**Kinome Profiling of Chondrosarcoma Reveals Src-Pathway Activity and Dasatinib as Option for Treatment**

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**Abstract**

Chondrosarcomas are notorious for their resistance to conventional chemotherapy and radiotherapy, indicating there are no curative treatment possibilities for patients with inoperable or metastatic disease. We therefore explored the existence of molecular targets for systemic treatment of chondrosarcoma using kinome profiling. Peptide array was performed for four chondrosarcoma cell lines and nine primary chondrosarcoma cultures with GIST882, MSCs, and colorectal cancer cell lines as controls. Activity of kinases was verified using immunoblot, and active Src- and platelet-derived growth factor receptor (PDGFR) signaling were further explored using imatinib and dasatinib on chondrosarcoma in vitro. The AKT1/GSK3B pathway was clearly active in chondrosarcoma. In addition, the PDGFR pathway and the Src kinase family were active. PDGFR and Src kinases can be inhibited by imatinib and dasatinib, respectively. Although imatinib did not show any effect on chondrosarcoma cell cultures, dasatinib showed a decrease in cell viability at nanomolar concentrations in seven of nine chondrosarcoma cultures. However, inhibition of phosphorylated Src (Y419) was found both in responsive and nonresponsive cells. In conclusion, using kinome profiling, we found the Src pathway to be active in chondrosarcoma. Moreover, we showed in vitro that the inhibitor of the Src pathway, dasatinib, may provide a potential therapeutic benefit for chondrosarcoma patients who are not eligible for surgery. [Cancer Res 2009;69(15):6216–22]

**Introduction**

Curative treatment of chondrosarcoma of bone is restricted to surgery because this tumor is reported to be extremely resistant against conventional therapeutic modalities (1–3). Therefore, there is not much to offer with curative intent to patients with metastatic disease or with tumors at inoperable sites. Whereas low-grade chondrosarcomas (grade I) are treated by marginal or intralesional excision, followed by margin improvement by application of fenol or cryosurgery (3, 4), high-grade chondrosarcoma is treated by, often mutilating, large en-bloc resection or amputation. The metastatic rate of chondrosarcoma is directly related to histologic grade (5), currently being the only predictor of outcome, although histological grade is highly subjected to interobserver variability (6, 7). Grade I chondrosarcomas almost never metastasize, whereas metastases occur in 10% of grade II chondrosarcomas and in 71% of grade III chondrosarcomas (8). The 10-year survival for patients with high-grade chondrosarcoma is poor: 64% for grade II and only 29% for grade III tumors.

Few recurrent genetic alterations were found in chondrosarcoma, pointing toward a loss of cell cycle control of chondrosarcoma, such as gain of CDK4 (9, 10) and loss of p16 (11, 12). Decreased cell viability was shown after restoration of p16 expression or the knockdown of CDK4 by shRNA in chondrosarcoma cells in vitro (10). Thus far, there is little evidence for a role for kinase inhibitors in chondrosarcoma treatment. In addition to CDK4 (10), AKT (13) and Flk-1/KDR, PDGFRB (14), and FGFR1 (15) were shown to be activated and suggested as possible targets in the treatment of chondrosarcoma.

In the present study, we explored new treatment options for chondrosarcoma using kinome profiling. Kinases are enzymes that phosphorylate tyrosine/serine or threonine residues on other proteins. They play a major role in signaling cascades that determine cell cycle entry, cell survival, and differentiation fate, which are often deregulated in tumors. Kinases are excellent targets for anticancer therapy because they work as a molecular switch; their regulation is reversible, rapid (merely in seconds), and does not require new protein synthesis (reviewed in ref. 16).

Kinome profiling allows the detection of kinase activity in cell lysates by detecting the level of substrate phosphorylation. This produces a comprehensive description of cellular signal transduction in a particular sample, which can be assigned to specific pathways, as has been shown by Diks and colleagues (17). Kinome profiling identified the AKT1/GSK3B pathway, PDGFRB and the Src pathway, as potential targets for chondrosarcoma treatment. We showed that inhibition of the Src pathway by dasatinib indeed resulted in decreased cell viability in seven of nine chondrosarcoma cell cultures in vitro.

