Activity of Dasatinib, a Dual SRC/ABL Kinase Inhibitor, and IPI-504, a Heat Shock Protein 90 Inhibitor, against Gastrointestinal Stromal Tumor – Associated PDGFRAD842V Mutation

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Abstract Purpose: Activating mutations in platelet-derived growth factor receptor-α (PDGFRα) have been reported in ~5% to 10% of patients with gastrointestinal stromal tumors (GIST). Imatinib efficiently inhibits the juxtamembrane PDGFRα mutations, whereas many tyrosine kinase domain activation loop PDGFRα mutations confer primary resistance to imatinib. In this study, we compared the efficacy of second-line tyrosine kinase inhibitors such as dasatinib, sorafenib, and nilotinib against two GIST-related PDGFRα mutants, PDGFRαD842V and PDGFRαΔDIM842-844. In addition, we sought to investigate the inhibitory effect of the heat shock protein 90 inhibitor, IPI-504, on these mutants.

Experimental Design: Primary imatinib-resistant tumor cells and cell lines expressing imatinib-resistant PDGFRαD842V or imatinib-sensitive PDGFRαΔDIM842-844 mutants were treated with different concentrations of dasatinib, sorafenib, nilotinib, and IPI-504. The effect of treatment on proliferation, survival, and signaling was determined.

Results: All inhibitors tested exhibited a high efficacy toward the PDGFRαΔDIM842-844 mutant. In contrast, ex vivo and in vitro assays revealed that only dasatinib potently inhibited the PDGFRαD842V isoform with an IC50 value of 62 nmol/L. Sorafenib and nilotinib were significantly less efficacious against this mutation, inhibiting the PDGFRα kinase activity at >1,000 and >5,000 nmol/L, respectively. IPI-504 treatment potently inhibited PDGFRα kinase activity by inducing the degradation of PDGFRαD842V mutant with an IC50 value of 239 and 1,310 nmol/L, respectively. IPI-504 treatment potently inhibited PDGFRα kinase activity by inducing the degradation of PDGFRαD842V and PDGFRαΔDIM842-844 at 256 and 182 nmol/L, respectively.

Conclusions: Treatment with dasatinib or the heat shock protein 90 inhibitor IPI-504 may provide a therapeutic alternative for GIST patients whose tumors carry the imatinib-resistant PDGFRαD842V mutant isoform.

Gastrointestinal stromal tumors (GIST) comprise the largest subset of mesenchymal stromal tumors that develop along the gastrointestinal tract (1, 2). Constitutive activation of the KIT tyrosine kinase through oncopgenic mutations is an early and probably initiating event in the majority of GISTS (3, 4). Alternatively, a subset of GISTS express a constitutively activated platelet-derived growth factor receptor-α (PDGFRα) mutant, a tyrosine kinase homologous to KIT (5). Activating KIT and PDGFRα mutations in GISTS differ in type and affect different receptor domains (6). Therapeutic inhibition of KIT or PDGFRα by the small-molecule inhibitor imatinib gives a clinical response in the majority of advanced GISTS (3). However, early resistance has been reported in 10% to 20% of patients. It is well known that sensitivity to imatinib depends on the location of the KIT and PDGFRα gene mutation (3). Based on in vitro studies and subsequent clinical trials, it has been shown that a subset of GISTS harboring KIT and PDGFRα mutations in the second tyrosine kinase domain (activation loop domain) do not respond well to imatinib treatment (3, 7–10). The oncogenic PDGFRα mutations detected in GISTS mainly involve exons 12, 14, or 18, and account for ~5% to 10% of all GIST mutations. Wild-type PDGFRα is sensitive to imatinib (11), but only a portion of the constitutively activated PDGFRα isoforms identified in GISTS are potently inhibited by imatinib (3). The missense PDGFRαD842V mutation resulting in the substitution of aspartic acid to valine at codon 842 accounts for ~60% of all PDGFRα mutations known in GISTS (5). It confers primary resistance to imatinib in vitro and is a driver of resistance to sunitinib in vitro (3, 8, 12), resistance to sunitinib in vitro (13), and resistance to nilotinib in vitro and in vivo (14).
Conversely, we have previously shown that PKC412 efficiently inhibited the PDGFRA\textsuperscript{D\textsubscript{842V}} mutant at the concentration of 1 \(\mu\)mol/L (12).

