Abstract

Purpose: To determine the efficacy of AZD0530, an orally active small molecule Src inhibitor, in human pancreatic cancer xenografts and to seek biomarkers predictive of activity.

Experimental Design: Sixteen patient-derived pancreatic cancer xenografts from the PanXenoBank collection at Johns Hopkins were treated with AZD0530 (50 mg/kg/day, p.o.) for 28 days. Baseline gene expression profiles of differently expressed genes in 16 tumors by Affymetrix U133Plus2.0 gene array were used to predict AZD0530 sensitivity in an independent group of eight tumors using the K-Top Scoring Pairs (K-TSP) method.

Results: Three patient tumors of 16 were found to be sensitive to AZD0530, defined as tumor growth ≤50% compared with control tumors (100%). Western blot and/or immunohistochemistry results showed that AZD0530 administration resulted in the down-regulation of Src, FAK, p-FAK, p-paxillin, p-STAT-3, and XIAP in sensitive tumor xenografts compared with control tumors. The K-TSP classifier identified one gene pair (LRRC19 and IGFBP2) from the 16 training cases based on a decision rule. The classifier achieved 100% and 83.3% of sensitivity and specificity in an independent test set that consists of eight xenograft cases.

Conclusions: AZD0530 treatment significantly inhibits the tumor growth in a subset of human pancreatic tumor xenografts. One gene pair (LRRC19 and IGFBP2) identified by the K-TSP classifier has high predictive power for AZD0530 sensitivity, suggesting the potential for this gene pair as biomarker for pancreatic tumor sensitivity to AZD0530.

A number of studies have shown that Src tyrosine kinase activity is frequently activated and elevated in many human tumor types where it seems to correlate with disease stage and patient survival (1, 2). The major consequence of increased Src activity is to promote an invasive tumor phenotype characterized by breakdown of cell-cell adhesion, increased cell-matrix adhesion, and formation of focal adhesions (3, 4). Accordingly, inhibition of Src activity in preclinical models restores cell-cell adhesion, inhibits cell migration and invasion, and reverses the Src-modulated invasive phenotype (5, 6).

Although a direct role of Src in regulating the cell cycle has been suggested, there is compelling evidence that Src affects cell adhesion, invasion, and motility in cancer cells during the later stages of cancer progression (4). Clearly, Src does not act alone but works in concert with a large number of substrates to orchestrate the process of cancer development and tumor progression (7). Src activates focal adhesion kinase (FAK) and signal transducers and activators of transcription 3 (STAT-3) and their linked activities act to control cell migration through the turnover of focal adhesions and the suppression of cell-cell contacts (8–10). Also, Src is an important mediator of many downstream effects of receptor tyrosine kinases including the epidermal growth factor receptor family (11). In addition, there are extensive data supporting the influence of Src on the development of a metastatic phenotype (12). Collectively, these findings linking Src to multiple processes that determine the clinical outcome of a tumor, have served as an impetus for the development of Src inhibitors, several of which are currently in clinical trials for treatment of a variety of human cancers (13–15).

Pancreatic cancer has the worst prognosis of any major malignancy, and the annual death rate due to this disease approximates its annual incidence rate (16). At advanced metastatic stages, pancreatic cancer can almost never be controlled by any of the available therapeutic options, as reflected by an extremely low estimated 5-year survival rate of <2% (17). Clinical benefit of gemcitabine as a systemic agent...
for the treatment of advanced pancreatic cancer results in a median survival of <6 months (18). Improvements in therapy have been modest with the addition of erlotinib to gemcitabine in combination, resulting in improved median survival in the order of weeks (19). The paucity of breakthroughs in treatment regimens and continued poor survival shows an acute need for improvement in therapy for this lethal malignancy.

The extensive presence of activated/overexpressed Src in pancreatic cancer and its potential role in tumor development and progression makes Src an appealing target for pancreatic cancer drug discovery (20, 21). In the present study, we explored the in vivo efficacy and pharmacodynamic effects of AZD0530, an orally active small molecule Src inhibitor, (22), in a collection of patient-derived pancreatic tumor xenografts (PancXenoBank; ref. 23). In addition, we attempted to identify potential predictive biomarkers of AZD0530 using K-Top Scoring Pairs (K-TSP) of baseline gene expression profiles of tumors as well as pancreatic cancer cell lines.

