Preclinical Development

Characterization of an Oxaliplatin Sensitivity Predictor in a Preclinical Murine Model of Colorectal Cancer

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Introduction

Colorectal cancer is the third most common cancer in the world with approximately 150,000 new cases in the United States each year and ranks second only behind lung cancer as the leading cause of cancer-related deaths (1). At initial diagnosis approximately 20% of patients will have distant metastasis, and another 25% to 30% of patients with early stage disease will develop metastasis (2, 3). Currently, the use of chemotherapy in the metastatic setting is predominantly for disease control and palliation of symptoms. If left untreated, patients with metastatic colorectal cancer have an overall survival of 6 to 9 months, but with combination therapy, survival can be improved to greater than 20 months (4–6). Although the prolonged survival with current combination therapy represents a significant achievement, metastatic colorectal cancer still remains an incurable disease, and new therapeutic approaches are required to improve clinical outcomes.

Therapy based upon the biology of an individual’s tumor rather than established histopathologic and anatomic classification is an approach that promises to optimize the use of existing therapies and identify novel targets for future therapies. Currently, either a single gene or a small collection of genes is used to determine response to therapeutic agents. Gene expression analysis offers the potential to measure genome-wide gene activity, which can be used to complement currently available clinical and biochemical markers to identify discrete clinically and biologically relevant phenotypes to help characterize a disease (7, 8). As a result, clinical medicine becomes a data-intensive, quantitative genomic science, and such data can be used to uncover patterns and trends that can distinguish between biologic phenotypes to help guide existing therapies and discover new therapeutic targets (9–11).

The ability to create a predictive model that can determine which patient may derive the most benefit from a particular agent is the first step in guiding therapy. Previous studies have shown that the NCI-60 cell line panel can be used to create predictive therapeutic models (12–14); however, it remains unclear whether or not...
responses to therapeutic agents in vitro are predictive of clinical response. Therefore, similarly to the incorporation of new therapeutic agents in the clinical setting, predictive biomarkers must be assessed for their therapeutic potential in preclinical models.

In the past, mouse xenografts have been developed to screen new cancer drugs (15). Initially, athymic mice (nu/nu) and SCID mice were used to establish xenografts from human tumor cell lines to test their response to cancer drugs (16). More recently, the direct transplantation of resected human tumors into mice to study sensitivities to therapeutic agents in gastrointestinal cancers has been carried out (17, 18). However, it remains unclear whether or not responses to therapeutic agents in vivo are predictive of clinical responses; thus, the need for a clinically relevant preclinical model arises.

In this study, we have developed a predictor of sensitivity to oxaliplatin to identify patients who would derive the most benefit from oxaliplatin-based therapy along with a preclinical murine model of patient-derived colorectal cancer explants (PDCCEs) to validate our predictive signature. Together, these approaches describe a widely applicable system that facilitates the preclinical development and characterization of therapeutic agents alone and in combination to maximize response to chemotherapeutic drugs and change the current paradigm of clinical cancer therapy evaluation in colorectal cancers.

Materials and Methods

Development of in vitro oxaliplatin sensitivity predictor

An oxaliplatin sensitivity signature was generated as follows. Briefly, the concentration needed to reduce the growth of treated cells to half that of untreated cells (GI50), tumor growth inhibition (TGI), and concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared with that at the resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared with that at the time point.

Development of PDCCEs

Colon tumor tissue specimens were obtained from patients (n = 14) with histologically confirmed colorectal cancer who had undergone complete surgical resections at the Duke University Medical Center (Durham, NC) between November 15, 2007, and August 27, 2009. This investigation was approved by the Institutional Review Board of the Duke University Medical Center, and all patients provided informed consent. All specimens were sectioned, stained with hematoxylin and eosin (H&E), and examined by microscopy by a board certified pathologist. Colorectal tumors resected at the time of surgery were washed with PBS and then minced into 2- to 3-mm cubes. The samples were then placed in an enzyme medium [RPMI media containing collagenase IV (6 mg/mL), hyaluronidase (1 mg/mL), and deoxyribonuclease (0.25 mg/mL; Sigma)] and agitated at room temperature for 18 to 24 hours. After agitation, the cells were centrifuged at 2,000 rpm for 15 minutes at room temperature, washed with PBS, and passed through a 70 μm cell strainer (BD Biosciences). After washing with PBS, the cells were again centrifuged at 2,000 rpm for 15 minutes at room temperature, resuspended in serum-free RPMI/Matrigel mixture (1:1 volume), and then injected into the flanks of 4-week-old female JAX NOD.CB17-Prkdcscid-J mice.