**Materials and Methods**

**Reagents.** Imatinib mesylate (Glivec/Gleevec, STI571) was obtained from Novartis and dasatinib (Sprycel, BMS-354825) from Bristol-Myers Squibb. Both drugs were dissolved in DMSO.

**Cell culture.** Chondrosarcoma cell lines, chondrosarcoma primary cultures (Table 1), and a gastrointestinal stromal tumor cell line, GIST882 (18), were cultured in RPMI 1640 (Life Technologies, Invitrogen LifeTechnologies), supplemented with 10% heat-inactivated fetal calf serum (Life Technologies). GIST882, a gastrointestinal stromal tumor cell line carrying a homozygous exon 13 missense mutation (K642E) in KIT (18) and known to be imatinib sensitive, was used, treated and untreated, as a proof of principle of the Pepchip technique. Two cell cultures of normal bone marrow–derived mesenchymal stem cells (MSC; L2361 and L2370) were used as nonneoplastic counterpart control. In addition, an independent set
of five colorectal carcinoma cell lines were tested (HT29, RKO, LS180, SW480, SW837) to estimate specificity.

Cells were grown at 37°C in a humidified incubator with 95% air and 5% CO2. The cartilaginous phenotype of the chondrosarcoma cultures was confirmed by reverse transcription-PCR, showing mRNA expression of collagens I, II, 3B, and 10, Aggrecan and SOX9 (19).

**Kinome array analysis.** A kinase substrate peptide array was used containing 1,024 different kinase substrates spotted in triplicate with 16 negative and 16 positive controls (Pepchip Kinomics, Pepscan Presto). Cells were harvested during their exponential growth phase. Cells were washed thrice with cold PBS. Cell lysis buffer (Cell Signaling Technology) supplemented with 1 mmol/L phenylmethylsulfonyl fluoride (PMSF) was used. Lysates were processed through a QIAshredder (Qiagen) for 10 min and a filter (Millipore) for 5 min both at 4°C to retain optimal kinase activity. For concentration of the lysates and discarding of the lysis buffer, a 10 kDa spin column (Millipore) was used for 30 min at 4°C. Concentration of the protein lysate was measured using the DC Protein Assay (Bio-Rad).

For kinase array analysis, 50 µg of the protein were diluted in kinase buffer (Cell Signaling Technology) in the presence of complete mini EDTA free (Roche) and 1 mmol/L PMSF. To 62.5 µL of the lysate, 12.5 µL activation mix (20 mmol/L TrisHCl (pH 7.5), 10 mmol/L MgCl2, Glycerol 5%, Brij-35 0.01%, 0.05 mg/mL bovine serum albumin, 5 µmol/L ATP, and 20 µCi [γ-32P]ATP) was added. A 24 × 60-mm coverslip was used. The slides were incubated in a humid chamber for 2 h at 37°C. Subsequently, slides were rinsed in PBS/Triton X-100 1% twice, then washed twice in NaCl 2.0M/Triton X-100 1% for 15 min, followed by washing in distilled water. All buffers were used at 37°C and washing was performed in a humid stove at 37°C. Subsequently, the slides were dried in a 50 µL tube in a centrifuge at 2,000 rpm. The measurement of the 32P signal was performed using a Biomex reader for real-time digital imaging of radioisotopes (Biomex). At least 1 × 106 hits were collected.