Materials and Methods

In vivo efficacy studies. All animal experiments were conducted following approval by the Johns Hopkins University Animal Care and Use Committee. Fresh pancreatic cancer tissues operated from patients at Johns Hopkins Hospital during Whipple procedure (pancreatoduodenectomy) were propagated as subcutaneous tumors in 6-wk-old female athymic nude mice (Harlan) as a live PancXenoBank according to an Institutional Review Board–approved protocol (23). The patients had not undergone chemotherapy or radiation therapy before Whipple procedure. Xenografts derived directly from patients were reported to have better retention of the morphologic and molecular markers of the source tumors, despite serial passing across several generations of mice (24–26). Tumors from each treatment case were profiled using Affymetrix U133 Plus 2.0 gene arrays at least in duplicates. Therefore, the gene expression profiles of baseline tumors as well as pancreatic cancer cell lines.

Tumor tissues (50-75 mg/mouse) from control and AZD0530-treated mice for 28 d were minced on ice in prechilled lysis buffer (20 mmol/L sodium phosphate buffer, 150 mmol/L NaCl, 5 mmol/L EDTA, 1% Triton X-100, and 0.5% sodium deoxycholate) in the presence of EDTA-free protease and phosphatase inhibitors (Roche Molecular Biochemicals) and sodium orthovanadate (1 mmol/L; pH 7.4). The minced tissue was homogenized using a Dounce homogenizer and centrifuged at 16,000 × g at 4°C for 10 min. Protein content in the supernatants was measured using the Pierce Protein Assay kit using bovine serum albumin as a standard. Forty micrograms of sample were electrophoresed on 4% to 12% Bis-Tris precast gels (Bio-Rad Laboratories, Inc.). After electrophoresis to Immobilon-P membranes (Millipore), membranes were blocked at room temperature with TBS [10 mmol/L-Tris-HCl (pH 7.5), 0.5 mol/L NaCl, and 0.1% (v/v) Tween 20] containing 5% nonfat milk (Pierce) for 1 h. Primary antibodies for Src, p-Src Tyr416, FAK, STAT-3, p-STAT-3 Tyr705, Akt, p-Akt Ser473, mitogen-activated protein kinase, p-MAPK Thr202/Tyr204, epidermal growth factor receptor, c-ParP, XIAP, matrix metalloproteinase-2 (Cell Signaling Technology), and p-FAK Tyr925 (BioSource International) were diluted at 1:1,000 in TBS containing 5% protease-free bovine serum albumin (Sigma-Aldrich), and the membranes were incubated with primary antibodies overnight at 4°C with rocking. After washing thrice with TBS, the membranes were incubated for 2 h at room temperature with enhanced chemiluminescence (Amer sham) anti-rabbit/mouse IgG horseradish peroxidase–conjugated antibody (GE Healthcare) at a final dilution of 1:2,000 in TBS containing 0.01% Tween 20 and 5% nonfat dry milk. After washing thrice with TBS, bound antibodies were detected by enhanced chemiluminescence (GE Healthcare).

Immunohistochemistry. Tumor tissues from control and AZD0530-treated mice were fixed in formalin immediately after surgical excision and processed in to paraffin wax blocks. Sections were deparaffinized using standard histologic procedures, and an antigen retrieval method (pressure cooker and high EDTA buffer) was used to ensure optimal antigen integrity and expression. A suite of immunohistochemical (IHC) biomarkers across several biological effect areas (Table 1) was screened against these tissue sections, using the Envision+ (Dako) detection system. IHC staining was scored by eye (from a minimum of 1,000 cells for the biomarkers) by a blinded pathology concordance panel.

Gene expression profiles. RNA isolated from baseline (untreated) tumors were profiled using Affymetrix U133 Plus 2.0 gene arrays at least in duplicates. This gene array has ~ 54,000 probes comprising ~ 20,000 unique transcripts. By using K-TSP, we considered a gene having a high discriminative score relative to the baseline gene expression profiles of untreated tumors as a potential predictive biomarker for AZD0530 sensitivity. For assessing relative tumor growth index (TGI), tumor size was measured twice per week throughout the study and tumor volume was calculated using the formula: tumor volume = (length × width²)/2.