All mouse experiments were carried out in accordance with the animal guidelines and with the approval of the Institutional Animal Care and Use Committee (IACUC) at the Duke University Medical Center.

In vivo oxaliplatin sensitivity assay of PDCCEs

To test the sensitivity of oxaliplatin in the PDCCEs, colorectal cancer cells extracted from previously generated, earlier passaged explants (passages 4–8) were injected subcutaneously into the flanks of 5 JAX NOD.CB17-Prkdcscid-J mice (four-week-old female) and measured every 2 to 3 days with a vernier caliper until the volume of the tumor (V = L × W² × 0.5 where L = longest diameter, W = shortest diameter) reached approximately 500 mm³. The mice were then randomized and treated either with oxaliplatin at a standard dose of 10 mg/kg weekly via intraperitoneal injection or with saline for 2.5 weeks with each group containing 5 mice each. Tumors were then measured at least 2 × /wk with a vernier caliper, and both tumor volume and TGI ratio [TGI% = 1 – (average tumor volume of oxaliplatin group)/average tumor volume of control group) × 100%] were calculated at each time point. At the end of 3 weeks, the tumors from both groups were harvested and placed immediately in optimal cutting temperature (OCT) compound (Sakura Finetek) and frozen on dry ice or placed in formalin overnight and paraffin embedded the next day.

Sample processing: fresh-frozen samples

Frozen PDCCE samples were sectioned at 8 μm and placed onto histologic slides. An initial section was stained with H&E (Sigma) for histologic characterization of the tissue, and the sample was subsequently...
macrodissected to ensure more than 80% tumor. Approximately 100 μg of tissue was macrodissected, and total RNA was isolated from the homogenized tissue by the RNAase Isolation Kit (Qiagen). RNA were quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies), and quality was assessed by spectrophotometric analysis on an Agilent 2100 Bioanalyzer conductor by the RNA 6000 Nano Assay Kit (Agilent Technologies).

Four μg of total RNA was used to generate gene expression data. Briefly, first strand cDNA synthesis was generated using a T7-linked oligo-dT primer followed by second strand synthesis. An in vitro transcription reaction was carried out to generate the cRNA-containing biotinylated UTP and CTP, which is subsequently chemically fragmented at 35 minutes. The fragmented, biotinylated cRNA was hybridized in MES buffer containing 0.5 mg/mL acetylated bovine serum albumin to Affymetrix GeneChip Human U133A 2.0 arrays at 45°C for 16 hours, according to the Affymetrix protocol (Affymetrix). Generated CEL files are available at GEO (GSE28691).

Formalin-fixed, paraffin-embedded samples
Tumors from the PDCCEs were fixed in formalin overnight and paraffin embedded the following day. Formalin-fixed, paraffin-embedded (FFPE) PDCCE samples were sectioned at 10 μm and placed onto histologic slides. An initial section was stained with H&E for histologic characterization of the tissue to ensure more than 80% tumor. RNA was then isolated from 8- to 10-μm FFPE sections by the RecoverAll-MagMAX Custom Kit and protocol (Applied Biosystems) with the following modifications: RNA isolation digestions were incubated at 50°C for 15 minutes and then at 80°C for 15 minutes; lysis-binding solution was reconstituted with 22 mL of 100% isopropanol (Mallinckrodt Chemicals); wash solution 1H was reconstituted with 12 mL of 100% isopropanol; and wash solution 2 was reconstituted with 44 mL of 100% ethanol (Pharmco-Aaper).

RNA was amplified according to the MessageAmp Premier protocol (Ambion). Affymetrix DNA microarray analysis was prepared according to the manufacturer’s instructions, and targets were hybridized to the Human U133A 2.0 GeneChip (Affymetrix). Generated CEL files are available at GEO (GSE28691).

Validation of oxaliplatin sensitivity signature
To validate the accuracy of the Bayesian probit regression model, first, the RMA-normalized gene expression data of the training data set (NCI-60 oxaliplatin sensitivity signature) and validation data set (PDCCE FFPE samples) were merged together using an in-house program, File Merger (22). Next, the model was used to estimate the relative probabilities and associated measures of uncertainty for each sample in the validation set as described previously (23). Samples scoring below 0.5 were considered belonging to the oxaliplatin-resistant class, whereas samples scoring above 0.5 were considered belonging to the oxaliplatin-sensitive class. The associations between the oxaliplatin sensitivity predictor and PDCCE TGIs were evaluated using Pearson correlation coefficients and 2-sided P values. Complete details are in the Supplementary Material and Methods.