**Data analysis.** Biomex Readback V3.6 and Biosplit software (Biomex) were used to create a list with intensities using a grid. For further data mining, R-packages Affyio and Limma were used. Quality of the triplicates and distribution of the data were assessed, and quantile normalization (Affyio) was performed. Phosphorylated substrates in chondrosarcoma cultures were compared with those in MScs using Limma, which provides functions to summarize results using a linear model to perform hypothesis tests and adjust the P values for multiple testing (20). In addition, the data set of 13 chondrosarcoma cell cultures was compared with an independent set of five colorectal carcinoma cell lines using Limma. Subsequently, phosphorylation signals of all chondrosarcoma cultures were averaged and the top 100 (the common denominators) were imported for core analysis in Ingenuity Pathway Analysis (IPA). IPA is a literature-based program that calculates the probability of involvement of identifiers and, in this case, combinations of kinases, in 74 known canonical pathways.

**Immunoblotting.** Ten micrograms of each sample, stored in kinase buffer at −20°C, were run on SDS-PAGE. Proteins were transferred onto polyvinylidene difluoride membranes (Immobilon-P; Millipore) using electrophoresis. Membranes were preincubated with 5% skimmed milk in PBS-Tween 0.05%. After incubation with first and secondary antibodies, the membranes were developed with enhanced chemiluminescence Western blotting detection reagent (Amersham Biosciences) and visualized by exposure to X-ray films (Hyperfilm ECL; Amersham Biosciences).

Rabbit monoclonal antibodies against phospho-c-Raf (Ser338; 56A6), phospho-MEK1/2 (Ser217/221; 41G9), phospho-p44/42 mitogen-activated protein kinase (Erk1/2; Thr202/Tyr204; D13.14.4E), phospho-p90RSK (Ser82; 9D9), phosphorylated Akt (Ser473; D9E), phosphorlyated AKT (Thr308; C31E5), pan AKT (C67E7), GSK3B (27C10) and rabbit antibodies against phosphorlyated c-Raf (Ser259), and phosphorylated GSK3B (Ser9) were obtained from Cell signaling Technology. Jurkat cells, treated with LY294002 or Calyculin A, were used as a negative and positive control for AKT phosphorylation, respectively. Polyclonal antibody to phosphorylated Src (Y419) was obtained from R&D Systems. Monoclonal antibody to total Src (clone GD11) was obtained from Upstate Biotechnology. Pro-caspase-3 (37 kDa) and β-tubulin antibodies were from Cell Signaling Technology and Sigma Aldrich, respectively.

**In vitro proliferation assays.** Response of chondrosarcoma primary cultures to escalating doses of imatinib (range, 1.0–100 µmol/L) was measured by cell count using a Bürker chamber. GIST882 was used as a positive control. Response of chondrosarcoma cell lines and primary cultures to escalating doses dasatinib (range, 5.0 nmol/L–1.0 µmol/L) was measured either by tritium incorporation assay (OMUS27, CH2879, SW1353, L835, L869, L846) or WST-1 colorimetric assay (Roche Diagnostics GmbH; L1081, L1250, L2252, L2388), which measures mitochondrial activity as described previously (21). C842, L821, and L835 did not reach an adequate proliferation rate to allow inhibition experiment. Results were compared with a poor responding (ALL CR) and a well-responding acute lymphoblastic leukemia (ALL CM) cell line, as well as to GIST882, known to respond to dasatinib at −10 µmol/L (22). In brief, 20,000 cells were seeded

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**Table 1. Chondrosarcoma cultures**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type</th>
<th>Grade</th>
<th>Gender</th>
<th>Age</th>
<th>Passage</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>OUMS27 (39)</td>
<td>III</td>
<td>M</td>
<td>Na</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>CH2879 (40)</td>
<td>III</td>
<td>F</td>
<td>35</td>
<td>58</td>
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<td>F</td>
<td>72</td>
<td>53</td>
</tr>
<tr>
<td>4</td>
<td>C3842 (41)</td>
<td>II</td>
<td>M</td>
<td>38</td>
<td>16</td>
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<td>L784</td>
<td>Primary culture</td>
<td>II</td>
<td>M</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>L821</td>
<td>Primary culture</td>
<td>I</td>
<td>F</td>
<td>53</td>
</tr>
<tr>
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<td>L835</td>
<td>Primary culture</td>
<td>III</td>
<td>M</td>
<td>55</td>
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<td>L869</td>
<td>Primary culture</td>
<td>II</td>
<td>M</td>
<td>52</td>
</tr>
<tr>
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<td>F</td>
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<td>13</td>
<td>L2388</td>
<td>Primary culture</td>
<td>I</td>
<td>M</td>
<td>42</td>
</tr>
</tbody>
</table>

Abbreviation: Na, not available.

