Pharmacogenomic Modeling of Circulating Tumor and Invasive Cells for Prediction of Chemotherapy Response and Resistance in Pancreatic Cancer

Kenneth H. Yu1,2, Mark Ricigliano3, Manuel Hidalgo4, Ghassan K. Abou-Alfa1,2, Maeve A. Lowery1,2, Leonard B. Saltz1,2, Joseph F. Crotty1,2, Kristen Gary1,2, Brandon Cooper5, Rena Lapidus5, Mariola Sadowska5, and Eileen M. O’Reilly1,2

Abstract

Purpose: Despite a challenging prognosis, modern cytotoxic therapy can induce tumor responses and extend life in pancreatic adenocarcinoma (PDAC). Pharmacogenomic (PGx) modeling of tumor tissue can predict the efficacy of chemotherapeutic agents in preclinical cancer models. We hypothesized that PGx profiling of circulating tumor and invasive cells (CTIC) isolated from peripheral blood could predict tumor response, progression, and resistance.

Experimental Design: A PGx model was created and validated in preclinical models. A prospective clinical trial was conducted. Fifty patients with advanced PDAC were enrolled. Before treatment, 10 mL of peripherally drawn blood was collected. CTICs isolated from this blood sample were expression profiled and the PGx model was used to predict effective and ineffective chemotherapeutic agents. The treating physicians were blinded to PGx prediction.

Results: We found that CTICs could be reliably isolated, total RNA extracted and profiled from 10 mL of peripheral blood from patients with unresectable PDAC before chemotherapy treatment and at disease progression. Using previously created PGx models to predict chemotherapy sensitivity, we found that clinical benefit was seen for study participants treated with chemotherapy regimens predicted to be effective versus chemotherapy regimens predicted to be ineffective with regard to progression-free (10.4 mo vs. 3.6 mo; \( P < 0.0001; \) HR, 0.14) and overall survival (17.2 mo vs. 8.3 mo; \( P < 0.0249; \) HR, 0.29).

Conclusions: These findings suggest that PGx profiling of CTICs can predict treatment response.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) currently represents the fourth leading cause of cancer mortality in the United States. Of the five most lethal cancers, incidence and death rates are only increasing for PDAC. Therefore, it is estimated that by 2015, PDAC is likely to rise to the second leading cause of cancer death in the United States (1). Despite this, the emergence of active combination chemotherapy regimens during the past 3 years has led to incremental improvements in overall survival. FOLFIRINOX (2) and gemcitabine with nab-paclitaxel (3) represent clinically meaningful improvements over the prior standard of care, single-agent gemcitabine. As with most cancers, however, clinicians are without tools to help choose the most effective therapeutic agents for individual patients with PDAC. Biomarkers for choosing second-line therapy are similarly unavailable. Therefore, a biomarker capable of identifying upfront the most effective treatment regimen for each individual patient is greatly needed. Inevitably, PDAC develops resistance to treatment. A greater understanding of treatment resistance and development of a biomarker allowing physicians to anticipate and change treatment accordingly are both greatly needed.

Over the years, there have been multiple approaches to developing biomarkers for predicting drug effect. In general, the most actively sought strategy is to determine in the host tumor, biologic traits that are linked to drug response. Although this strategy has been useful in several settings, particularly when the biomarker is linked to the mechanism of action of the agent, i.e., Her2 expression and trastuzumab treatment, it has not been useful in PDAC. A recent example is human equilibrative nucleoside transporter-1 (hENT1), a
Translational Relevance

We present results of a validation study of an innovative pharmacogenomic tool to predict effective chemotherapeutic treatments for patients based on profiling of circulating tumor and invasive cells isolated from peripheral blood. This study provides proof of principle that an individualized approach to treating pancreatic cancer is feasible.

transporter protein thought important for cellular uptake of gemcitabine. Preliminary studies suggested that low expression of hENT1 could result in gemcitabine resistance; however, prospective validation did not confirm these findings in patients with advanced disease (4). Another approach to predict drug response is based in the connectivity map concept (5). Briefly, connectivity mapping hypothesizes that biologic systems with similar drug expression profiles might share biologic properties, including drug response. The connectivity mapping approach has been validated, for example, effectively predicting rapamycin-induced glucocorticoid sensitivity in acute lymphoblastic leukemia (6). Applied to PDAC, a tumor with a gene expression profile similar to a profile that predicts response to a drug in an experimental system could be sensitive to that drug. Indeed, gene expression profiles of response to anticancer agents can be created by comparing the expression profiles of model systems with divergent drug response. We call this approach pharmacogenomic drug sensitivity profiling (PGx).