Because resistance of GIST patients to imatinib is a continuous clinical challenge, multiple novel therapeutic strategies are under development. Novel small molecule compounds being tested in clinical trials include nilotinib, sorafenib, and dasatinib.

In addition, heat shock protein 90 (HSP90) inhibition, causing degradation of wild-type and imatinib-resistant KIT mutant proteins, has recently been validated in preclinical studies as an alternative therapeutic strategy to challenge the heterogeneity of KIT imatinib-resistant mutations in patients with GIST (15). The PDGFR protein is also subject to HSP90-mediated protection from proteasomal degradation in cancer cells (16) and HSP90 inhibition may cause effective PDGFR oncoprotein degradation, but the effect of HSP90 inhibitors on GISTs bearing PDGFRA mutations has thus far not been tested.

In this report, we examined the efficacy of second-generation tyrosine kinase inhibitors nilotinib, dasatinib, and sorafenib for the inhibition of PDGFRA\textsuperscript{D\textsubscript{842V}}-expressing and PDGFRA\textsuperscript{DIM842-844}-expressing cells. In addition, we tested the potency of the HSP90 inhibitor, IPI-504, to induce PDGFRA oncoprotein degradation as a therapeutic alternative for imatinib-resistant, PDGFRA mutant GISTS.

### Materials and Methods

**Inhibitors.** The inhibitors, imatinib mesylate (Glivec/Gleevec, Novartis), sorafenib (BAY-43-9006, Bayer AG), nilotinib (AMN107, Novartis), dasatinib (BMS-354825, Bristol-Meyers-Squibb), and IPI-504 were provided by Infinity Pharmaceuticals, Inc. and MedImmune Inc.
A 10 mmol/L stock solution of the inhibitors, dissolved in DMSO or a citric salt buffer (for IPI-504) was stored at -80°C. The inhibitors were diluted in culture medium just before use.

In vitro assays using transduced Ba/F3 cells. The retroexpression constructs pMSCV-PDGFRAD842V and pMSCV-PDGFRAD842VΔDIM842-844 were described previously (12). Retrovirally transduced Ba/F3 cells, stably expressing the above PDGFRα constructs, were maintained in culture at 1 × 10⁶ cells/mL. For the Ba/F3 dose-response assays, retrovirally transduced Ba/F3 cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum, with or without interleukin 3, in the presence of vehicle alone or with varying concentrations of the inhibitors. The number of viable cells was determined at the start and after 48 h, using the CellTiter 96 AQncious One Solution proliferation assay (Promega).

Induction of apoptosis on transduced Ba/F3 cells was evaluated by flow cytometry using Annexin-V-FLUOS Staining Kit according to the manufacturer’s instructions (18, 19). Both tests were conducted with a BD FACSCanto System, with application of BD FACS Diva software (BD Biosciences).

In addition, the response of the transduced Ba/F3 cells was assessed by Western immunobassays. Cells were exposed to a range of inhibitors in different concentrations, or to vehicle alone (DMSO, or citric salt buffer for IPI-504) and incubated for 2 h at +37°C. After a wash in ice-cold PBS, cells were lysed. Lysates were separated by SDS-PAGE electrophoresis and immunoblotted using anti–phospho-PDGFRα (Tyr754), anti-PDGFRα, and anti-actin antibodies. PAGE electrophoresis and immunoblotted using anti–phospho-PDGFRα (Tyr754), anti-PDGFRα, and anti-actin antibodies.

The 5-bromo-2′-deoxyuridine cell proliferation (Roche Applied Science) and sulforhodamine B survival (Sigma-Aldrich) assays were used to evaluate the proliferation rate and the survival of primary GIST cells, according to the manufacturer’s instructions (18, 19). Both tests were run simultaneously and in duplicate. In short, the adherent primary GIST cells were seeded in 96-well plates (10,000 cells per well) and incubated overnight at +37°C. On the second day, the medium was replaced and different inhibitors (or vehicle alone) were added for 72 h. Cell growth inhibition curves were plotted using curve-fitting Origin software.