*SW1353 was obtained from American Type Culture Collection (Manassas, VA).

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3 http://www.bioc conductor.org

4 http://www.ingenuity.com
in a 96-well plate and were allowed to incubate with the drugs for 72 h. \(^{3}H\) was added, and the following day, cells were harvested using the FilterMate system (Perkin-Elmer). \(^{3}H\)-thymidine incorporation was measured using a TopCount scintillation counter (Perkin-Elmer). All experiments were performed in duplicate and in the presence of a maximum of 0.1% DMSO. In vitro experiments were performed at least thrice. Graphs show data from one representative experiment. Error bars indicate the SE.

**Results**

**Identification of active kinases in chondrosarcoma cultures.**

Through kinome profiling of 13 chondrosarcoma cultures, we created a list of phosphorylated targets and their corresponding active kinases. The top 100 of chondrosarcoma targets was analyzed using IPA, ranking the importance of the corresponding kinases in chondrosarcomas. The specificity of this list of substrates for chondrosarcoma was verified by comparing the intensity of the signals with those for normal MSCs, using Limma (Supplementary Table S1). Based on the average spot intensity and how frequent their targets were present in the top 100 of substrates, 21 kinases were identified by IPA analysis (Table 2).

**Verification of kinome profiling.** Kinome profiling of untreated GIST882 revealed an active Ras/Raf/MEK/ERK pathway, which is activated by KIT (Supplementary Table S2). GIST882 carries an activating KIT mutation (18). Subtracting the average intensities of untreated GIST882 from the 1.0 \(\mu\)mol/L imatinib-treated GIST882 revealed that indeed the Ras/Raf/MEK/ERK pathway was targeted by imatinib, which was confirmed by analysis using IPA (data not shown). Inhibition of the Ras/Raf/MEK/ERK pathway in GIST882 by imatinib was verified by immunoblot, using the same lysates as hybridized on the kinase array. Staining for cRaf, MEK1/2, ERK1/2, and 90RSK was decreased after 1.0 \(\mu\)mol/L imatinib treatment (Fig. 1A), confirming that the Ras/Raf/MEK/ERK pathway was targeted by imatinib.

In addition, comparison of the substrate phosphorylation patterns of all substrates of 13 chondrosarcoma cell cultures and the two MSC cultures (Supplementary Table S2), and the five colorectal carcinoma cell lines (Supplementary Table S3) was performed, revealing 167 and 175 differently phosphorylated spots, respectively (adjusted \(P < 0.01\)).

Phosphorylation of AKT in chondrosarcoma cell cultures could be verified by immunoblot at both the serine 473 and the threonine 308 position in all cultures (Fig. 2). Whereas total GSK3B was present in nearly all samples, phosphorylation levels of GSK3B at serine 9 were indeed very low in the chondrosarcoma cell cultures confirming active GSK3B. cRaf, which links the AKT pathway with the MEK/ERK pathway, was also detected by immunoblot in all primary cultures.