The study of circulating tumor cells (CTC) has distinct advantages over tumor tissues. Genomic aberrations present in tissue may not inform the behavior of cancer cells in transit or with high metastatic potential. Serial sampling of tumor tissue during the course of treatment is technically challenging and not without risk. Approaches that allow for convenient sampling and characterization of CTCs have the potential for addressing these concerns. Using innovative and proprietary cell adhesion matrices, we have built a robust platform for capturing and preserving rare CTCs from 10 mL of heparinized blood drawn peripherally from patients with PDAC. This approach is built upon methods used to successfully isolate and study CTCs in breast (7) and prostate (8) cancers. In these studies, this assay has been shown to successfully capture cells with tumorigenic properties (CD45−, EpCAM−ESA−cytokeratin−, and ability to degrade and ingest collagenous matrices; refs. 7–9). Not all captured cells express these markers typical of classic tumor cells, but all cells isolated in this manner have the ability to invade into the cell-adhesion matrix. Thus, we have coined this population of cells circulating tumorigenic and invasive cells (CTIC).

The current study was performed to determine whether PGx profiling could be performed on CTICs isolated from patients with advanced PDAC, and to determine whether this approach could be used to predict effective chemotherapeutic regimens for treatment.

Materials and Methods

PGx model

In vitro drug sensitivity testing of the NCI-60 tumor cell line collection against each chemotherapeutic in the panel was performed, with two biologic replicates, by the National Cancer Institute’s Developmental Therapeutics Program as previously described (10). The NCI-60 cell lines have been authenticated previously by DNA fingerprinting (11). Following standardization of the IC50 values, we consider cell lines with a standardized GI50 score (z-score) > 0.75 as sensitive to the chemotherapeutic in question. Normalized log2 mRNA expression data for the non–drug-treated NCI-60 tumor cell line was obtained and filtered for 416 genes that comprise the ABC, SLC, and CYP family of genes. Supplementary Table S1 lists a series of genes that were used to create the pharmacogenomic models of 12 commonly used chemotherapy treatment regimens for the subsequent enrichment analysis based on GI50 values for each chemotherapeutic and corresponding expression profiles of the filtered gene sets.

Study design

An Institutional Review Board (IRB)–approved observational study was conducted at Memorial Sloan Kettering Cancer Center (registered at Clinicaltrials.gov, identifier NCT01474564). Enrollment occurred from November 2011 to October 2012. The primary objective of the study was to assess the feasibility of (1) obtaining and characterizing CTICs, and (2) using the resulting microarray analysis to generate a treatment profile for patients with PDAC. Target accrual for the study was between 30 and 60 patients, and a total of 50 study participants were ultimately enrolled (see Fig. 3). At the time of the current analysis, 35 study participants were evaluable for treatment response, as they have met criteria for progression of disease or death. Key eligibility criteria included histologic or cytologic confirmation of pancreatic adenocarcinoma, the patient was deemed eligible for chemotherapy treatment and an ECOG performance status of 0, 1, or 2.

Following written informed consent and before the initiation of chemotherapy treatment, a 10 mL blood sample was obtained in a heparinized Vacutainer (Becton Dickinson) tube from each study participant using standard clinical procedures. Blood samples were collected by venipuncture or by accessing an indwelling catheter normally used for phlebotomy.

Cell enrichment

Coded and deidentified samples were shipped at 4°C overnight to CellPath Therapeutics, Inc. for CTIC isolation and enrichment. A collagen-adhesion matrix (CAM) in a modified cell invasion assay was used to capture invasive cells. Three-milliliter aliquots of whole blood were subjected to enrichment in a CAM-coated modified cell invasion assay (Vita-Cap; Vitatex) and cultured for 2 hours in the Cancer Cell Culture (CCC) media (1:1 mixture of Dulbecco’s modified Eagle medium and RPMI-1640 medium...
supplemented with 10% calf serum, 10% Nu-serum, 2 mmol/L L-glutamine, 1 U/mL penicillin and 10 μg/mL streptomycin). Captured cells were then washed and lysed in situ.

Microarray data analysis
Lysed CAM-adherent cells that were directly isolated from the invasion assay were used in mRNA microarray analyses. Specifically, total RNA from lysed CAM-bound cells was purified by the RNeasy Mini Kit (Qiagen) and then subjected to DNA microarray analysis. Generation of ssDNA and labeling were performed (NuGen Pico; NuGen, Inc.) with subsequent hybridization and scanning of the Affymetrix high-density 3' IVT oligonucleotide microarray HG_U133_Plus_2 chip (containing 54,675 gene probes) according to the manufacturer’s specifications (Affymetrix).