Statistical analysis
Expression estimates were obtained from the Affymetrix CEL files using MAS5 and RMA (24). To check for sample outliers and batch effects, 3-dimensional (3D) principal components analysis of the global gene expression was conducted. Batch effects were normalized using the ComBat algorithm (25). Unsupervised hierarchical clustering of the human tumors and matching PDCCEs was carried out on the 20% of genes with the greatest coefficient of variation (CV). Agglomerative clusters were generated using the Pearson correlation coefficient and complete linkage. To determine whether clusters were statistically robust, the AU (approximate unbiased) and BP (bootstrap probability) values were calculated by 10,000 resamples using the R package pvclust. The associations between cell line phenotypes and genomic predictors are evaluated using Spearman correlation coefficients and 2-sided P values.

Results
Development of oxaliplatin sensitivity signature
For patients with metastatic colorectal cancer, standard-of-care first-line treatment options are either oxaliplatin or irinotecan-based therapies. However, response rates for either drug regimen range between 40% and 45% (26). As a first step in the goal to optimize therapy for colon cancer and to determine which patients would benefit from oxaliplatin-based therapy, we used expression data from NCI-60 cell lines (20) with known sensitivities to oxaliplatin to develop a binary Bayesian model to predict oxaliplatin response. Genes whose expression was most highly correlated with sensitivity to oxaliplatin were identified, and these genes were then used to develop a predictive model that could differentiate between oxaliplatin sensitivity and resistance.

First, we identified NCI-60 cell lines that were most resistant or sensitive to oxaliplatin as defined by their oxaliplatin GI50 (growth inhibition of 50%) values while also taking into consideration their TGI and LC50 values. Cell lines with a GI50 less than 0.5 μmol/L were considered sensitive, and cell lines with a GI50 more than 20 μmol/L were considered resistant (Supplementary Table S1). From these cell lines, corresponding RMA-estimated gene expression array data were used for subsequent analysis. However, one sensitive cell line, MCF-7, was observed to be a single outlier by 3D principal components analysis of global expression values of all of the NCI-60 cell lines (Supplementary Fig. S1). Because of this, MCF-7 was omitted from the training set before developing the predictor (Table 1).
To tune parameters in the model to give the largest separation between binary phenotypes, a data-driven empirical approach was taken to select the optimal number of genes to include in the predictor. The discriminatory power was evaluated using the misclassification rate under leave-one-out performance (LOOP) with an \( a \ priori \) defined threshold of \( Pr > 0.5 \). Gene set sizes of 50 to 200 were considered. Note: because the LOOP is used to optimize the multigene models, no statistical inferences are drawn from the cross-validation, and an independent validation is required to fully assess their predictive value. After consideration, the final developed model consisted of 120 probes (Supplementary Table S2) based on prediction of oxaliplatin sensitivity and LOOP (Fig. 1).

**Development of PDCCEs**

The true value of a predictor lies in its ability to predict sensitivity in an independent \( in vivo \) setting. To generate a validation set to test our oxaliplatin sensitivity signature, a

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**Table 1.** List of NCI-60 cell lines in oxaliplatin sensitivity predictor with corresponding oxaliplatin sensitivity values