### Results

The imatinib-sensitive PDGFRAD842VΔDIM842-844 mutant Ba/F3 cells are highly sensitive to all second-generation tyrosine kinase inhibitors tested. As illustrated in Fig. 1, all second-line inhibitors tested are potent inhibitors of the growth of the PDGFRAD842VΔDIM842-844–expressing Ba/F3 cells in a dose-dependent manner. Imatinib, nilotinib, sorafenib, and dasatinib inhibited the cell growth of PDGFRAD842VΔDIM842-844 Ba/F3 cells with IC₅₀s of 20, 56, 17, and 10 mmol/L, respectively. Furthermore, significant induction of apoptosis of these cells was recorded at 100 mmol/L of imatinib, sorafenib, and dasatinib, and at 500 mmol/L of nilotinib (Fig. 1; Table 1). The Western immunobassays were in line with the dose-response experiments (Fig. 2). All four tyrosine kinase inhibitors tested significantly inhibited the phosphorylation of the PDGFRAD842VΔDIM842-844 Ba/F3 cells at low nanomolar concentrations.

These data indicate that all four second-generation tyrosine kinase inhibitors tested have a high activity towards the PDGFRAD842VΔDIM842-844–expressing Ba/F3 cells in vitro. Notably, dasatinib is even slightly more potent than imatinib in vitro.

The imatinib-resistant PDGFRAD842VΔDIM842-844–expressing Ba/F3 cells are responsive to dasatinib in vitro. As expected, PDGFRAD842VΔDIM842-844 expressing Ba/F3 cells were resistant to imatinib and nilotinib,

### Table 1.

Comparison of in vitro apoptosis induction of imatinib-sensitive PDGFRAD842VΔDIM842-844 and imatinib-resistant PDGFRAD842VΔDIM842-844 mutants expressed in the Ba/F3 cells

<table>
<thead>
<tr>
<th>PDGFRAD842VΔDIM842-844</th>
<th>Normal (%)</th>
<th>Necrotic (%)</th>
<th>Apoptotic (%)</th>
<th>PDGFRAD842VΔDIM842-844</th>
<th>Normal (%)</th>
<th>Necrotic (%)</th>
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<td>IPI-504</td>
<td>84.2</td>
<td>7.6</td>
<td>7.4</td>
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<td>Imatinib 0.1 µmol/L</td>
<td>71.8</td>
<td>15.1</td>
<td>12.9</td>
<td>IPI-504</td>
<td>85.9</td>
<td>6.8</td>
<td>6.6</td>
</tr>
<tr>
<td>Dasatinib 0.1 µmol/L</td>
<td>85</td>
<td>7.8</td>
<td>12.5</td>
<td>IPI-504</td>
<td>87.7</td>
<td>4.7</td>
<td>7.3</td>
</tr>
<tr>
<td>Sorafenib 0.1 µmol/L</td>
<td>73.8</td>
<td>13.4</td>
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<td>87.7</td>
<td>6.9</td>
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<td>IPI-504</td>
<td>88.4</td>
<td>5.7</td>
<td>5.2</td>
</tr>
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with IC_{50}s of 642 and 1,310 nmol/L, respectively, based on growth inhibition measurements (Fig. 1). The efficacy of sorafenib for PDGFRA\textsuperscript{D842V} inhibition was better than imatinib (IC_{50} of 239 nmol/L), but dasatinib was the most potent inhibitor of the PDGFRA\textsuperscript{D842V}-expressing Ba/F3, with an IC_{50} of 62 nmol/L. An apoptosis assay confirmed the significant induction of apoptosis in PDGFRA\textsuperscript{D842V} Ba/F3 transformants 48 h after treatment with 500 nmol/L of dasatinib (Fig. 1; Table 1). Moreover, dasatinib completely inhibited the PDGFRA\textsuperscript{D842V} phosphorylation at 500 nmol/L in Western immunoblot assay (Fig. 2). In line with proliferation and apoptosis experiments, imatinib, nilotinib, and sorafenib were much less efficient in reducing the tyrosine phosphorylation of the PDGFRA\textsuperscript{D842V} protein in Ba/F3 because micromolar concentrations were needed to inhibit PDGFRA kinase activity. The effect on tyrosine phosphorylation of the PDGFRA\textsuperscript{D842V} protein is shown in Fig. 2.