Enrichment analysis and determination of sensitivity score
The CEL file from the Affymetrix scanner was used to determine the intensity value of each probe, and then collapsed to a single maximum value resulting in an array of 20,606 genes (Expression Console; Affymetrix). Gene expression of study participant CTICs was then assessed for differentially expressed pharmacogenomic models using gene-set enrichment analysis (GSEA v2.0.12; Broad Institute, Cambridge MA) based on a panel of chemotherapeutics designed for first-line treatment of PDAC as the a priori defined gene sets (CellPath Therapeutics, Inc.). The result of the enrichment analysis was a normalized enrichment score (NES) for each chemotherapeutic regimen. To correct for multiple hypotheses testing and for selection bias in the enrichment analysis, only those chemotherapeutic regimens with a FDR <1% and a P-value <0.001 were considered to be significant. The NES for each chemotherapeutic regimen was further used to calculate an implied GI50 value, and a corresponding sensitivity score for each chemotherapeutic regimen in the panel based on the following formula:

\[
\text{SensitivityScore} = \begin{cases} 
-4, & \text{if } GI_{50} < -1.0; \\
-3, & \text{if } -0.99 \leq GI_{50} < -0.70; \\
-2, & \text{if } -0.69 \leq GI_{50} < -0.30; \\
-1, & \text{if } -0.29 \leq GI_{50} < 0; \\
1, & \text{if } 0 \leq GI_{50} < 0.29; \\
2, & \text{if } 0.30 \leq GI_{50} < 0.69; \\
3, & \text{if } 0.70 \leq GI_{50} < 0.99; \\
4, & \text{if } GI_{50} > 1.0.
\end{cases}
\]

The chemotherapeutic regimens are then assigned to a predicted response category as SENSITIVE, INTERMEDIATE, or RESISTANT based on the Sensitivity Scores.

Results
PGx model development and validation
To test our central hypothesis, we took advantage of the PanXenoBank collection from the Johns Hopkins Hospital. A set of 32 PDAC patient-derived tumor xenograft (PDX) models that had been treated with gemcitabine was selected for this work. As shown in Fig. 1A, these models display a range of response to gemcitabine. By comparing the gene expression profile of the highly sensitive PANC253 model with the resistant JH033 model using a predefined set of 450 genes involved in drug transport, distribution, and metabolism, we established a set of genes overexpressed in the sensitive model (Fig. 1B). We next tested if this PGx model of gemcitabine susceptibility predicted the response of this drug in the remaining 30 models using gene GSEA. The PGx model of gemcitabine susceptibility predicted the response of this drug in the remaining 30 models using gene GSEA. The PGx model of gemcitabine susceptibility predicted the response of this drug in the remaining 30 models using gene GSEA. The PGx model of gemcitabine susceptibility predicted the response of this drug in the remaining 30 models using gene GSEA.

PGx profiling, a prospective study
To further test our hypothesis in the clinical setting, a PGx model was developed for a number of chemotherapeutic regimens commonly used in PDAC. The following chemotherapy regimens were modeled: FOLFIRINOX (5-FU, irinotecan, and oxaliplatin), FOLFOX (5-FU and oxaliplatin), Gem-nab (gemcitabine + nab-paclitaxel), Gem-Ox (gemcitabine + oxaliplatin), Gem-Cap (gemcitabine + capecitabine), and GTX (gemcitabine, docetaxel, and capecitabine) from single-agent response data obtained from the NCI 60 cell line dataset. In addition, instead of using tumor biopsies to determine the patient gene expression profile, we decided to study CTICs. The rationale for this is provided above and was supported by preliminary work showing...
A strong correlation between tumor tissue and CTIC profiles (Supplementary Table S2 and Supplementary Fig. S1).

A total of 50 patients with advanced or locally advanced PDAC, whose pertinent demographic characteristics are listed in Table 1, were treated in a prospective clinical trial. The study was IRB-approved and registered at clinicaltrials.gov (identifier #NCT01474564). As shown in Fig. 3 and described in Materials and Methods, blood samples for CTIC were collected at baseline and at the time of progression. Patients were, for the most part, treated with one of the above-mentioned regimens as per physician discretion.