Translational Relevance

Given that Src inhibitors are in clinical trials, the ability to predict which patients would benefit most from Src inhibitor therapy would be a great clinical asset and may ultimately improve the grim prognosis of pancreatic cancer patients. To this end, we evaluated the in vivo efficacy and pharmacodynamic effect of AZD0530, a small molecule inhibitor of Src in a large collection of pancreatic tumor xenografts and used a novel approach to predict biomarkers for AZD0530 sensitivity. In this study, we show, for the first time, that the gene expression profiles of baseline tumors could be used to predict AZD0530 sensitivity in pancreatic tumors. Here, we used K-TSP algorithm, a novel machine learning approach, to identify one gene pair that has a high predictive power for AZD0530 sensitivity and that may have potential as a biomarker for predicting pancreatic tumor sensitivity to AZD0530 in the clinic.

Table 1. List of IHC markers and method used for scoring

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Biological effect area</th>
<th>Scoring method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase 3</td>
<td>Apoptosis</td>
<td>% Positive cells</td>
</tr>
<tr>
<td>M30</td>
<td>Apoptosis</td>
<td>% Positive cells</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Proliferation</td>
<td>% Positive cells</td>
</tr>
<tr>
<td>Phospho HH3</td>
<td>Proliferation</td>
<td>% Positive cells</td>
</tr>
<tr>
<td>MCM2</td>
<td>Proliferation</td>
<td>% Positive cells</td>
</tr>
<tr>
<td>CD31</td>
<td>Vascular</td>
<td>Microvessel density</td>
</tr>
<tr>
<td>CD105</td>
<td>Vascular</td>
<td>Microvessel density</td>
</tr>
<tr>
<td>Phospho FAK</td>
<td>Invasion</td>
<td>Total H-score</td>
</tr>
<tr>
<td>Phospho Paxillin</td>
<td>Invasion</td>
<td>Total H-score</td>
</tr>
<tr>
<td>Cyclin D</td>
<td>Cell cycle</td>
<td>% Positive cells</td>
</tr>
<tr>
<td>Cyclin E</td>
<td>Cell cycle</td>
<td>% Positive cells</td>
</tr>
<tr>
<td>Phospho H2AX</td>
<td>DNA damage</td>
<td>% Positive cells</td>
</tr>
</tbody>
</table>
genes. Sample preparation and processing procedure was done as described in the Affymetrix GeneChip Expression Analysis Manual (Affymetrix, Inc.). Gene expression levels were converted to a rank-based matrix and standardized (mean, 0; SD, 1) for each microarray.

**K-TSP classifier.** We used the K-TSP algorithm (28) to construct a discriminative classifier in predicting tumors sensitive to AZD0530. In brief, the algorithm exploits the information contained in the rank-based matrix by focusing on “marker gene pairs” \((i, j)\) for which there is a significant difference in the probability of the event \((R_i < R_j)\) across the \(N\) samples from class \(Y = 1\) (AZD0530 sensitive) to \(Y = -1\) (AZD0530 resistant), where the event \((R_i < R_j)\) is equivalent to the rank of gene \(i\) is less than the rank of gene \(j\) if and only if gene \(i\) is expressed less than gene \(j\) (relative expression). Here, the quantities of interest are \(p_{ij}(m) = \text{Prob}(R_i < R_j | Y = m)\), \(m = (18)\), i.e., the probabilities of observing \(R_i < R_j\) in each class. These probabilities are estimated by the relative frequencies of occurrences of \(R_i < R_j\) within profiles and over samples. Let \(\Delta_p\) denote the “score” of gene pair \((i, j)\), where \(\Delta_p = | p_{ij}(1) - p_{ij}(-1) |\). A score \(\Delta_p\) is computed for every pair of genes \(i, j \in \{1, \ldots, P\}\), \(i \neq j\). Gene pairs with high scores are viewed as most informative for classification. Using an internal leave-one-out cross-validation, the final k-TSP classifier uses the \(k\) disjoint pairs of genes, which achieve the \(k\) best scores from the training set. In this study, maximum number of pairs (\(k_{\text{max}}\)) was fixed as 10.