| Table 2. Results from the top 100 phosphorylated substrates (data not shown), their corresponding kinases and targeted drugs in chondrosarcoma cultures |
|-------|-------|-------|-------|
| Intensity | Kinase | Nr hits | Description |
| 1 | 681.09 | AKT1 | 9 | V-akt murine thymoma viral oncogene homologue 1 |
| 2 | 587.329 | GSK3B | 4 | Glycogen synthase kinase 3 b |
| 3 | 501.354 | TTN | 1 | Titin |
| 4 | 446.35 | RPS6KA5 | 5 | Ribosomal protein S6 kinase, 90 kDa, polypeptide 5 |
| 5 | 410.859 | FYN | 1 | FYN oncogene related to SRC, FGR, YES |
| 6 | 410.859 | LCK | 1 | Lymphocyte-specific protein tyrosine kinase |
| 7 | 376.366 | CDC2 | 4 | Cell division cycle 2, G1 to S and G2 to M |
| 8 | 360.768 | AURKB | 2 | Aurora kinase B |
| 9 | 343.572 | PAK2 | 1 | P21 (CDKN1A)-activated kinase 2 |
| 10 | 330.902 | PIP5K3 | 2 | Phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase, type III |
| 11 | 324.603 | CSNK2A1 | 6 | Casein kinase 2, \(\alpha\) 1 polypeptide |
| 12 | 288.843 | CAMK1D | 4 | Calcium/calmodulin-dependent protein kinase ID |
| 13 | 272.998 | EPHB2 | 1 | EPH receptor B2 |
| 14 | 269.754 | RH0A | 1 | Ras homologue gene family, member A |
| 15 | 268.982 | MAP2K1 | 2 | Mitogen-activated protein kinase kinase 1 |
| 16 | 267.397 | AKAP1 | 1 | A kinase (PRKA) anchor protein 1 |
| 17 | 262.579 | PRKACA | 1 | Protein kinase, cyclic AMP-dependent, catalytic, \(\alpha\) |
| 18 | 247.71 | CSNK1A1 | 1 | Casein kinase 1, \(\alpha\) 1 |
| 19 | 241.637 | CAMK2G | 3 | Calcium/calmodulin-dependent protein kinase (CaM kinase) II \(\gamma\) |
| 20 | 239.691 | PDGFRB | 1 | PDGFR, \(\beta\) polypeptide |
| 21 | 202.938 | SDK1 | 1 | Sidekick homologue 1, cell adhesion molecule (chicken) |

NOTE: Nr hits, how many times the kinases were present in the top 100 spots. For each kinase, only the highest average expression is shown here.
Chondrosarcomas do not respond to imatinib treatment in vitro. In Table 2, the currently used kinase inhibitors specific for the active kinases in chondrosarcoma are shown. Dasatinib and imatinib, targeting the Src kinase family and KIT/PDGFR pathway, respectively, were available to us. Sensitivity of chondrosarcoma cells to both drugs was tested. Whereas GIST882 showed a profound decrease of cell number relative to the DMSO control at lower dosages of imatinib, the chondrosarcoma primary cultures did not show any effect, only at high concentrations of imatinib, probably due to nonspecific toxicity, rather than on target effects (Fig. 1B).

Chondrosarcomas are responsive to dasatinib treatment in vitro. Seven of nine chondrosarcoma cell cultures responded to dasatinib treatment with a decrease in cell growth. Primary cultures L1081, L1250, L2252, and L2388 showed 20% decreased cell viability at 15 nmol/L and 40% to 50% decreased cell viability at 1,000 nmol/L of dasatinib treatment, as measured by either 3H incorporation or WST-1 assay (Fig. 1C). The strongest effect was observed in primary culture L869, with a 50% reduction in cell growth at 15 nmol/L treatment. D, cell lines OUMS27 and SW1353 showed a decrease in viability at 50 nmol/L dasatinib, whereas both primary culture L783 and cell line CH2879 showed a response only at low concentrations (5 and 15 nmol/L), whereas at higher concentrations, no effect was found. E, in positive controls, ALL CM and GIST882, a decrease in cell viability of >80% was observed, whereas a limited effect was found in dasatinib resistant leukemia cell line CR.
both primary culture L784 (Fig. 1C) and cell line CH2879 (Fig. 1D) did not show any effect. Positive controls ALL CM and GIST882, and negative control ALL CR are shown in Fig. 1E.