Table 2 shows representative profiles for three study participants. As detailed in Materials and Methods, a score is calculated for each one of the regimens based on the NES of each one of the individual agents.

All 3 patients in this example received FOLFIRINOX chemotherapy. In Patient A in whom the PGx profiling predicted sensitivity to FOLFIRINOX, the PFS with this regimen was 7.3 months. This is in contrast with patients B and C, in whom the profile predicted intermediate and low sensitivity to FOLFIRINOX; PFS was 2.1 and 1.7 months, respectively.

Adequate numbers of CTICs were captured and sufficient RNA was subsequently isolated for successful gene expression analysis and PGx profiling from all the 50 study participants. At the time of the current analysis, 35 study participants were evaluable for disease progression to first-line chemotherapy. Fifteen study participants were deemed not evaluable for response for a variety of reasons. Seven study participants with locally advanced PDAC were treated with concurrent chemoradiotherapy following their initial line of chemotherapy, before disease progression. Radiotherapy is a treatment modality not accounted for by the PGx model. Two study participants received investigational agents that were also not present in the PGx model. Two patients died of cancer-related causes, and another 2 patients died of cancer-unrelated causes, all before receiving any treatment. One patient went on to have the tumor resected. One patient had yet to progress on first-line treatment at the time of the analysis.

On the basis of the CTIC PGx profiling, the 35 evaluable study participants were classified into three groups: those whose chemotherapy regimen was predicted to be effective (“sensitive”), ineffective (“resistant”), or of intermediate effectiveness (“intermediate”). Sixteen (45.7%) of the evaluable study participants fell into the sensitive group and 12 (34.3%) into the resistant group. All evaluable study participants received combination chemotherapy in the first-line
setting. The majority of study participants, 22 (62.9%), received FOLFIRINOX chemotherapy and 10 (28.6%) received gemcitabine-based combination chemotherapy.

PGx profiling predicts treatment response

There was a statistically significant difference in median progression-free survival (PFS) among the three groups as predicted by PGx profiling, with those in the sensitive group responding the longest (10.4 months), those in the resistant group responding the shortest (3.6 months), and those in the intermediate group responding in between (7.8 months, Fig. 4A). These differences were statistically significant ($P = 0.0001$, log-rank test; $P < 0.0001$, log-rank test for trend). The HR for PFS comparing sensitive with resistant groups was 0.14. The PFS of 10.4 months seen in the sensitive group was numerically greater than the median PFS of 7.5 months seen in all evaluable patients; however, this did not reach statistical significance. Patients in the resistant group experienced significantly worse PFS when compared with all evaluable patients (3.6 vs. 7.5 months, $P = 0.0084$, Log-rank test).

An overall survival (OS) difference was seen among the three groups, median OS for the sensitive group was 17.2 months, compared with 13.8 months in the intermediate group and 8.3 months in the resistant group ($P = 0.083$, log-rank test; $P < 0.0304$, log-rank test for trend; Fig. 4B). Comparing OS between only the sensitive and resistant groups, a statistically significant difference was seen ($P = 0.0249$, log-rank test).

Although gender and treatment regimens were not significantly different among the three groups, the age of study participants in the resistant group was significantly older than those in the sensitive group ($P = 0.006$, Table 1). The age of study participants in the intermediate group was in between the sensitive and resistant groups. Despite this, there was not a statistically significant correlation between age and either PFS or OS. Baseline ECOG performance status, location and number of disease sites, presence of ascites, albumin level, CA 19-9, CEA, and total bilirubin
were compared and not found to be significantly different among the three groups.

An overall analysis of the treatment score assigned to the treatment regimen patients received and the magnitude of response was performed. The treatment score assigned by PGx profiling demonstrated a statistically significant positively correlated with both PFS ($r = 0.4583; P = 0.0056$) and OS ($r = 0.5152; P = 0.015$; See Supplementary Fig. S2).

The PPV of PGx profiling was determined for predicting PFS of 6 months or better. For study participants with PFS $> 6$ months, the PPV was 0.81. For study participants with PFS $< 6$ months, the NPV was 0.75 (Fig. 4C).

Interestingly, PGx profiles performed longitudinally in individual study participants showed changes following treatment (Table 2). In patient D, the initial PGx profile demonstrated treatment sensitivity to FOLFOX. The patient received this treatment and ultimately progressed and PGx profiling was repeated. At this point, the CTIC PGx profile has changed dramatically, predicting resistance to FOLFOX chemotherapy and increased sensitivity to a regimen such as gemcitabine and nab-paclitaxel. Analysis to determine the predictive ability of the PGx model for second-line treatment is under way.