**Gene set enrichment analysis.** Gene set analysis was done using the GSEA software Version 2.0.1 obtained from the Broad Institute. Genes represented by more than one probe were collapsed using the Collapse Probes utility to the probe with the maximum value. Gene set permutations were done 500 times for each analysis and the pathway/gene set list was sorted by the Normalized Enrichment Score. We used the 199 pathways defined by Kyoto Encyclopedia of Genes and Genomes (KEGG) database as the gene set in this study. Human pathway annotations were downloaded from KEGG (August 2007 release). The KEGG human pathways used in this study include metabolism, genetic information processing, environmental information processing, cellular processes, and human diseases. One hundred and sixty-six gene sets passed the gene set size filter criteria (min, 10; max, 500).

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**Fig. 1.** Efficacy of AZD0530 on the tumor growth of in vivo pancreatic adenocarcinoma xenografts. Patient-derived low passage pancreatic cancer xenografts were implanted in athymic mice. Animals with established tumors were treated with AZD0530 50 mg/kg/d by oral gavage for 28 d. Tumor size was evaluated twice per week by caliper measurements using the formula: tumor volume = [length × width²]/2. Relative TGI was calculated by relative tumor growth of treated mice divided by relative tumor growth of control mice × 100. Cases with a TGI of ≤50% were considered sensitive, TGI of ≥50% were considered resistant to AZD0530. A, antitumor effect of AZD0530 on the tumor growth of 16 xenografts. Points, mean (\(n = 8\) to 10 tumors per group); bars, SE. Dotted line, 50% TGI. *Significance (\(P < 0.01\)) compared with vehicle-treated tumors. B, growth curves representative of tumor sensitive to AZD0530 (Panc410) and resistant (Panc253) to AZD0530 treatment.

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http://www.broad.mit.edu/gsea
Quantitative real-time reverse transcription-PCR analysis. Total RNA was extracted from baseline tumors of predicted cases (A6L, Panc140, Panc194, Panc294, JH010, JH069, and JH131) using the RNeasy Mini kit (Qiagen). cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad), following the manufacturer’s instructions. cDNA (25 ng) from each sample was amplified with specific primers for LRRC19 and IGFBP2, and the housekeeper gene ubiquitin from Applied Biosystems Taqman probes using an iCycler iQ real-time PCR detection system (Bio-Rad). Accumulation of the specific PCR products was detected as an increase in fluorescence that was plotted against cycle number to determine the cycle threshold (CT) values. CT reflects the cycle number at which the fluorescence generated within a reaction crosses the threshold. Relative expression of the mRNA analyzed was estimated using the formula: $2^{-\Delta\Delta CT}$, where $\Delta CT = C_{T}$ (mRNA) - $C_{T}$ (Housekeeper). Samples were analyzed in a blinded manner and plotted as log-scale.

Statistical analysis. All error bars are represented as the SE. Significance levels for comparison between groups were analyzed using unpaired Student’s $t$ test and Kruskal Wallis statistic. The differences were considered significant when $P$ value was <0.05.

Results

AZD0530 slowed the growth of pancreatic tumor xenografts. AZD0530 exhibited a range of in vivo tumor growth indices between 40% to 142% in the 16 human pancreatic xenografts (Fig. 1A). Using TGI <50% as a cutoff value for sensitive, three cases (Panc291, Panc410, and Panc420) showed sensitivity to AZD0530 treatment (Fig. 1A). AZD0530 did not result in tumor regression even in the most sensitive cases. Rather, the established tumors showed decreased growth compared with controls in sensitive cases (Fig. 1B). This is consistent with previously published studies of AZD0530 in prostate cancer models (22) as well as other Src inhibitors in colorectal, breast, and pancreatic cancer mouse models (28–30).

Protein expression by Western blot and immunohistochemistry. Src is an integral part of several signaling pathways and signaling through Src can mediate mitogenic, proinvasive, anti-apoptotic, and proangiogenic effects. We therefore analyzed whether AZD0530 treatment affected tumor proliferation, invasion, apoptosis, and angiogenesis in vivo in xenografts using Western blot and quantitative IHC techniques. AZD0530 administration clearly down-regulated Src, FAK, p-FAK, and p-STAT3 expression in the sensitive tumor (Panc410) compared with control tumors (Fig. 2). In addition, AZD0530 administration resulted in the down-regulation of XIAP as evidenced by Western blot analysis.

