inhibited Bcr-Abl phosphorylation at a concentration of 2.5 μmol/L; AMN107 achieved the same effect at 125.0 nmol/L, indicating ~20 times higher potency (Fig. 3A). In KBM5-STI571$^{R1.0}$ and KBM7-STI571$^{R1.0}$ cells, imatinib failed to completely inhibit Bcr-Abl phosphorylation even when cells were exposed to concentrations up to 10 μmol/L; AMN107 inhibited Bcr-Abl phosphorylation at 2.5 μmol/L (KBM5-STI571$^{R1.0}$) or 10 μmol/L (KBM7-STI571$^{R1.0}$; Fig. 3B), again indicating higher potency of AMN107 compared with imatinib.

***Effect of AMN107 on human chronic myelogenous leukemia cells* in vivo.** As previously established for the SCID mouse model of advanced blastic phase CML (31), mice developed ascites, macroscopically visible tumors in the peritoneum or at the site of inoculation or both, and dissemination of leukemia into organs. Mice tolerated administration of AMN107 well, without overt evidence of toxicity. Animals in the control group died between 23 and 32 days after tumor inoculation (median, 27 days). Median survival times for groups treated with AMN107 ranged from 39 to 49 days (Fig. 4). AMN107 was effective at all doses tested and the increases in survival times were dose related (Fig. 4; Table 2).

Discussion

In the study reported here, we evaluated AMN107, a novel aminopyrimidine inhibitor of Bcr-Abl, in imatinib-sensitive and imatinib-resistant CML cell lines and CML-derived cell lines growing as xenografts in nude mice. The resistant cell lines we chose manifested two of the best appreciated *BCR-ABL* – dependent mechanisms of imatinib resistance: (*a*) increased amounts of Bcr-Abl tyrosine kinase associated with amplification of the *BCR-ABL* gene (KBM7-STI571$^{R1.0}$ cells; refs. 19, 20 – 22) 22) and (*b*) a point mutation in the *BCR-ABL* domain coding for the ATP-binding site of the Bcr-Abl tyrosine kinase (KBM5-STI571$^{R1.0}$ cells; refs. 16, 19, 34).

In MTS assays, AMN107 was more effective in KBM7 cells, which express a low number of *BCR-ABL* gene copies and transcripts, than in KBM5 cells, which have a high degree of *BCR-ABL* gene amplification. AMN107 was 60 times more potent than imatinib in KBM7 cells and 43 times more potent in KBM5 cells. Given the large difference in the number of gene copies in these two cell lines, the sensitivity of the cells to AMN107 does not seem to be directly related to the extent of *BCR-ABL* gene amplification. In both these imatinib-naïve cell lines, AMN107 completely inhibited the autophosphorylation of the Bcr-Abl tyrosine kinase, with a potency ~20 times that of imatinib. Although resistance to imatinib was associated with a decrease in the effectiveness of AMN107, AMN107 remained more potent than imatinib in both resistant cell lines: It was 26 times more potent than imatinib in KBM7-STI571$^{R1.0}$ cells and thrice as potent in KBM5-STI571$^{R1.0}$ cells. AMN107 was also more effective than imatinib in inhibiting autophosphorylation of Bcr-Abl kinase in imatinib-resistant cells.

The resistant cell lines used in our study had similar degrees of resistance to imatinib (resistance index ~13). AMN107 was more effective in KBM7-STI571$^{R1.0}$ cells, with resistance presumed due to *BCR-ABL* gene amplification (19), than in