**Discussion**

PDAC remains among the most challenging malignancies to diagnose and treat. The development of more effective therapies has, nevertheless, made it possible to improve the treatment and prolong the life of affected patients. As more effective cytotoxic and targeted agents become

---

Table 1. Patient demographics

<table>
<thead>
<tr>
<th></th>
<th>All study participants</th>
<th>Nonevaluable</th>
<th>Evaluable</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>50</td>
<td>15</td>
<td>35</td>
<td>12</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Mean age</td>
<td>66.2</td>
<td>66.5</td>
<td>66.0</td>
<td>70.9$^*$</td>
<td>66.1</td>
<td>62.3$^*$</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>6</td>
<td>16</td>
<td>5</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>9</td>
<td>19</td>
<td>7</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>38</td>
<td>30</td>
<td>11</td>
<td>5</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOLFIRINOX</td>
<td>22</td>
<td></td>
<td>5</td>
<td>3</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>FOLFOX</td>
<td>3</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>GemOx</td>
<td>6</td>
<td></td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gem-Cap</td>
<td>3</td>
<td></td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTX</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

NOTE: Statistically significant values are represented by $^*$.  

---

Figure 3. CONSORT diagram; circulating tumor and invasive cells (CTICs).
available, development of a clinical assay capable of predicting optimal therapy becomes increasingly important. In a more indolent disease, there may be time to try a number of different regimens to find an effective approach. In PDAC, upfront treatment with a regimen effective for the individual patient is critical as opportunities for second and subsequent lines of therapy are often limited. Developing such an assay that is not contingent on tissue acquisition is particularly attractive in PDAC, as the primary tumor is not convenient for biopsy, and serial biopsies pose both risk along with inconvenience and expense. Furthermore, tumor cells in active vascular transit represent a population of tumor cells of particular interest.

The current approach offers advantages to prior PGx approaches to guide cancer therapy. For example, a recently developed approach termed COXEN (CO-eXpression ExtrapolatioN; ref. 12) similarly uses in vitro gene expression profiles to model drug responses. The in vitro model is then used to predict treatment responses in vivo by profiling tumor tissue. One advantage of our approach that is not contingent on tissue acquisition is particularly attractive in PDAC, as the primary tumor is not convenient for biopsy, and serial biopsies pose both risk along with inconvenience and expense. Furthermore, tumor cells in active vascular transit represent a population of tumor cells of particular interest.

The current approach offers advantages to prior PGx approaches to guide cancer therapy. For example, a recently developed approach termed COXEN (CO-eXpression ExtrapolatioN; ref. 12) similarly uses in vitro gene expression profiles to model drug responses. The in vitro model is then used to predict treatment responses in vivo by profiling tumor tissue. One advantage of our approach that is not contingent on tissue acquisition is particularly attractive in PDAC, as the primary tumor is not convenient for biopsy, and serial biopsies pose both risk along with inconvenience and expense. Furthermore, tumor cells in active vascular transit represent a population of tumor cells of particular interest.

The current study provides proof of principle that PGx profiling of CTICs can effectively predict treatment response in patients with advanced PDAC. A number of questions are raised, which will require further study. Because of the

<table>
<thead>
<tr>
<th>Patient</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS (mo)</td>
<td>7.3</td>
<td>2.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Regimen</td>
<td>Score</td>
<td>Regimen</td>
<td>Score</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>0.69</td>
<td>FOLFOX</td>
<td>0.65</td>
</tr>
<tr>
<td>FOLFIRINOX</td>
<td>0.59</td>
<td>Gem-Cap</td>
<td>0.56</td>
</tr>
<tr>
<td>Gem-Cap</td>
<td>0.25</td>
<td>GemOx</td>
<td>0.53</td>
</tr>
<tr>
<td>GemOx</td>
<td>0.55</td>
<td>Gem-nab</td>
<td>0.15</td>
</tr>
<tr>
<td>Gem-nab</td>
<td>0.90</td>
<td>Gem-nab</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Patient D (pretreatment)

<table>
<thead>
<tr>
<th>PFS (mo)</th>
<th>3.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen</td>
<td>Score</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>1.02</td>
</tr>
<tr>
<td>FOLFIRINOX</td>
<td>0.74</td>
</tr>
<tr>
<td>Gem-nab</td>
<td>0.22</td>
</tr>
<tr>
<td>Gem-Cap</td>
<td>0.07</td>
</tr>
<tr>
<td>GemOx</td>
<td>0.24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PFS (mo)</th>
<th>3.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen</td>
<td>Score</td>
</tr>
<tr>
<td>Gem-nab</td>
<td>1.27</td>
</tr>
<tr>
<td>Gem-Cap</td>
<td>0.63</td>
</tr>
<tr>
<td>GemOx</td>
<td>0.07</td>
</tr>
<tr>
<td>Gem-nab</td>
<td>0.07</td>
</tr>
<tr>
<td>FOLFIRINOX</td>
<td>2.02</td>
</tr>
</tbody>
</table>

NOTE: Shaded values highlight drug regimens of interest discussed in the article.