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Source</th>
<th>GI(_{50}) µmol/L</th>
<th>TGI, µmol/L</th>
<th>LC(_{50}) µmol/L</th>
<th>Classification(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADR-RES</td>
<td>Ovarian</td>
<td>0.0317</td>
<td>30.4</td>
<td>100</td>
<td>Sensitive</td>
</tr>
<tr>
<td>SW-620</td>
<td>Colon</td>
<td>0.0469</td>
<td>83.75</td>
<td>100</td>
<td>Sensitive</td>
</tr>
<tr>
<td>RPMI-8226</td>
<td>Leukemia</td>
<td>0.0527</td>
<td>91.2</td>
<td>100</td>
<td>Sensitive</td>
</tr>
<tr>
<td>MOLT-4</td>
<td>Leukemia</td>
<td>0.1365</td>
<td>15.63</td>
<td>100</td>
<td>Sensitive</td>
</tr>
<tr>
<td>HT29</td>
<td>Colon</td>
<td>0.1754</td>
<td>8.24</td>
<td>72.44</td>
<td>Sensitive</td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>Leukemia</td>
<td>0.3499</td>
<td>100</td>
<td>100</td>
<td>Sensitive</td>
</tr>
<tr>
<td>HCT-116</td>
<td>Colon</td>
<td>0.3784</td>
<td>100</td>
<td>100</td>
<td>Sensitive</td>
</tr>
<tr>
<td>NCI-H460</td>
<td>Lung</td>
<td>0.4355</td>
<td>38.28</td>
<td>100</td>
<td>Sensitive</td>
</tr>
<tr>
<td>TK-10</td>
<td>Renal</td>
<td>21.18</td>
<td>73.96</td>
<td>100</td>
<td>Resistant</td>
</tr>
<tr>
<td>HOP-92</td>
<td>Lung</td>
<td>25.47</td>
<td>73.96</td>
<td>100</td>
<td>Resistant</td>
</tr>
<tr>
<td>NCI-H322M</td>
<td>Lung</td>
<td>35.32</td>
<td>100</td>
<td>100</td>
<td>Resistant</td>
</tr>
<tr>
<td>HOP-62</td>
<td>Lung</td>
<td>46.13</td>
<td>100</td>
<td>100</td>
<td>Resistant</td>
</tr>
<tr>
<td>SK-OV-3</td>
<td>Ovarian</td>
<td>66.22</td>
<td>100</td>
<td>100</td>
<td>Resistant</td>
</tr>
<tr>
<td>EKVX</td>
<td>Lung</td>
<td>88.72</td>
<td>100</td>
<td>100</td>
<td>Resistant</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Breast</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>Resistant</td>
</tr>
<tr>
<td>HS 578T</td>
<td>Breast</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

\(^a\)Cell lines with a GI\(_{50}\) less than 0.5 µmol/L were classified as sensitive; cell lines with a GI\(_{50}\) more than 20 µmol/L were classified as resistant.

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**Figure 1.** NCI-60 oxaliplatin signature Bayesian regression Heatmap and LOOP. Subset of cell lines that represent the extremes of sensitivity to oxaliplatin were identified from the NCI-60 panel. Left, expression plot for genes selected for discriminating the oxaliplatin sensitive and resistant cell lines from the NCI-60 set based on the expression levels of the 120 genes selected during the Bayesian binary regression analysis. Blue represents lowest expression and red highest. Each column represents an individual cell line and each row one of the discriminating genes in the oxaliplatin predictor. Right, from these 120 genes, phenotype discriminatory power from sample misclassification was optimized using LOOP. The accuracy of the predictor on each samples in the LOOP are displayed with 95% CI (confidence interval).
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Table 2. PDCCEs with human origin and chemotherapy history

<table>
<thead>
<tr>
<th>Tumor ID</th>
<th>Primary site</th>
<th>Metastatic site</th>
<th>Neoadjuvant chemotherapy</th>
<th>Adjuvant chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRC007</td>
<td>Colon</td>
<td>Liver</td>
<td>None</td>
<td>FOLFIRI</td>
</tr>
<tr>
<td>CRC010</td>
<td>Colon</td>
<td>Liver</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>CRC012</td>
<td>Colon</td>
<td>Liver</td>
<td>None</td>
<td>N/A</td>
</tr>
<tr>
<td>CRC025</td>
<td>Rectal</td>
<td>Lung</td>
<td>None</td>
<td>XELOX</td>
</tr>
<tr>
<td>CRC028</td>
<td>Colon</td>
<td>Liver</td>
<td>None</td>
<td>FOLFOX + bevacizumab</td>
</tr>
<tr>
<td>CRC034</td>
<td>Colon</td>
<td>Colon</td>
<td>N/A</td>
<td>XELOX</td>
</tr>
<tr>
<td>CRC039</td>
<td>Colon</td>
<td>Liver</td>
<td>FOLFOX + bevacizumab</td>
<td>None</td>
</tr>
<tr>
<td>CRC054</td>
<td>Colon</td>
<td>Lung</td>
<td>Xeloda</td>
<td>FOLFOX + bevacizumab</td>
</tr>
<tr>
<td>CRC057</td>
<td>Colon</td>
<td>Liver</td>
<td>None</td>
<td>FOLFIRI + bevacizumab</td>
</tr>
<tr>
<td>CRC059</td>
<td>Colon</td>
<td>Colon</td>
<td>N/A</td>
<td>None</td>
</tr>
<tr>
<td>CRC067</td>
<td>Colon</td>
<td>Liver</td>
<td>FOLFOX + bevacizumab</td>
<td>Xeloda + bevacizumab</td>
</tr>
<tr>
<td>CRC075</td>
<td>Colon</td>
<td>Liver</td>
<td>None</td>
<td>FOLFOX + bevacizumab</td>
</tr>
<tr>
<td>CRC102</td>
<td>Colon</td>
<td>Liver</td>
<td>XELOX + bevacizumab</td>
<td>XELIRI + bevacizumab</td>
</tr>
<tr>
<td>CRC105</td>
<td>Colon</td>
<td>Liver</td>
<td>FOLFIRI</td>
<td>None</td>
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</table>