Table 2. Summary of the statistical significance in the level of expression of each biomarker between control and AZD0530-treated samples

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Control N</th>
<th>AZD0530 N</th>
<th>Fixed effect test $P$</th>
<th>Kruskal Wallis statistic</th>
<th>Kruskal Wallis $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD105</td>
<td>18</td>
<td>18</td>
<td>0.855</td>
<td>0.07</td>
<td>0.788</td>
</tr>
<tr>
<td>CD31</td>
<td>18</td>
<td>18</td>
<td>0.369</td>
<td>1.6</td>
<td>0.205</td>
</tr>
<tr>
<td>Caspase 3</td>
<td>18</td>
<td>18</td>
<td>0.242</td>
<td>1.61</td>
<td>0.204</td>
</tr>
<tr>
<td>Cyclin D</td>
<td>18</td>
<td>18</td>
<td>0.845</td>
<td>0.26</td>
<td>0.613</td>
</tr>
<tr>
<td>Cyclin E</td>
<td>18</td>
<td>17</td>
<td>0.105</td>
<td>2.06</td>
<td>0.151</td>
</tr>
<tr>
<td>Ki67</td>
<td>18</td>
<td>18</td>
<td>0.051</td>
<td>3.25</td>
<td>0.071</td>
</tr>
<tr>
<td>M30</td>
<td>18</td>
<td>17</td>
<td>0.808</td>
<td>0.47</td>
<td>0.495</td>
</tr>
<tr>
<td>MCM2</td>
<td>18</td>
<td>18</td>
<td>0.572</td>
<td>0.13</td>
<td>0.716</td>
</tr>
<tr>
<td>pH2AX</td>
<td>18</td>
<td>18</td>
<td>0.778</td>
<td>0</td>
<td>0.961</td>
</tr>
<tr>
<td>paxillin</td>
<td>18</td>
<td>18</td>
<td>0.613</td>
<td>0.18</td>
<td>0.666</td>
</tr>
<tr>
<td>pFAK</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>7.75</td>
<td>0.005</td>
<td>0.003</td>
</tr>
<tr>
<td>paxillin</td>
<td>17</td>
<td>16</td>
<td>0.001</td>
<td>8.91</td>
<td>0.003</td>
</tr>
</tbody>
</table>

NOTE: Deparaffinized sections from control and AZD0530-treated xenografts (2-3 samples each) from Panc291, Panc420, Panc185, Panc265, JH033, and JH024 were used for the IHC. Values of all six cases were pooled together for each biomarker to calculate the significance. AZD0530 treatment resulted in the significant down-regulation of pFAK and pPaxillin. Entries in bold have statistical significance.
by the immunoblot of Panc410 (Fig. 2). However, there were no obvious differences in the protein expression in the AZD0530-treated resistant case (Panc265) compared with controls (Fig. 2).

There was a very clear treatment effect from AZD0530 on both p-FAK and p-paxillin as shown by IHC (Table 2; Fig. 3A, B, and C). Boxplots of the levels of expression of these biomarkers showed clear differences (Fig. 3A), with well-separated median
H-scores in each arm. There was a down-regulation of proliferative (Ki-67) and vascular markers (CD31) with AZD0530 treatment (Fig. 3A). However, the values are not statistically significant (Table 2). There were no significant differences in the expression of caspase3, M30, p-HH3, MCM2, p-H2AX, CD105, Cyclin D, and Cyclin E with AZD0530 treatment (Table 2).

Global analysis of gene expression profiles. Baseline whole-genome transcriptome profiles of the 24 xenografts were obtained by microarray. By comparing the gene expression profiles between the AZD0530-sensitive and AZD053-resistant xenografts, a clear pattern of differentially expressed genes was observed. Figure 4A depicts the heatmap of the top 100 differentially expressed genes between these phenotypes selected by signal-to-noise metric. These data suggest that we can identify a predictor for AZD0530 sensitivity from baseline gene expression profiles.

Training K-TSP classifier for predicting AZD0530 sensitivity. One of the goals of this work is to construct an accurate classifier in predicting AZD0530 sensitivity. We used the 16 tumors treated with AZD0530 as the training set. Tumors with TGI of <50% were considered as sensitive ($n = 3$), and tumors with TGI of >50% were considered as resistant to AZD0530 ($n = 13$). We used the K-TSP algorithm in this study as it has proven to be a discriminative classifier in various studies (31). From this training step, the K-TSP classifier identified a gene pair (LRRC19, IGFBP2) as the most discriminative features in predicting AZD0530 sensitivity (Fig. 4B). The decision rule for the classifier is as follows: If $LRRC19 > IGFBP2$ then sensitive to AZD0530, otherwise resistant to AZD0530. The interpretation of this rule is that the relative expression of LRRC19 higher than IGFBP2, the query case was predicted as sensitive to AZD0530, otherwise the cases were predicted as resistant to AZD0530 (Fig. 4C). The estimated leave-one-out cross-validation accuracy for the K-TSP classifier based on the training set is 97.8%.