Fig. 2. Western blot analyses of, respectively, PDGFRA\textsuperscript{D842V}-transduced and PDGFRA\textsuperscript{D842V}-transduced Ba/F3 cells after treatment with imatinib (A and E), nilotinib (B and F), sorafenib (C and G), and dasatinib (D and H) for 90 min. Total cell lysates were analyzed with anti-PDGFRA or anti-phospho-PDGFRA (Tyr\textsuperscript{754}) antibodies.
tyrosine kinase again correlated nicely with the dose-dependent inhibition of cell growth. These data show that dasatinib is a potent inhibitor of PDGFRAD842V activity in Ba/F3 cells.

**The imatinib-resistant PDGFRAD842V mutant GIST cells are responsive to dasatinib ex vivo.** As we found that dasatinib was a potent inhibitor of PDGFRAD842V-expressing Ba/F3 cells in vitro, we investigated whether it had comparable efficacy towards human GIST tumor cells carrying the same PDGFRAD842V mutation ex vivo, using cultures of primary tumor cells from an imatinib-naïve patient with PDGFRAD842V mutant GIST. We measured the effect of dasatinib and sorafenib on the proliferation rate and on the survival of primary GIST cells using 5-bromo-2′-deoxyuridine and sulforhodamine B assays (Fig. 3A). The proliferation of the PDGFRAD842V mutant GIST primary tumor cells was almost completely inhibited at a 1,000 nmol/L concentration of dasatinib, with an IC₅₀ of 47 nmol/L. Moreover, ~60% of PDGFRAD842V-expressing primary tumor cells died at 1,000 nmol/L of dasatinib (IC₅₀ of 513 nmol/L for cell survival).

Sorafenib was almost 10 times less effective than dasatinib in inhibiting the proliferation of PDGFRAD842V mutant GIST primary tumor cells, with an IC₅₀ of 425 nmol/L. After 72 hours of treatment, no more than 50% of the PDGFRAD842V-expressing primary cells underwent apoptosis by sorafenib in doses of up to 5 μmol/L.
Western immunoblotting on PDGFRAD842V mutant primary GIST cells showed that 500 nmol/L of dasatinib efficiently inhibited the tyrosine kinase activity of PDGFRAD842V (Fig. 3B). Sorafenib also inhibited the phosphorylation of the PDGFRAD842V protein in a dose-dependent manner, but at 10-fold higher concentrations compared with dasatinib to obtain the same level of inhibition (Fig. 3C). In contrast, imatinib was not able to significantly reduce the ex vivo tyrosine kinase activity of the PDGFRAD842V primary GIST cells at doses of up to 5 µmol/L (Fig. 3D).

**PDGFRAD842V**-expressing and **PDGFRAD842V**-expressing Ba/F3 cells are sensitive to the HSP90 inhibitor IPI-504. As illustrated by the proliferation curves, IPI-504 efficiently inhibited the growth of both PDGFRAD842V-expressing and PDGFRAD842V-expressing Ba/F3 cells with IC50 values of 182 and 256 nmol/L, respectively (Fig. 4A and B). Furthermore, IPI-504 induced pronounced apoptosis of both PDGFRAD842V-expressing and PDGFRAD842V-expressing Ba/F3 cells at 500 nmol/L (Fig. 4C and D; Table 1).

Both PDGFRAD842V mutant Ba/F3 cell lines were treated with increasing concentrations of IPI-504 for 6 hours and analyzed by Western blotting (Fig. 5). For comparison, both cell lines were also treated with 1 µmol/L of imatinib for 2 hours. For both mutants, the PDGFRAD842V tyrosine kinase activity was significantly inhibited by 500 nmol/L of IPI-504. In a similar fashion, the total PDGFR expression levels were significantly lowered at this concentration. Notably, PDGFR inactivation preceded total protein degradation, with the mature (glycosylated) form of PDGFR more substantially degraded in the PDGFRAD842V deletion mutant. In addition, the signaling pathways of both mutants were investigated. There was a lack or only minor activation of the AKT signaling pathway in PDGFRAD842V and PDGFRAD842V, respectively, whereas activation of mitogen-activated protein kinase (MAPK) was clearly visible in both mutant forms. Interestingly, treatment of PDGFRAD842V mutant Ba/F3 cells with imatinib led to increased phosphorylation of MAPK. Conversely, a decrease of MAPK activation was observed in PDGFRAD842V mutants after imatinib treatment, which is consistent with the imatinib phenotype—sensitivity of this mutant. Similarly, treatment of PDGFRAD842V and PDGFRAD842V mutants with IPI-504 led to total and partial inhibition of MAPK phosphorylation levels at 0.5 and 1.0 µmol/L, respectively, being in line with the suppressive effect of the drug on the PDGFR activation levels.

