Bcr-Abl resistance screening predicts a limited spectrum of point mutations to be associated with clinical resistance to the Abl kinase inhibitor nilotinib (AMN107)

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Two of the authors (PWM+JM) are employed by a company (Novartis) whose product was studied in the present work.
Abstract

In advanced-phase CML, resistance to imatinib mesylate is associated with point mutations in the Bcr-Abl kinase domain. A new generation of potent Abl kinase inhibitors is undergoing clinical evaluation. It is important to generate specific resistance profiles for each of these compounds, which could translate into combinatorial and sequential treatment strategies. Having characterized nilotinib (AMN107) against a large panel of imatinib-resistant Bcr-Abl mutants, we investigated which mutants might arise under nilotinib therapy using a cell based resistance screen. In contrast to imatinib mesylate, resistance to nilotinib was associated with a limited spectrum of Bcr-Abl kinase mutations. Among these were mutations affecting the P-loop and T315I. Rarely emerging resistant colonies at a concentration of 400 nM nilotinib exclusively expressed the T315I mutation. With the exception of T315I, all of the mutations that were identified were effectively suppressed when the nilotinib concentration was increased to 2000 nM, which falls within the peak – trough range in plasma levels (3.6 – 1.7 µM) measured in patients treated with 400 mg bid. Our findings suggest that nilotinib might be superior to imatinib in terms of the development of resistance. However, our study indicates that clinical resistance to nilotinib may be associated with the predominant emergence of T315I.

Introduction

The tyrosine kinase inhibitor imatinib mesylate (Gleevec; Novartis Pharma) fundamentally changed the standard of care for the treatment of chronic myeloid leukaemia (CML). In the International Randomized Interferon versus STI571 Study (IRIS) trial, imatinib achieved a complete cytogenetic response rate of 84% in newly diagnosed CML patients after a 42-months follow-up. However, in advanced-phase CML and Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ ALL), responses to imatinib may be of a short
Clinical resistance to imatinib has been attributed to amplification of the Bcr-Abl gene, clonal evolution, and most importantly, Bcr-Abl gene mutations that obviate binding to the drug target. Consequently, alternative Bcr-Abl kinase inhibitors, such as PD166326, nilotinib (AMN107) or dasatinib (BMS-354825) have been developed. Nilotinib and dasatinib already entered clinical trials and displayed promising activity in patients with imatinib-resistant chronic and advanced phase CML, and imatinib-resistant Ph+ ALL, respectively. These compounds are more potent than imatinib in suppressing Bcr-Abl tyrosine kinase activity and cell growth of Bcr-Abl-transformed cells, and are capable of suppressing imatinib-resistant, mutant forms of Bcr-Abl. This is in line with structural data, suggesting different binding properties for imatinib and alternative Abl kinase inhibitors such as PD173955 and nilotinib to the Bcr-Abl kinase domain. Thus, specific resistance profiles for different therapeutically used Abl kinase inhibitors can be expected to emerge in treated patients, and it may be important to know these resistant profiles upfront in order to guide therapeutic decisions. To fully characterize nilotinib, we evaluated it against a panel of Ba/F3 cell lines expressing imatinib-resistant Bcr-Abl. In order to identify specific resistance mutations in Bcr-Abl which could emerge under therapy with this new agent, we then evaluated nilotinib in the cell-based screening method which we have previously established.

**Materials and methods**

**Inhibitors**

Nilotinib (AMN107) was dissolved in dimethyl sulfoxide (DMSO) to give a 10 mM stock solution which was and stored at -20°C. Imatinib mesylate was dissolved in water and 10 mM stock solutions were stored at -20°C.
Cell culture and DNA constructs

Ba/F3 cells were maintained, transfected and transformed as described previously.33 Point mutations that were either identified in the nilotinib-screen or that are known to be associated with imatinib resistance in patients with CML or Ph+ ALL were introduced in Mig EGFP Bcr-Abl p18534,35 using the QuickChange mutagenesis kit (Stratagene, Amsterdam, the Netherlands). Mutant p185 was then subcloned as described before.33 Total RNA was extracted using TRizol reagent (Invitrogen, Carlsbad, CA). Bcr-Abl-specific nested reverse-transcription-polymerase chain reaction of the Bcr-Abl kinase domain was performed as described before.33