K-TSP predictor achieves high accuracy in predicting AZD0530 sensitivity. To evaluate the predictive power of the two-gene
were enriched in the AZD0530-resistant phenotype (Supplementary Table S1A). In contrast, basal cell carcinoma, cell sensitive phenotype were mostly metabolic pathways (Supplementary Table S1B). The top 10 pathways enriched in the AZD0530-profiles of the xenografts by comparing the 5 sensitive to the 19 resistant cases. The top 10 pathways enriched in AZD0530-sensitive and -resistant cases. Gene set enrichment analysis was done on the expression microarray data that are highly accurate in predicting AZD0530 sensitivity; and the relative expression of the two genes can be rapidly tested by RT-PCR in the clinic without costly microarray gene expression profiling.

**Gene set analysis reveals the pathways enriched in AZD0530-sensitive cases.** Finally, we sought to identify the underlying pathways enriched in AZD0530-sensitive and -resistant cases. Gene set enrichment analysis was done on the expression profiles of the xenografts by comparing the 5 sensitive to the 19 resistant cases. The top 10 pathways enriched in the AZD0530-sensitive phenotype were mostly metabolic pathways (Supplementary Table S1A). In contrast, basal cell carcinoma, cell cycle, and hedgehog signaling pathways per KEGG definition were enriched in the AZD0530-resistant phenotype (Supplementary Table S1B).

## Discussion

Predictive biomarkers, which would assist in patient selection for clinical trials, would be of utmost importance in the development of a potentially effective drug. Given that at least four Src inhibitors are in active clinical trials, the ability to predict which patients would benefit most would be a great clinical asset. This is especially true in pancreatic cancer, which has an extremely poor prognosis and a paucity of active therapeutic agents. To this end, we evaluated the in vivo efficacy and pharmacodynamic effects of AZD0530, a small molecule Src inhibitor in pancreatic tumor xenografts, and used a novel approach to predict biomarkers for AZD0530 sensitivity in pancreatic cancer.

Src is an integrator of divergent signal transduction pathways that has been implicated in oncogenesis from proliferation to invasion and metastatic spread (32). Observations that Src expression increases with disease progression suggests that Src may be more active in invasion and metastasis than in tumor initiation (2). The contribution of Src in pancreatic tumor progression has been firmly established in an orthotopic implantation model done using human pancreatic tumor cells, in which Src expression was down-regulated by siRNA, resulting in a significant reduction in the incidence of metastases (26). Given its well-defined role in cancer growth, therapeutic targeting of Src kinase has been an area of intense investigation. Recently, Src inhibition with a Src family kinase inhibitor alone or in combination with gemcitabine, inhibited growth and metastases of orthotopically implanted human pancreatic carcinoma cells and reduced tumor cell proliferation, tumor microvessel density, and increased apoptosis in vivo (6). Evidence from several lines of promising preclinical research has suggested that Src kinase activity influences a plethora of mechanisms that allow tumor cells to proliferate and migrate (33). Consequently, strategies to target Src kinase activity alone and in combination with cytotoxic agents are undergoing investigation in most major tumor types including breast, lung, prostate, pancreatic, and colorectal carcinomas (34).
The present preclinical study used large number pancreatic tumor xenografts to test the effect of Src inhibitor in pancreatic cancer. In this study, we show, for the first time, that the gene expression profiles of baseline tumors could be used to predict AZD0530 sensitivity in pancreatic tumors. Here, we used the K-TSP algorithm, a novel machine learning approach that used a small number of genes in its decision rules. The classification is based on comparing the relative expression of gene pairs, which is relatively easy to interpret and facilitates translational work as shown by RT-PCR validation in this study (Fig. 5B) and others (35).