NOTE: FOLFOX = oxaliplatin + 5-fluorouracil (5-FU)/leucovorin; XELOX = oxaliplatin + xeloda; FOLFIRI = irinotecan + 5-FU/leucovorin; XELIRI = irinotecan + xeloda.

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<td>Liver</td>
<td>None</td>
<td>None</td>
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<td>Colon</td>
<td>Liver</td>
<td>None</td>
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</tr>
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<td>Lung</td>
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<td>Colon</td>
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<td>Colon</td>
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<td>Liver</td>
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<tr>
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<td>Colon</td>
<td>Liver</td>
<td>None</td>
<td>FOLFOX + bevacizumab</td>
</tr>
<tr>
<td>CRC102</td>
<td>Colon</td>
<td>Liver</td>
<td>XELOX + bevacizumab</td>
<td>XELIRI + bevacizumab</td>
</tr>
<tr>
<td>CRC105</td>
<td>Colon</td>
<td>Liver</td>
<td>FOLFIRI</td>
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NOTE: FOLFOX = oxaliplatin + 5-fluorouracil (5-FU)/leucovorin; XELOX = oxaliplatin + xeloda; FOLFIRI = irinotecan + 5-FU/leucovorin; XELIRI = irinotecan + xeloda.

A murine model was developed by generating human metastasis-derived colorectal cancer explants (PDCCE) in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice. Following surgical resection and pathologic assessment, excess tissue to be discarded was immediately processed to generate PDCCEs as described earlier. A total of 20 resected tumors were injected into SCID mice, and 14 PDCCEs (Table 2) have been established for an uptake rate of 70% (14 of 20). Of these patients, 9 of 14 did not receive any chemotherapy before surgical resection (neoadjuvant) and 9 of 14 received chemotherapy after surgical resection (adjuvant).

Figure 2A revealed that sections stained with H&E were consistent with adenocarcinoma and that immunohistochemistry stains with carcinoembryonic antigen (CEA) were consistent with a colorectal cancer.

To determine the extent to which the underlying biology of a resected colorectal cancer metastatic tumor is maintained when explanted into a murine model, global gene expression analysis between the matched resected patient colorectal tumor and PDCCE was conducted. Initial 3D principal components analysis between the patient tumors and the corresponding explants revealed batch effects; therefore, to minimize these effects, the 2 sets were subsequently normalized using ComBat. An unsupervised hierarchical clustering was then carried out on CV-filtered expression data to generate a Heatmap of clustered gene expression (Fig. 2B), which revealed that 10 of 14 (71%) of the matched patient tumor samples and corresponding PDCCEs clustered together with greater than 95% probability under resampling (Fig. 2C), suggesting that the global biology between the matched samples are similar.

In vivo validation of the oxaliplatin predictor

To identify oxaliplatin-sensitive tumors, the 14 PDCCEs were treated with oxaliplatin as described earlier, and TGI was monitored and recorded for each PDCCE during treatment (Supplementary Table S3). Figure 3A shows photographs taken at time of PDCCE extraction to illustrate the difference between oxaliplatin-sensitive (e.g., CRC025) and oxaliplatin-resistant (e.g., CRC039) PDCCEs. The cutoff point for sensitivity was defined as the arithmetic mean of the TGI values (mean = 0.665).

From these studies, 7 PDCCEs were identified as sensitive (TGI% < mean) and 7 PDCCEs were identified as resistant (TGI% > mean).