The dynamics of IPI-504-mediated PDGFR inhibition were determined in a time course experiment in which both PDGFRAD842V-expressing and PDGFRAD842V-expressing Ba/F3 cell lines were treated with 1 µmol/L of IPI-504 for different time intervals or with 1 µmol/L of imatinib for 2 hours. Under IPI-504 inhibition of PDGFRAD842V, phosphorylation was observed within 2 hours, followed by protein degradation 6 hours or later (Fig. 6A). Similarly, PDGFRAD842V was partially and completely inactivated after 2 and 6 hours of IPI-504 treatment, respectively (Fig. 6B). As a reference, the PDGFRAD842V mutant in Ba/F3 cells was resistant to treatment with 1 µmol/L of imatinib after 2 hours of treatment, whereas the PDGFRAD842V mutant was clearly sensitive, confirming the differences in sensitivity to imatinib of these two mutants.

**Imatinib-resistant PDGFRAD842V mutant GIST is responsive to IPI-504 ex vivo.** To test the sensitivity of primary GIST cells from a PDGFRAD842V-expressing GIST tumor to the inhibitor IPI-504, the cells were cultured and treated with different concentrations of IPI-504. The proliferation rate of the PDGFRAD842V mutant GIST primary tumor cells was almost completely inhibited at 100 nmol/L of IPI-504, with an IC50 of 21 nmol/L (Fig. 7A). Moreover, after 72 hours,
PDGFRAD842V-expressing primary tumor cells died dose-responsively with an IC50 of 21 nmol/L for cell survival, and with ~50% of PDGFRAD842V primary cells killed at 5 μmol/L of IPI-504 (Fig. 7B).

Western immunoblotting of PDGFRAD842V mutant primary GIST cells (Fig. 7C) showed that IPI-504 completely inhibited the tyrosine kinase activity of PDGFRAD842V at 0.5 μmol/L. The PDGFRAD842V protein (mature and immature forms) expression levels were both significantly lowered at IPI-504 concentrations of 1 μmol/L. Both, AKT and MAPK were activated in PDGFRAD842V mutant primary GIST cells, albeit AKT to a lesser degree. Treatment of these cells with IPI-504 resulted in a clear inhibition of both signaling pathways at 0.5 μmol/L, and parallel to PDGFRA tyrosine kinase activity inhibition. These results indicate that IPI-504 is also a potent inhibitor of PDGFRAD842V activity in human primary GIST cells ex vivo.

**Discussion**

Although most patients with GIST initially respond to therapy, 10% to 20% of patients still exhibit primary resistance to imatinib. Primary resistant tumors either carry no mutations in KIT or PDGFRα, or harbor specific activation loop mutations in KIT or PDGFRα, including the PDGFRAD842V mutation (3, 5, 8). The D842V mutation is the most common activating PDGFRα mutation in GIST, which has also been identified as a mechanism of secondary resistance to imatinib (12).

The development of primary and secondary resistance of GIST toward imatinib is emerging as a significant clinical challenge. Therefore, it is important to validate novel therapeutic strategies for the specific KIT and PDGFRα genotypes.

Second-generation tyrosine kinase inhibitors, nilotinib and dasatinib, inhibit the kinase activity of the wild-type PDGFRα (7, 14, 20, 21). The activity of sorafenib in this regard is unknown. Given the lack of in vivo models for PDGFRα mutant isoforms, in vitro experiments using transformed Ba/F3 mutant cells as well as primary tumor GIST cells are the approach of choice for efficacy testing. In this report, we compared the efficacy of nilotinib, sorafenib, and dasatinib toward two GIST-related PDGFRα mutants, PDGFRAD842V and PDGFRAD842V, which confer differential sensitivity to imatinib (12).