Resistance screen

Screening for inhibitor-resistant colonies was performed as described previously.33 Briefly, Ba/F3 Mig EGFPp185 wild-type cells were cultured in 96-well plates at a density of 4 x 10^5 cells per well in the presence of nilotinib at 50, 100, 150, 200, 300 and 400 nM. Culture supernatants were replaced by fresh medium containing inhibitor after 48 h. Cell colonies that became visible were picked, expanded and analyzed. Resulting inhibitor-resistant sublines were cultured in the presence of inhibitor at a concentration corresponding to that used during the screen.

Effects of imatinib and nilotinib on Bcr-Abl autophosphorylation

The phosphorylation status of Bcr-Abl in cell lysates was determined with a capture ELISA: Ba/F3 cells were seeded at 1-2 x 10^5 cells in 50 µL/well in 96-well round bottom tissue culture plates. Compounds (serial dilutions) were added to the cells and incubated for 1.5 h, (37°C, 5% CO_2). Untreated cells were used as controls. After centrifugation, the cell pellets were lysed by addition of 150 µL cold lysis buffer (50 mM Tris/HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1 mM EGTA, 1% NP-40, 2 mM Na_3VO_4, 1 mM PMSF, 50 µg/mL aprotinin and 80 µg/mL leupeptin). 50 µL/well of the lysates were transferred to black ELISA plates
(Packard HTRF-96 black plates) that had been coated (4°C, overnight) with the capture Ab anti-Abl-SH3 domain Ab 06-466 from Upstate (50 ng/well) and blocked (4 h, 22°C) with PBST, 3% TopBlock (Juro). After 3-4 h incubation at 4°C, phosphorylation of the captured Bcr-Abl was detected with PY20(AP) diluted in blocking buffer to 0.1 to 0.33 µg/mL. Finally, chemiluminescence was quantified after 45 min incubation with 90 µL per well of the AP substrate (CDPStar RTU with Emerald II from Applied Biosystems (#T2388C) by measuring counts per second (CPS) with a Packard Top Count Microplate Scintillation Counter (Top Count). Compound effects were calculated as percent reduction of the readout for the positive controls. From the dose-response curves, IC50 values were calculated by graphical extrapolation.

Effects of imatinib and nilotinib on cell growth

Effects on cell viability (and proliferation) were determined using an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)-based method (absorption of formazan at 490 nm; CellTiter 96; Promega, Madison, WI) with measurements taken in triplicates after 24 and 48 h culture. Alternatively, the luminescent ATPLiteTM kit (Perkin Elmer Life Sciences, #6016947) was used according to the instructions of the suppliers. After 72 h incubation in absence or presence of drug, light emission (luminescence) was measured with a Packard TopCount. After subtraction of the background signal, compound effects were calculated as percent reduction of the control signal. TGI50 values were determined from the dose-response curves by graphical extrapolation.

Western blot

Ba/F3 cells were cultured for 2.5 h without and in the presence of inhibitor at the indicated concentrations. Cell lysis, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotting were done as described previously. Abl antibodies were obtained from Pharmingen (8E9) (BD Biosciences, Heidelberg, Germany) and Calbiochem-
Novabiochem (Ab3) (Schwalbach, Germany). Antibodies to phosphotyrosine were purchased from Upstate Biotechnology (4G10) (Biozol, Eching, Germany) and Transduction (PY20) (BD Biosciences). Bands were visualized using the enhanced chemoluminescence system (ECL) system (Amersham, Braunschweig, Germany).