The accuracy of the oxaliplatin sensitivity predictor was then determined using drug sensitivity data derived from the PDCCEs treated with oxaliplatin. Using the defined cutoff point for sensitivity as described earlier, the oxaliplatin sensitivity predictor was then applied to each PDCCE (Table 3) and was found to have an accuracy of 92.9% (sensitivity = 87.5%, specificity = 100%; Fig. 3B). Figure 3B shows that there is a statistically significant correlation between predicted probability of oxaliplatin sensitivity and TGI% to oxaliplatin treatment (P = 0.002).

In vivo validation of the oxaliplatin predictor in FFPE samples

Finally, although microarray analyses are best conducted using minimally degraded RNA from freshly collected cell lines or tumor tissue, the challenge of incorporating a genomic signature into the clinical setting is that fresh tissue samples can be limited and therefore constrains our ability to take these studies forward to broad validations of the initially identified predictive...
profiles. As a result, the ultimate use of these profiles in a clinical diagnostic setting may best be done on standard pathologic samples, including FFPE, and an ability to assay gene expression patterns making use of FFPE samples would clearly represent a major advance and an opportunity to validate the initial signatures.

The oxaliplatin sensitivity predictor was then applied to the FFPE-derived PDCCE tumors as described earlier to predict their sensitivity to oxaliplatin and was found to have an accuracy of 84.6% (sensitivity = 83.3%, specificity = 85.7%; Fig. 3C). One FFPE PDCCE sample, CRC037, was omitted from analysis due to not meeting...
quality control standards (Supplementary Fig. S2). The mean TGI of the 13 remaining samples was 0.643, and this number was used as the cutoff point for classification of sensitivity (Supplementary Table S4). Figure 3C shows that there is a statistically significant correlation between predicted probability of oxaliplatin sensitivity and TGI to oxaliplatin ($P = 0.025$).

Discussion

Recent advances in molecular profiling technologies such as gene expression profiling, proteomic profiling, and genetic analysis are currently being used to tailor medical care to an individual’s needs. In medical oncology, the challenges are to first, develop a method to predict which patient would derive the most benefit from these specific therapies and second, develop a preclinical model to test these therapies. This form of personalized medicine requires the ability to assay tumors for molecular features associated with responsiveness to the proposed therapy along with the development of a reliable preclinical model to test drug sensitivities.

Recent advances in the use of microarrays to assess the entire complement of the expressed genome have documented the power of this method to identify characteristics unique to an individual patient’s tumors (8, 10, 11). This information has the potential to best match existing therapeutic agents to individual patient tumors as well as to identify novel therapeutic agents that could be used to treat individual patients. In metastatic colorectal cancer, cytotoxic chemotherapy with an oxaliplatin or irinotecan-based regimen remains the backbone of therapy (26, 27). However, the use of oxaliplatin-based therapy as a standard-of-care first-line therapy results in only a 50% to 60% response rate (4, 6). Thus, the challenge is develop a predictive marker of oxaliplatin therapy.

Several mechanisms have been proposed to mediate oxaliplatin resistance, including increasing drug efflux and decreased cellular uptake, drug detoxification, apoptosis, and DNA repair (28, 29). In colorectal cancer, in vitro models have implicated genes involved in apoptosis, drug transport, and DNA repair as potential predictive biomarkers of oxaliplatin sensitivity (30–32). ERCC1 mRNA level and polymorphic variants within the ERCC1 gene have been shown to be prognostic markers of colorectal patients treated with oxaliplatin (33, 34). However, it remains unclear whether proteins involved in nucleotide excision repair such as ERCC1 can serve as predictive markers for oxaliplatin sensitivity in patients with colorectal cancer.

In this study, we have used a genomic-based assay to develop a gene signature of oxaliplatin sensitivity.
However, genes identified in the predictor did not include previously identified genes known to be involved in oxaliplatin resistance such as ERCC1 and other repair proteins, but instead consisted of genes known to be involved in oncogenesis [epidermal growth factor receptor (EGFR), insulin-like growth factor–binding protein (IGFBP7), platelet-derived growth factor C (PDGFC), and CD44]. These genes were found to have lower expression in oxaliplatin sensitive cell lines and are consistent with studies that show low levels of VEGF-A and VEGF-C in oxaliplatin-sensitive cell lines (35), and that cetuximab, which targets the EGFR pathway, can overcome oxaliplatin resistance (36).