In line with previous reports, we have found that PDGFRADIM842-844-expressing Ba/F3 cells were efficiently inhibited by imatinib (with an IC50 of 20 nmol/L). Correspondingly, nilotinib, sorafenib, and dasatinib also effectively inhibited the cell growth of PDGFRADIM842-844-transduced Ba/F3 cell lines, with IC50s of 56, 17, and 10 nmol/L, respectively. In contrast, the PDGFRAD842V mutant showed varying degrees of sensitivity to these compounds. Thus, nilotinib had a minor effect on the PDGFRAD842V-transduced Ba/F3 cells, displaying an IC50 of 1.31 μmol/L, and leading to only a modest decrease in levels of PDGFRα phosphorylation at high 5.0 μmol/L concentrations. Similar findings were reported by Weisberg and coworkers (22), who showed similar IC50 values of PDGFRAD842V for both nilotinib and imatinib in the 0.5 to 1.0 μmol/L range. The KIT and PDGFRα missense mutations in the second kinase domain keep the activation loop of the tyrosine kinases in an “open” or active conformation.
Dasatinib (BMS-354825) is an orally active, multitarget inhibitor of BCR-ABL and SRC family kinases with a log2 increased potency relative to imatinib (26). This drug was developed for patients with chronic myelogenous leukemia, who were either intolerant to or who developed resistance to imatinib. Dasatinib is also a potent inhibitor of many other protein kinases, including KIT and PDGFs. Studies of dasatinib in GIST and solid tumors are ongoing and preliminary data have suggested some antitumor activity (27). It was recently shown that dasatinib inhibits the kinase activity of the imatinib-resistant KIT D816V mutation with an IC50 between 50 and 100 nmol/L (24). The PDGFRAD842V mutation investigated in this study is analogous to the D816V mutation in KIT. Therefore, we hypothesized that dasatinib might also potently inhibit the kinase activity of the PDGFRAD842V mutant. We have found that dasatinib completely inhibits the kinase activity of the PDGFRAD842V mutant isoform at nanomolar concentrations in vitro, albeit at somewhat higher concentrations than needed for the inhibition of wild-type PDGFR, as evidenced by experiments done on A10 (rat VSMC cells) and human AoSMC cell lines, in which PDGF-stimulated wild-type PDGFR tyrosine phosphorylation was completely blocked by 50 nmol/L of BMS-354825 (28). Using PDGFRAD842V mutant primary GIST cells, we have shown that dasatinib nearly totally inhibits the phosphorylation of PDGFRAD842V at 0.5 μmol/L ex vivo. This is in the same range as the IC50 found for the analogous KITD816V mutant in systemic mastocytosis models (24). Furthermore, at 0.5 μmol/L of dasatinib, numerous PDGFRAD842V-expressing primary GIST tumor cells and significantly inhibits their proliferation. Pharmacokinetic studies done during a phase I dose-escalation study of dasatinib have shown that high nanomolar concentrations of the compound can be safely achieved in humans (24). Given the fact that dasatinib is well tolerated, and that PDGFRAD842V is inhibited by 500 nmol/L of dasatinib, treatment with dasatinib is a potential alternative for the patients with this mutation.

Sorafenib is an orally active multikinase inhibitor which targets tumor cell growth and angiogenesis by potently inhibiting, respectively, the RAF family of serine/threonine kinases and the tyrosine kinase receptors VEGFR-2 (KDR) and VEGFR-3 (FLT-4). Sorafenib also has selectivity for the tyrosine kinases PDGFRB, KIT, FLT-3, and RET (29, 30). Sorafenib is approved for the treatment of advanced renal cell carcinoma by the Food and Drug Administration and European Medicines Agency (31). Recently, the efficacy of sorafenib in the treatment of patients with hepatocellular carcinoma was shown (25). In patients, a dose of 100 mg/d of sorafenib could result in steady-state serum drug concentrations of up to 4 μmol/L (32–34).