Results

Activity of nilotinib against known imatinib-resistant Bcr-Abl mutants

The autophosphorylation activity of the Bcr-Abl fusion protein in cells was quantified with a capture ELISA using a c-Abl-specific capture antibody, together with an enzyme-labeled anti-phosphotyrosine antibody and a luminescent substrate. The background tyrosine kinase activity of c-Abl was too low to be detected. The effects of imatinib and nilotinib on Ba/F3 cell lines expressing wild-type or imatinib-resistant Bcr-Abl are shown in Table 1. Imatinib and nilotinib inhibited wild-type Bcr-Abl autophosphorylation with mean IC50 values of 208 and 49 nM respectively. In confirmation of previous reports, the Y253H, E255K and E255V mutants were inhibited with IC50 values between 200 – 800 nM, and the T315I mutant was insensitive to nilotinib at concentrations < 10000 nM (data not shown). In addition, a number of less frequently observed imatinib resistance mutations were potently inhibited by nilotinib with mean IC50 values in the 30-200 nM range (Table 1). These effects were highly correlated with those on cell proliferation with nilotinib inhibiting the growth of wild-type Bcr-Abl-expressing Ba/F3 cells with a mean IC50 value of 28 nM (imatinib 430 nM) and inhibiting mutant cell lines with mean IC50 values in the 20-300 nM range.

Screening for nilotinib-resistant Ba/F3 clones

Employing a cell-based screening system, we generated a total of 234 Ba/F3 cell clones displaying nilotinib-resistance. The frequency of resistant cell clones emerging in the presence of nilotinib at 50 nM, which corresponds to 2.5 times the IC50 value, was 1.9 per
million cells (Fig. 1), and decreased with increasing nilotinib concentrations to 0.05 per million at 400nM (20-fold the IC50 value). Thus, an increase of nilotinib concentration by a factor of eight reduced the frequency of nilotinib resistant Ba/F3 cell clones by a factor of 38. We then investigated whether or not the nilotinib resistant Ba/F3 cell clones harbored Bcr-Abl kinase domain mutations which were reduced in their sensitivity to the drug. Mutant forms of Bcr-Abl were not detected in cell clones surviving 50 nM and 100 nM nilotinib. However, from cell lines growing in the presence of 150, 200, 300 and 400 nM nilotinib, 6/48, 6/37, 37/37 and 2/2 resistant clones, respectively, had Bcr-Abl kinase domain mutations. This corresponded to a frequency of mutant clones of 0.16, 0.16, 0.27, and 0.05 per million cells at 150, 200, 300 and 400 nM, respectively (Fig. 1). For all concentrations, the P-loop contained four out of nine different exchanges identified within the Bcr-Abl kinase domain, including Q252H, Y253H, E255K, and E255V. P-loop mutations predominated at lower nilotinib concentrations (150 and 200 nM, Fig. 2). When inhibitor concentrations were increased from 150 to 200 and 300 nM, respectively, we observed a shift from the low-grade resistant P-loop mutant Q252H to more resistant ones (Y253H and E255K/V). At the same time, the ratio of P-loop versus T315I changed in favor of T315I, constituting 88 and 100 per cent of mutations at 300 nM and 400 nM, respectively (Figure 2). T315I represented the most abundant single exchange, which was present in 33 out of 51 mutant clones. Less frequently observed mutations at lower concentrations included F311I, a Q252H/S349L double mutant, and F359I (Fig. 2). An exchange at F359 to isoleucine and an exchange at S349 have thus far not been described in imatinib resistant patients. Compared to the cell-based resistance screen with imatinib, which we have reported recently,33 nilotinib produced a lower frequency of inhibitor resistant clones. A frequency of 0.56 per million cells at 4 μM imatinib (0.1 per million/wild-type and 0.46 per million/mutant)33 opposed to 0.05 per million (mutant only) at 400 nM nilotinib (Fig. 1). In addition, nilotinib produced a limited spectrum of Bcr-Abl kinase domain mutations when compared to imatinib. For all inhibitor concentrations, the nilotinib-screen
produced nine exchanges at seven different positions, as opposed to 26 exchanges at 21 positions with imatinib (Fig. 3A and 33). At 400 nM nilotinib, resistant clones exclusively contained T315I, whereas at 4 µM imatinib, there were still 12 exchanges at 11 different positions (Figure 3B).