The genes in the K-TSP predictor are the leucine rich repeat containing protein 19 (LRRC19) and insulin-like growth factor binding protein 2 (IGFBP2; Fig. 4A). Global analysis also identified LRRC19 and IGFBP2 in the top 100 differentially expressed genes. These two genes have been implicated to play essential roles in the progression of various cancer types (36, 37). LRRC19 encodes a leucine rich surface protein where up-regulation is associated with hypoxia (36). Recently, using a glial-specific transgenic mouse model, IGFBP2 has been implicated to play a key role in promoting the development and progression of glioma. Most importantly, activation of two separate pathways (combined expression of IGFBP2 or Akt with K-ras) is necessary for glioma tumorigenesis (37). Overexpression of IGFBP2 may indicate activation of the cell cycle via the Akt pathway, and may make the tumor less responsive to AZD0530. This complex interaction between IGFBP2 and the cell cycle was revealed by the two-gene predictor and gene set analysis. This indicates that interpretation of a predictor also plays an important aspect in building an accurate classifier, which is often neglected by many bioinformatics analyses. From this study, the higher relative expression of LRCC19 to IGFBP2 indicates AZD0530 sensitivity (Fig. 4B and C). This may indicate that the K-TSP algorithm helps to identify key genes that important cellular processes in response to AZD0530.

The two-gene predictor achieved 83% overall accuracy in the validation experiments. Panc159, which was predicted sensitive (TGI, <50%) based on the predictor turned out to be resistant (TGI, 71%) in the test validation. Differences in response based on predictive biomarkers among patient tumors may be attributed to a wide variety of tumor factors including the sequestration or efflux of drugs, down-regulation of apoptotic pathways that likely act downstream of drug targets and the stimulation of growth/survival pathways (38). In addition, continued exposure of tumor cells to chemotherapy agents can often result in selective amplification of specific genes whose products promote drug resistance (38). The present study used a small sample size for test and prediction. Significantly larger sample sizes will be required to further validate the prediction before translating the prediction to select patients in the clinic.

Western blots of sensitive tumor xenografts indicated that AZD0530 led to significant down-regulation of FAK and p-FAK (Fig. 2). It has been reported that Src and FAK cross-activate, and enhanced migratory activity is linked to increased FAK expression and activation (39). Src activates FAK and STAT-3 and their linked activities act to control cell migration through the turnover of focal adhesions and the suppression of cell-cell contacts (8–10). p-STAT-3 inhibition observed in this study with AZD0530 is also consistent with the p-STAT-3 inhibition reported with other Src kinase inhibitors (40). Interestingly, it has recently been shown that Src-dependent induction of vascular endothelial growth factor expression is mediated via STAT-3 (41). AZD0530 treatment down-regulated XIAP, a member of the apoptosis inhibitor family (Fig. 2). These results are consistent with the reported results of AZD0530 in an ovarian tumor model, where AZD0530 treatment inhibited the expression of survivin, another member of the apoptosis inhibitor family (42).

Immunohistochemistry results clearly show a treatment effect for AZD0530 on the Src substrates key to the invasive phenotype. There were significant decrease in the posttreatment levels of expression of both p-FAK and p-paxillin compared with controls for both sensitive and resistant tumors. This work in pancreatic explants agrees with and reinforces the clinical work in man with the same biomarkers (43). There was a very clear treatment effect of AZD0530 on both p-FAK and p-paxillin with high values of the test statistics. These data provide evidence supportive of earlier clinical results in man demonstrating the effect of AZD0530 on these biomarkers (43).

In summary, we show that the Src inhibitor AZD0530 displays preclinical activity in a subset of pancreatic tumor xenografts. Antiproliferative activity of AZD0530 results in the specific down-regulation of Src, FAK, XIAP, p-FAK, p-paxillin, and p-STAT-3. Finally, our study identified one gene pair (LRRC19 and IGFBP2) that has a high predictive power for AZD0530 sensitivity and that may have potential as a biomarker for predicting pancreatic tumor sensitivity to AZD0530 in the clinic.

Disclosures of Potential Conflicts of Interest

M. Hidalgo, commercial research support, Astra Zeneca. C. Womack, H. Wombwell, S. Morgan, J. Walker, and T.P. Green, employees, Astra Zeneca. The other authors declare no potential conflicts of interest.

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Antitumor Effects and Biomarkers of Activity of AZD0530, a Src Inhibitor, in Pancreatic Cancer

N.V. Rajeshkumar, Aik Choon Tan, Elizabeth De Oliveira, et al.