Pharmacogenomic Modeling in Pancreatic Cancer

Table 2. PGx profiles of three study participants (patients A–C) before treatment, and comparison of PGx profiles in 1 study participant (patient D) before and after treatment
timing of this study, the majority of patients received 5-FU-based chemotherapy; in particular, FOLFIRINOX was most commonly used. Although there is no reason to believe that PGx profiling is more effective at predicting response to 5-FU-based regimens compared with other regimens, such as gemcitabine, a prospective study is in progress to specifically study the patients receiving gemcitabine and nab-paclitaxel to validate the utility of PGx profiling in this particular regimen. Although our matrix invasion approach has been shown in numerous studies to isolate classically defined circulating tumor cells, we are likely capturing a heterogeneous population of cells. Current work is under way to define the cell types composing this population of invasive cells and to characterize their contributions to the overall PGx profile. It is certainly possible that some of the profiled cells are malignant cells undergoing epithelial–mesenchymal transition (EMT) or nonmalignant cells that contribute to the treatment response and resistance in other ways. For example, a robust stromal response is a hallmark feature of PDAC (18), a response in which PBMCs play a crucial role (19). A recent global gene expression profiling study demonstrated a rich set of differentially expressed genes in PBMCs from patients with PDAC compared with healthy controls (20). Another study previously demonstrated that mononuclear cells protect PDAC cells from chemotherapy induced apoptosis. (21) Therefore, profiling a diverse variety of circulating cells may be more informative than restricting the analysis to circulating cells expressing a classic CTC phenotype.

The current study provides the first evidence that PGx profiling of a specific invasive subset of cells found in peripheral blood, CTICs, can be used to predict treatment response in PDAC. The test is convenient, requiring a single tube of blood drawn peripherally, and reliable; adequate profiles were generated for all 50 study participants before treatment and in 22 study participants at progression. Importantly, PGx profiling of CTICs accurately stratified treatment response of study participants based on the treatment regimen they received. Study participants treated with chemotherapeutic agents predicted to be effective by PGx profiling experienced significantly longer PFS and OS.

Figure 4. (A), PFS and (B) OS of study participants, grouped by treatment response as predicted by PGx profile. (C), performance of PGx model for predicting treatment sensitivity as reflected by long PFS (> 6 months), versus resistance as reflected by short PFS (< 6 months).
compared with the study participants treated with chemotherapeutic agents predicted to be ineffective. This study is the first of its kind to predict treatment response in PDAC. Such an approach would be highly valuable in guiding front-line therapy in PDAC. As data mature, we may be able determine utility in predicting response in the second-line setting. Digging deeper into the gene expression data has yielded insights into pathways that may predict prognosis and drug resistance. A detailed discussion of these findings will be presented separately. Two active regimens, FOLFIRINOX and gemcitabine with nab-paclitaxel, are currently offered to patients with advanced PDAC; a prospective study using our PCx assay to guide front-line therapy is warranted. Further studies are warranted to expand applicability to other malignancies or as a tool to choose effective targeted agents and clinical trials for patients.

Disclosure of Potential Conflicts of Interest

K.H. Yu is an uncompensated consultant/advisory board member for CellPath Therapeutics. M.J. Ricigliano is an employee of and has ownership interest (including patents) in CellPath Therapeutics. M. Hidalgo has ownership interest (including patents) in CellPath Therapeutics. No potential conflicts of interest were disclosed by the other authors.

References

Clinical Cancer Research

Pharmacogenomic Modeling of Circulating Tumor and Invasive Cells for Prediction of Chemotherapy Response and Resistance in Pancreatic Cancer

Kenneth H. Yu, Mark Ricigliano, Manuel Hidalgo, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-14-0531

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2014/08/16/1078-0432.CCR-14-0531.DC1

Cited articles
This article cites 19 articles, 8 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/20/20/5281.full.html#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/20/20/5281.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.