Analysis of nilotinib resistance mutations identified in the screen

Mutations identified in the nilotinib-screen were recreated using site-directed mutagenesis and expressed in Ba/F3 cells. In cell growth assays, dose-response curves indicated that all these mutations mediate resistance to nilotinib (Fig. 4, Table 2). P-loop mutations shifted IC90 values from 87 nM for wt Bcr-Abl to concentrations ranging from 383 nM (Q252H) to 2640 nM (Y253H). However, proliferation of clones expressing weak (Q252H, E255K), and also strong P-loop mutations (Y253H, E255V) was effectively inhibited when the nilotinib concentration was increased to 4000 nM (Fig. 4). F311I caused a moderate resistance to nilotinib (IC50: 121 nM, IC90: 467 nM). In contrast, in line with previous reports,30,37 T315I caused a complete resistance to nilotinib at concentrations of 4000 nM and above (Fig. 4, Table 2). The double mutant Q252H/S349L displayed a cooperative reduction of inhibitor sensitivity compared to both single mutations (Fig. 4, Table 2). Both exchanges at F359 were completely inhibited by 500 to 1000 nM nilotinib (Fig. 4). In accordance with growth inhibition assays, Bcr-Abl autophosphorylation was suppressed in Ba/F3 cells expressing wild-type Bcr-Abl (Fig. 5A). All mutants except T315I displayed complete suppression of Bcr-Abl autophosphorylation at concentrations ranging from 250 nM (F311I, F359V) to 4000nM (Y253H).

Discussion

The availability of small molecule kinase inhibitors specifically acting on different oncogeneic tyrosine kinases undoubtedly inaugurated new therapeutic options in a variety of neoplastic disorders. The Abl kinase inhibitor imatinib induces impressive and durable responses in chronic myeloid leukaemia, with a low risk of relapse and only a small
percentage of patients being unable to tolerate the drug. However, the development of resistance constituted a major drawback in the treatment of advanced-phase CML. Resistance to imatinib in CML often is associated with specific point mutations in the Bcr-Abl kinase domain that affect binding of imatinib to its target. Specific mutations mediating imatinib resistance were also detected in FIP1L1-PDGFRalpha in patients with hypereosinophilic disorders, and in the c-Kit kinase domain in patients with gastrointestinal stromal tumors. This phenomenon is not specific for imatinib, since a resistance mutation in the FLT3 kinase domain has been described in a patient who received PKC412 for acute myeloid leukaemia, and a T790M mutation in the epidermal growth factor gene (EGFR), which is homologous to the T315I mutation in Bcr-Abl, has been reported in patients with lung cancer resistant to the EGFR inhibitor gefitinib (Iressa).

For imatinib, cellular IC50 values in Bcr-Abl-transformed cell lines are reported to be in the range of 200 to 400 nM. In contrast, cellular IC50 values of alternative Abl kinase inhibitors are below 10 nM for the pyridopyrimidine PD166326, 14 nM for the trisubstituted purine analog AP23464, 20-60 nM for the aminopyrimidine nilotinib (AMN107), and below 2.5 nM for the disubstituted pyrimidine derivative dasatinib (BMS-354825). In addition, these novel compounds are capable of suppressing imatinib-resistant mutant forms of Bcr-Abl, with the exception of T315I. Nilotinib and dasatinib have entered clinical trials and displayed promising activity in patients with imatinib-resistant chronic and advanced phase CML, and imatinib-resistant Ph+ ALL, respectively. However, data from analyses of resistant patients, especially in the setting of chronic-phase CML, will not be available until a large number of patients have been treated within clinical trials. As Abl kinase inhibitors based upon different chemical scaffolds exhibit distinct modes of binding to the Abl kinase domain, each inhibitor class or even each single compound may display a distinct profile of amino-acid residues critical for binding and inhibition.
Here we show that nilotinib, both in terms of kinase inhibition as well as Bcr-Abl-dependent cell proliferation, potently inhibits 21 known imatinib-resistant mutant forms of Bcr-Abl, with IC50 values in the 20 – 300 nM range. As previously reported, the P-loop mutants Y253H, E255K and E255V displayed moderate resistance to nilotinib with IC50 values in the 200-800 nM range, and nilotinib did not significantly inhibit T315I. Since in Phase I clinical studies in CML patients, oral administration of nilotinib at 400 mg BID provided maximal and trough plasma drug concentrations of 3.6 and 1.7 µM respectively, only the T315I Bcr-Abl mutant is expected to be insensitive to nilotinib therapy. However, this does not address potential new mutations which might emerge under treatment with nilotinib.