In our study, although sorafenib was only slightly less potent than dasatinib in inhibiting the kinase activity of PDGFRAD842V, it was markedly less potent than dasatinib in inhibiting the imatinib-resistant PDGFRAD842V mutation. Hence, a dose-dependent decrease of proliferation with an IC50 of 425 nmol/L, and incomplete inhibition of the PDGFR tyrosine kinase activity at a concentration 1 μmol/L was observed. In line with this observation, it was recently shown that sorafenib inhibits imatinib-resistant PDGFRB gatekeeper mutant T681I (IC50 of 110 nmol/L), but is less active (IC50 of 1.17 μmol/L) against the activation loop mutant of PDGFRAD850V, which is analogous with the PDGFRAD842V investigated in the present study (35). Moreover, the KITD816V isoform, analogous with the PDGFRAD842V isoform, is resistant...
to sorafenib (36). These differences may reflect a preference for sorafenib to bind to the inactive form of the PDGFR as has been shown for binding of sorafenib to RAF kinase (37).

In the second part of this study, we examined the potency of IPI-504, a HSP90 inhibitor, towards PDGFRA oncoproteins, as an alternative treatment option for imatinib-resistant PDGFRA mutant GISTs. HSP90 is an emerging therapeutic target of interest for the treatment of cancer. HSP90 is a chaperone molecule that maintains the structure and activity of certain key signaling proteins within cancer cells. 17-Allylamino-18-demethoxy-geldanamycin (17-AAG) is a geldanamycin derivative that binds a conserved pocket in the HSP90 NH2-terminal domain and prevents HSP90 from stabilizing clients (38). Concentrations of 1 µmol/L of 17-AAG can be achieved in vivo in the blood as shown by preliminary data from phase I clinical trials (39). A study by Fumo et al. (40) showed that 17-AAG led to rapid degradation of the imatinib-resistant KITD816V mutant, with a more profound effect at concentrations of >500 nmol/L. It was recently shown that 17-AAG can selectively promote the degradation of HSP90-dependent PDGFRA proteins in transformed cells (16). We could expect that PDGFRA containing activating mutations would even become more dependent on HSP90 chaperoning.

IPI-504 is a novel, highly soluble analogue of 17-AAG. It is an active metabolite of 17-AAG, and it is slightly more potent than 17-AAG (41). IPI-504 preferentially targets and accumulates in tumor tissues. IPI-504 was recently evaluated in a murine model of chronic myelogenous leukemia and might be a new therapeutic agent for the treatment of imatinib-resistant BCR-ABL–induced leukemia (42). Multiple phase I and phase II clinical trials with IPI-504 are ongoing. Preliminary data from an ongoing dose escalation phase I clinical trial in patients with imatinib-resistant metastatic GISTs showed stable disease in 76% of patients (43).

In the present study, we showed the potency of IPI-504 to inactivate and degrade imatinib-resistant PDGFRAD842V and imatinib-sensitive PDGFRA WM842 oncoproteins in Ba/F3 cell lines within 6 hours at a concentration of 500 nmol/L.
Moreover, IPI-504 induced a pronounced apoptosis of the tested cells after 48 hours of treatment. Secondly, we provided \textit{ex vivo} evidence of significant activity of IPI-504 against PDGFR\textsubscript{A\&V} primary GIST cells in the high nanomolar range. Finally, we have shown that 500 nmol/L of IPI-504 inhibited imatinib-sensitive and imatinib-resistant PDGFR\textsubscript{A} oncogenes, with totally diminished PDGFR\textsubscript{A} phosphorylation and significantly reduced total PDGFR\textsubscript{A} expression after 2 and 6 hours, respectively. Given the possible broad heterogeneity of secondary KIT or PDGFR\textsubscript{A} resistance mutations during imatinib treatment in a given patient, the HSP90 inhibitor IPI-504 might be more effective in the clinic compared with any other single second-line tyrosine kinase inhibitor because it induces the degradation of constitutively activated oncproteins irrespective of the specific mutational activation mechanisms.

We thus conclude that treatment with the second-generation tyrosine kinase inhibitor dasatinib or with the HSP90 inhibitor IPI-504 may provide a therapeutic alternative option for patients with GIST whose tumors carry the imatinib-resistant PDGFR\textsubscript{A\&V} mutant isoform. Clinical use of these drugs as first-line treatment should be considered in patients with the primary imatinib-resistant PDGFR\textsubscript{A} mutant GISTS.

Disclosure of Potential Conflicts of Interest

M. Debiec-Rychter is a member of the speakers’ bureau.

References

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