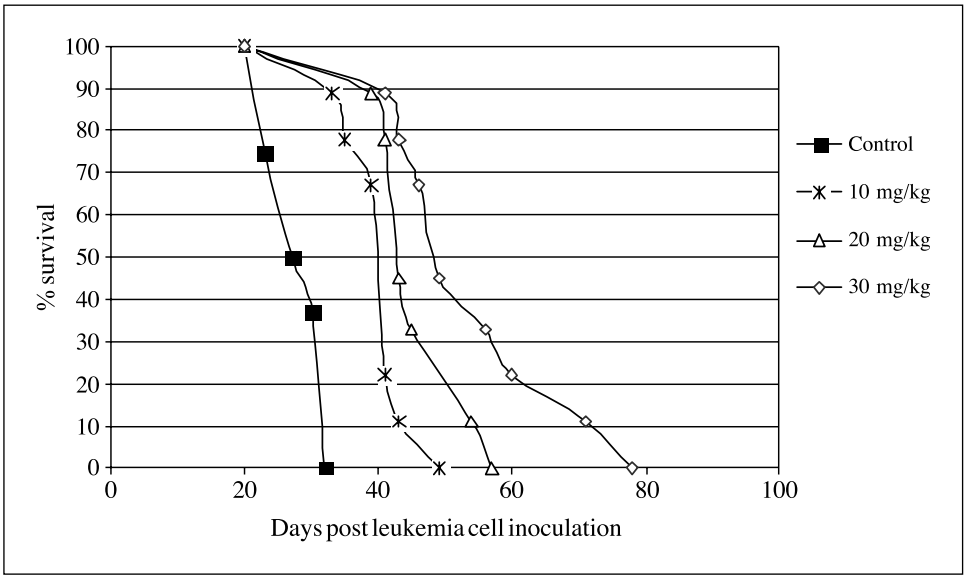

Fig. 4. Survival of SCID mice bearing KBM5 cells treated with AMN107. Irradiated female 5-week-old SCID mice were injected i.p. with 2.4×10^7 KBM5 cells on day 0. Starting on day 20, AMN107 was delivered i.p. daily for 20 days at a dose of 10, 20, or 30 mg/kg. Groups consisted of eight mice (control group) or nine mice (all experimental groups).

KBM5-STI571$^{R1.0}$ cells, characterized by T315I mutation (19, 34). This finding suggests that AMN107 has superior activity against cells with imatinib resistance due to quantitative rather than qualitative changes in the Bcr-Abl protein, perhaps because AMN107 interferes more effectively with activation of the native Bcr-Abl kinase than with activation of the kinase modified by T315I mutation in the activation site(s). The T315I mutation involves the ATP-binding pocket of the Abl kinase and confers a high degree of clinical resistance to imatinib. Of 15 *BCR-ABL* isoforms transfected into Ba/F3 cells, T315I was the only mutation that conferred complete resistance to a new inhibitor of SRC-family kinases, BMS-354825 (30). BMS-354825 treatment of animals grafted with Ba/F3 cells harboring the T315I mutant *BCR-ABL* failed to improve survival (30). These and our findings document the unique impact of this mutation on the interaction of Bcr-Abl with a variety of small-molecular inhibitors. Therefore, even the modest activity of AMN107 in this highly imatinib-resistant human CML model is of interest for future clinical studies and for elucidating the impact of subtle structural changes in Bcr-Abl on the binding and inhibitory activity of new agents.

In experiments focused on cell cycle analysis and caspase-3 activity, the cell cycle distribution and apoptotic responses induced by imatinib and AMN107 seemed generally similar, although in KBM5-STI571$^{R1.0}$ cells, treatment with AMN107 was associated with a more discernible increase in apoptosis than was treatment with imatinib.

In addition to a higher potency *in vitro*, AMN107 had significant antileukemia activity at a range of doses in the KBM5 xenograft model (31). The survival time of SCID mice bearing KBM5 cells treated with AMN107 was extended over that of controls. SCID mouse models of leukemia have been an effective aid in the development of subsequent clinical studies (35–38). We were less successful, however, in evaluating activity of AMN107 in a similar xenograft model using imatinib-resistant KBM5 STI571$^{R1.0}$ cells, the main difficulty being an inconsistent engraftment of resistant cells in the strain of SCID mice and experimental conditions used. Therefore, the weight of our report is with the *in vitro* data, which now seems to be supported by experience in the phase I clinical trial.

In conclusion, the currently reported data provided a rationale for clinical studies of AMN107 in patients with CML who are resistant to or intolerant of imatinib. The preliminary results of phase I studies of AMN107 in advanced stages of chronic myelogenous leukemia support the prediction of our *in vitro* studies by showing documented activity of AMN107 in imatinib-resistant patients (39).

Table 2. *In vivo* antitumor activity of AMN107 in SCID mice bearing KBM5 CML cells

Treatment	No. mice per group	Survival time, range (d)	Median survival time (d)	Treated survival as percentage of control survival*
Control	8	23-32	27	—
AMN107, 10 mg/kg	9	33-49	39	144%
AMN107, 20 mg/kg	9	39-57	43	159%
AMN107, 30 mg/kg	9	41-78	49	182%

*Median survival time of treated mice as percentage of median survival time of control mice.

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Clin Cancer Res 2005;11:4941-4947.