We have recently reported results of a cell-based strategy that allows generation of imatinib-resistant Ba/F3 cell clones in a high frequency. The pattern of mutations identified in these clones corresponded to the pattern observed in patients displaying imatinib resistant CML. Our findings with nilotinib reported here point to significant advantages over imatinib that may translate in superior activity of nilotinib in the clinic. When compared to imatinib, nilotinib produced a lower frequency of resistant cell clones and a reduced spectrum of mutations. The proportion of T315I to P-loop mutations changed in favour of T315I with increasing concentrations of nilotinib. There were only few clones expressing mutations other than P-loop or T315I. At 400 nM nilotinib, resistant cell clones emerged at a low frequency of one per 20 million cells, and these clones exclusively contained T315I. Although bone marrow concentrations are not available, the steady-state trough plasma concentration of 1700 nM following oral administration of 400 mg nilotinib BID, is four-times higher than the maximum concentration of 400 nM used in this screen. In marked contrast, imatinib at 4000 nM, which approximates to trough concentrations achieved in plasma of treated patients, resulted in a frequency of resistant cell clones of one per 1.8 million cells. These imatinib-resistant clones still contained a variety of different resistance mutations. Thus, in comparison to imatinib, nilotinib induced resistant cell clones at a frequency that was by the factor of 10
lower at a 10-fold lower concentration. Consequently, the plasma concentrations of nilotinib achieved in patients treated with 400 mg BID might prevent the selection and expansion of resistant disease clones. Moreover, in contrast to imatinib, clinical resistance to nilotinib is predicted to be associated with the predominant emergence of T315I. Resistance mutations that may arise in the presence of suboptimal nilotinib concentrations include P-loop mutations that mediate a higher degree of nilotinib resistance such as Y253H and E255V. However, since nilotinib plasma levels of up to 3600 nM were measured in phase I/II studies,20,21 P-loop mutations may emerge less frequently in patients treated with nilotinib, provided that sufficient plasma concentrations are achieved. Also, nilotinib is expected to induce responses in cases where P-loop mutations cause resistance to imatinib.

Taken together, our findings indicate that nilotinib should be efficacious in the majority of cases where Bcr-Abl point mutants cause imatinib-resistance, and suggest that nilotinib may be superior to imatinib in terms of the development of resistance. Specific resistance profiles for different Abl kinase inhibitors will guide therapeutic decisions with respect to drug combinations and sequential treatment strategies, and will allow the determination of critical plasma concentrations that have to be reached to prevent or overcome specific resistance mutations. This or similar resistance screening approaches can be translated to other neoplastic diseases, where rational drug targets are identified and therapeutic kinase inhibitors are available, such as gastrointestinal stromal tumor (GIST), hypereosinophilic syndrome, acute myelogenous leukaemia (AML), or lung cancer.

**Acknowledgements**

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Figure 1. Frequency of resistant clones decrease with increasing nilotinib concentrations. Ba/F3 Mig EGFP Bcr-Abl p185 wild-type cells were cultured in 96-well plates in the presence of nilotinib at the indicated concentrations. Resistant colonies were picked and analyzed for presence of point mutations within the Bcr-Abl kinase domain. Wild-type (open bars) and mutant (filled bars) sublines are shown separately. The frequency is shown as resistant clones per million cells at the beginning of culture.

Figure 2: Occurrence of P-loop mutations and T315I change inversely with increasing nilotinib concentrations. The relative abundance of different mutations is depicted for each nilotinib concentration that was used in the screen. P-loop mutations included Q252H, Y253H, E255K, and E255V. A Q252H/S349L double mutant is shown separately.

Figure 3: Nilotinib produces a limited set of Bcr-Abl kinase domain mutations. The position of identified mutations within the Bcr-Abl kinase domain is shown for imatinib (above) and nilotinib (below). Point mutations that were identified in imatinib-resistant clones were described previously and are shown for comparison.33 (A) 26 exchanges affecting 21 different positions were identified in cell clones resistant to 1, 2 or 4µM imatinib (above). With nilotinib at 150, 200, 300 or 400 nM, nine exchanges at seven positions were found (below). S349L was identified in conjunction with Q252H. (B) Resistance mutations identified using imatinib at 4µM (above) compared to 400nM nilotinib (below).