**Discussion**

Chondrosarcomas are highly resistant to conventional chemotherapy and radiotherapy, and as a consequence, there is no curative treatment option for patients with inoperable or metastatic disease. Kinome profiling was used to search for drugable targets in chondrosarcoma.

A major caveat in large scale phosphorylation studies is the promiscuity of kinases in the absence of in vivo regulation signals, which may lead to false-positive results. Ideally, by comparing kinase profiles with and without a certain stimulus, for instance, a kinase inhibitor, kinases being activated or deactivated upon the stimulus can be detected, as was previously reported (17). However, because there are no compounds available that are known to have any effect in chondrosarcoma, we averaged 13 chondrosarcoma cultures to get an impression of the most active kinases and the pathways they are involved in.

Using GIST882, we showed that the Pepchip kinase profiling platform correctly identified the pathways that are known to play an important role in GIST and that their activity is decreased by imatinib treatment. Using MSCs and an independent set of colorectal carcinoma cell lines, we also showed tumor specificity and tumor type specificity, respectively.

Analyzing kinome profiles of 13 chondrosarcoma cell cultures identified several drugable targets. Validation of the results by immunoblot as well as the inhibition of chondrosarcoma cell viability in vitro by dasatinib show that this is an elegant approach to identify targets for treatment of tumors for which thus far no systemic treatment options are available.

Analyzing the top 100 activated substrates of the 13 chondrosarcoma cell cultures revealed 21 active kinases, of which AKT and GSK3B were shown to be the most active. The phosphorylation of AKT was confirmed by immunoblot. This pathway can be blocked by Enzastaurin, a PKCB-selective inhibitor, which has been shown to suppress angiogenesis and induces apoptosis in colorectal cancer and glioblastoma xenografts, by targeting AKT and GSK3B (24). In contrast to AKT, GSK3 is constitutively active and becomes functionally inactivated after phosphorylation. GSK3 has a central function in physiologic (i.e., transcription, apoptosis, and cell cycle progression) and pathologic (i.e., diabetes mellitus, Alzheimer, and carcinogenesis) processes (reviewed in ref. 25). In chondrosarcoma cell cultures, we showed the absence of phosphorylation at serine 9, indicative for active GSK3B. An important role for the AKT kinase in chondrosarcoma survival was previously suggested by Jang and colleagues (13).

Kinome profiling of chondrosarcoma also revealed an active Src pathway. Src plays a role in the regulation of embryonic development and cell growth (26). Mutations in Src are involved in the malignant progression of colorectal cancer (27).
A decrease in pro-caspase-3 could be observed.

... levels of pSrc decrease gradually, although both bands of the staining are present. A decrease of pro-caspase 3 could be seen in the dasatinib sensitive cell culture, levels of pSrc decrease gradually, although levels of total Src do not decrease upon dasatinib treatment. In contrast, Klenke and colleagues (15) showed SU6668, which inhibits tyrosine kinases PDGFRB, Flk-1/KDR, and FGFR1, to repress chondrosarcoma growth via antiangiogenesis in vivo. One must take into account that we studied the effect of dasatinib and imatinib in chondrosarcoma in vitro, and that an additional effect in vivo through the inhibition of angiogenesis may be possible, as has been described for dasatinib (37).

Also Flavopiridol and AZD-1152, inhibitors of CDC2 and Aurora kinase activity, respectively, were suggested for chondrosarcoma treatment, by our Pepchip approach (Table 2). Flavopiridol is a pan cyclin-dependent kinase inhibitor, not only targeting CDC2, also as cyclin-dependent kinase 1, but also cyclin-dependent kinase 2 and 4. Previously, we reported the amplification of 12q13 (9), the locus of CDK4, increased expression of CDK4 and a decrease in cell viability using shRNA to knock down CDK4 expression in vitro (10). We suggested the use of CDK4 inhibitors in the treatment of chondrosarcoma, which is now being emphasized accordingly in the present study. Also RPS6kinase was found to be active in chondrosarcoma, which was found to predict the response to mammalian target of rapamycin inhibitors in sarcoma (38). RPS6 kinase is responsible for the phosphorylation of ribosomal protein S6, which we previously found to be deleted and down-regulated in a subset of chondrosarcomas (9). This suggests that the tumor cells may try to overcome this deletion by phosphorylation. No array CGH data were available for the cell cultures described here.