A frequently used strategy for identifying and testing potentially effective drugs is to administer them to immunodeficient mice that have been implanted with well-characterized cancer cell lines that mimic human malignancies. However, these cell lines, having been derived and repeatedly cultured over many years, may have little resemblance to tumors growing in patients. Thus, a more reliable model is needed. Recently, the direct transplantation of resected gastrointestinal human tumors in mice and observed that the biology of the metastasis-derived colorectal cancer explants were similar to their corresponding patient tumors based on gene expression profiling. It was, however, observed that 2 of the PDCCEs (CRC067 and CRC039) did not cluster with the rest of the samples, suggesting that these tumors may have different biology, but this may be a result of these 2 patients receiving more chemotherapy [FOLFOX (oxaliplatin/5-FU) + bevacizumab] than the other 12 patients before resection of their cancer (Table 2). As a whole, we feel that these findings provide greater reassurance that the observed antitumor effects in our murine model would be similar to those observed in patients and thus provide a preclinical model to study drug sensitivities and mechanisms.

Our study has now shown both the ability to develop a genomic based predictor for oxaliplatin sensitivity and also the capability to validate the accuracy of the predictor in a preclinical model. Furthermore, we have also shown that our genomic based predictors can also be applied to FFPE samples, which are much more prevalent in the clinical setting. However, we do realize the limitations of our study. First, in using the NCI-60 cell lines as our training set, there were only 2 colon cancer cell lines that were classified as sensitive to oxaliplatin and no colon cancer cell lines that were classified as resistant to oxaliplatin. This raises the question as to whether or not a more robust colorectal cancer oxaliplatin sensitivity predictor could be generated if only colorectal cancer cell lines are used. To attempt this, we treated 25 colorectal cancer cell lines with oxaliplatin but found that there was only a 10-fold difference in oxaliplatin IC$_{50}$ values between the most sensitive and most resistant cell lines (data not shown), which is consistent with another published report (31). Furthermore, when attempting to develop an oxaliplatin sensitivity signature from these colorectal cancer cell lines, this signature was not able to predict response to oxaliplatin in the PDCCEs (data not shown). The discrepancy between the performance of the NCI-60 oxaliplatin signature and the colorectal cancer cell line signature in predicting oxaliplatin response in the PDCCE model is most likely due to the greater than 100-fold difference in oxaliplatin IC$_{50}$ values in the NCI-60 cell lines as opposed
to the 10-fold difference in the purely colorectal cancer cell lines, suggesting that to develop a reliable and accurate chemotherapy sensitivity predictor, the difference in sensitivity to a drug must be greater than 2 logs.

Second, we must also be very careful in extrapolating results from a preclinical model to potential patient outcome. In our preclinical model, the PDCCEs were only treated for 2.5 weeks, and outcome was measured by response to drug. Although, there are studies in colorectal cancer suggesting that response rate can be a surrogate for survival (37), response rate is still not an accepted end point in clinical trials. In addition, although single-agent oxaliplatin is not typically used in the initial treatment of colorectal cancer due to poor response rates (2%-10%), it must be noted that these trials were mainly small phase II trials (38–40). However, given these limitations, we must be careful with interpreting these results within a clinical setting.

Nevertheless, while this only serves as a proof-of-concept study, it is still a crucial first step in bringing a predictive biomarker to the clinic. However, before a predictive biomarker can become clinically relevant, it must undergo rigorous preclinical testing to gauge its accuracy, reliability, and reproducibility. The next crucial step is the retrospective validation of our oxaliplatin sensitivity predictor in patient samples, and this must be carried out on multiple patient samples to further validate the signature’s predictive capabilities before it can finally be prospectively tested in a clinical trial. Thus, the strength of our study lies in the power of our preclinical murine model coupled with gene expression technology to identify and test novel combinations of therapeutic agents and also to develop both predictive and prognostic biomarkers that can then be systematically brought forth into the clinical setting. More importantly, this now lays the foundation for the development and validation of future genomic based biomarkers in a preclinical model before clinical assessment.

Finally, the capacity of a genomic based signature to predict response in preclinical models begins to define a strategy for personalized medicine and also presents the ability to identify cytotoxic agents that best match individual patients with advanced colorectal cancer. Although these strategies will need to be eventually validated in clinical trials, this model is the first step in evaluating the performance of genomic signature-based selection in the individualized treatment strategy for patients with metastatic colorectal cancer.

Disclosure of Potential Conflicts of Interest
M. Datto is a consultant/on advisory board of Affymetrix. No potential conflicts of interest were disclosed by the other authors.

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