Figure 4: Of all mutations identified, T315I was the only exchange mediating full resistance to nilotinib. Mutations identified in the nilotinib-screen were recreated in Bcr-Abl p185 using site-directed mutagenesis and expressed in Ba/F3 cells. After incubation for 24 and 48 hours without and in the presence of nilotinib at the indicated concentrations, proliferation was measured using an MTS-based method. At least two independent
experiments were performed for each construct. Representative results of one experiment after 48 hours of incubation are shown. OD indicates optical density. Values are expressed as mean of triplicates. Bars: ± SE. S349L, although not identified in the screen, was included for comparison with Q252H and the Q252H/S349L double mutant.

**Figure 5: Inhibition of autophosphorylation in Bcr-Abl-mutant, nilotinib-resistant cell lines.** Mutations that emerged in the nilotinib-screen were reengineered and expressed in Ba/F3 cells. Cells were incubated without and in the presence of nilotinib at the indicated concentrations. Cell lysates were subjected to SDS-PAGE. Blots were probed for phosphotyrosine (left panel) and Abl (right panel). (A) Results for resistance mutations displaying cellular IC50 values of less than 500nM and, (B) for mutants causing strong resistance towards nilotinib with cellular IC50 values of 500nM or above.

**Table 1:** Ba/F3 cells were engineered to express mutant forms of Bcr-Abl p185 which are known to mediate resistance to imatinib, in extension to frequent clinical isolates already studied. A cell-capture enzyme linked immunosorbant assay was used to evaluate drug effects on Bcr-Abl kinase activity, and an ATLite™ assay (Perkin Elmer) used to evaluate effects on cell growth. Results are expressed as mean IC50 values (nM) ± SEM (number of replicates).

**Table 2:** Ba/F3 cells were transfected with engineered mutant forms of Bcr-Abl and treated as stated in Figure 4. IC90 values and fold increase in comparison to Bcr-Abl wild-type-expressing Ba/F3 cells were calculated using the resulting growth curves.
References


17. Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, et al. Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. Blood. 2002;100:1014-1018.


Table 1: Effects of imatinib and nilotinib on known imatinib-resistant mutant forms of Bcr-Abl.

<table>
<thead>
<tr>
<th>Bcr-Abl form (Construct)</th>
<th>Imatinib</th>
<th>Nilotinib</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Autophosph</td>
<td>Proliferation</td>
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<tr>
<td>wild-type p185</td>
<td>208 (2)</td>
<td>430 ± 11 (9)</td>
</tr>
<tr>
<td>M237I</td>
<td>399 (2)</td>
<td>1545 (2)</td>
</tr>
<tr>
<td>L248V</td>
<td>1011 (2)</td>
<td>2081 (2)</td>
</tr>
<tr>
<td>G250A</td>
<td>313 (2)</td>
<td>1269 (2)</td>
</tr>
<tr>
<td>G250V</td>
<td>489 (2)</td>
<td>624 (2)</td>
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<td>E255D</td>
<td>754 (2)</td>
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<td>E255R</td>
<td>1877 (2)</td>
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<td>E275K</td>
<td>1038 (2)</td>
<td>583 (2)</td>
</tr>
<tr>
<td>D276G</td>
<td>1284 (2)</td>
<td>2486 (2)</td>
</tr>
<tr>
<td>E281K</td>
<td>584 (2)</td>
<td>1601 (2)</td>
</tr>
<tr>
<td>E285N</td>
<td>919 (2)</td>
<td>1284 (2)</td>
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<td>1480 (2)</td>
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</tr>
<tr>
<td>F317C</td>
<td>1090 (2)</td>
<td>694 (2)</td>
</tr>
<tr>
<td>F317V</td>
<td>544 ± 47 (3)</td>
<td>549 ± 173 (4)</td>
</tr>
<tr>
<td>D325N</td>
<td>584 (2)</td>
<td>887 (2)</td>
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<tr>
<td>S348L</td>
<td>553 (2)</td>
<td>1370 (2)</td>
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<tr>
<td>E355A</td>
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<td>L387F</td>
<td>530 (2)</td>
<td>172 (2)</td>
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<tr>
<td>M388L</td>
<td>517 (2)</td>
<td>525 (2)</td>
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### Table 2. Cellular IC90 values of Bcr-Abl resistance mutations identified in the nilotinib screen