In the present study, we report the kinome profiling of 13 chondrosarcoma cell cultures, and by averaging the profiles, we identified activity of the Src pathway. Accordingly, Src inhibitor dasatinib decreased cell viability in seven of nine chondrosarcoma cell cultures. Our experiments suggest that dasatinib is a potential treatment option in chondrosarcoma treatment. Future studies in vivo should be performed to confirm these data and to investigate the combination with conventional chemotherapy and possible additional effects through the inhibition of angiogenesis.

identified activity of Fyn and Lck in chondrosarcoma, which are, together with Yes, Fgr, Hck, Blk, Lyn, and Frk, members of the Src family. The Src pathway can be targeted by dasatinib. Dasatinib is well-known for its efficacy in the treatment of chronic myelogenous leukemia and Philadelphia chromosome-positive ALL (28), in which dasatinib inhibits the Abl-kinases (29).

Recently, dasatinib has also been shown to be effective in the treatment of cells derived from solid tumors, i.e., prostate cancer (30) and head and neck squamous cell carcinoma (31). In previous research, also expression of Abl kinase was shown in chondrosarcoma by immunohistochemistry with interestingly, a negative correlation with histologic grade (32). We show decreased cell viability after dasatinib treatment in the majority (7 of 9) of chondrosarcoma cell cultures, although a maximum of 60% of inhibition of cell growth was reached, whereas the effect in GST882 and the leukemia cell lines were more profound. This difference may be explained by secondary events, which stimulate cell growth in chondrosarcoma, i.e., the loss of cell cycle inhibition, which was previously shown to occur in 96% of the tumors (10). Likewise, GISTs have been shown to become refractory to initial successful response to imatinib due to loss of cell cycle control (33).

Strikingly, dose-dependent inhibition of Src kinase phosphorylation by dasatinib, as measured by autophosphorylation at Y419, was found both in a responsive (L869) and in a nonresponsive (CH2879) cell culture. This suggests that growth inhibition induced by dasatinib might be independent of Src kinase phosphorylation. Thus, dasatinib might exert its function via other pathways in chondrosarcoma, i.e., by inhibition of Abl kinases, fibroblast growth factor receptor kinases or PDGFR kinases (34) or AKT (35). Activity of the latter two is shown in the present study.

Although dasatinib was suggested to induce caspase-3–mediated apoptosis in the control GST882 cell line, this was not observed in chondrosarcoma cell cultures. This suggests that dasatinib inhibits chondrosarcoma cell growth through other mechanisms, for example, by inducing G1 arrest. However, immunoblotting for pSrc and caspase-3 was performed on one responsive and one nonresponsive cell line only and extrapolating these results to all chondrosarcomas should be done with caution.

More experiments are needed to further explore the mechanism underlying growth inhibition and whether the effects of dasatinib on chondrosarcoma growth can be increased by combination with another cytostatic compound to reach higher growth inhibition rates.

Despite the finding of PDGFRB activity using the Pepchip and the fact that PDGFRα protein expression (36) and activity of the α and β receptor were reported previously in the absence of gain-of-function mutations (14), we were not able to decrease cell viability of chondrosarcoma cell cultures by imatinib treatment. In contrast, Klenke and colleagues (15) showed SU6668, which inhibits tyrosine kinases PDGFRB, Flk-1/KDR, and FGFR1, to repress chondrosarcoma growth via antiangiogenesis in vivo. One must take into account that we studied the effect of dasatinib and imatinib in chondrosarcoma in vitro, and that an additional effect in vivo through the inhibition of angiogenesis may be possible, as has been described for dasatinib (37).

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Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References

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