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Nilotinib, nM</th>
<th>Fold, IC90 wt</th>
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<tbody>
<tr>
<td>wild-type p185</td>
<td>87</td>
<td>-</td>
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<tr>
<td>Q252H</td>
<td>383</td>
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</tr>
<tr>
<td>Y253H</td>
<td>2640</td>
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<tr>
<td>E255K</td>
<td>644</td>
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<td>E255V</td>
<td>1928</td>
<td>22.1</td>
</tr>
<tr>
<td>F311I</td>
<td>467</td>
<td>5.4</td>
</tr>
<tr>
<td>T315I</td>
<td>&gt;&gt;4000</td>
<td>&gt;&gt;45</td>
</tr>
<tr>
<td>S349L</td>
<td>119</td>
<td>1.4</td>
</tr>
<tr>
<td>S349L+Q252H</td>
<td>887</td>
<td>10.2</td>
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<tr>
<td>F359I</td>
<td>433</td>
<td>5</td>
</tr>
<tr>
<td>F359V</td>
<td>682</td>
<td>7.8</td>
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Fig. 1

Resistant clones per $10^6$ cells - Nilotinib

IC$_{50}$ x 2.5 5 7.5 10 15 20

<table>
<thead>
<tr>
<th>Concentration (nM)</th>
<th>Resistant clones ($10^6$ cells)</th>
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<tr>
<td>50 nM</td>
<td>1.9</td>
</tr>
<tr>
<td>100 nM</td>
<td>0.96</td>
</tr>
<tr>
<td>150 nM</td>
<td>1.09</td>
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<tr>
<td>200 nM</td>
<td>0.81</td>
</tr>
<tr>
<td>300 nM</td>
<td>0.16</td>
</tr>
<tr>
<td>400 nM</td>
<td>0.05</td>
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</tbody>
</table>
Fig. 2

[Chart showing occurrence (%) of various mutations at different AMN concentrations: AMN 150nM, AMN 200nM, AMN 300nM, AMN 400nM.]
Fig. 3A

Imatinib screen

E255D/K/R/V
Y253F/H
Q252H
G250E
L248V
M237I
M244V
E275K
D276G
F311V
F317L
K285N
T315I
M351L
S348L
F359C/V
M388L
SH2
A
P
C
F311I
S349L*
T315I
F359I/V

Nilotinib screen

Q252H
Y253H
E255K/V

* Q252H/S349L double mutant

Fig. 3B

Imatinib screen

4 μM

E255R/V
Q252H
L248V
E281K
D276G
F311V
F317L
T315I
M351L
S348L
E355G
SH2
A
P
C
T315I

Nilotinib screen

0.4 μM
Fig. 4

Graph showing the OD490 [%] for different samples under various concentrations. Key: parental, WT, Q252H, Y253H, E255K, E255V, F311I, T315I, S349L, S349L+Q252H, F359I, F359V, DMSO, 12.5nm, 25nm, 50nm, 125nm, 250nm, 500nm, 1000nm, 2000nm, 4000nm.
Fig. 5

A

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<tr>
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<th>αPtyr</th>
<th>αAbl</th>
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<td>wt</td>
<td><img src="image1.png" alt="Image" /></td>
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<td>Q252H</td>
<td><img src="image3.png" alt="Image" /></td>
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<td>Q252H+</td>
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<td>S349L</td>
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B

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Bcr-Abl
Bcr-Abl resistance screening predicts a limited spectrum of point mutations to be associated with clinical resistance to the Abl kinase inhibitor nilotinib (AMN107)

Nikolas von Bubnoff, Paul W Manley, Jurgen Mestan, Jana Sanger, Christian Peschel and Justus Duyster