Integrated Patient-Derived Models Delineate Individualized Therapeutic Vulnerabilities of Pancreatic Cancer

Graphical Abstract

Highlights
- Only a few genetic events in pancreatic cancer are currently sensitive to therapeutic targeting
- Patient-derived models can serve as the basis for empirically defined drug sensitivities
- There are few strong responses to single agents in patient-derived models
- Drug combination screens reveal diverse therapeutic sensitivities that are patient selective

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In Brief
Pancreatic cancer is therapy recalcitrant, and new approaches to therapeutic intervention are needed. Witkiewicz et al. used a large panel of patient-derived models to address the ability to use genetics or empirically determined sensitivities to guide treatment. These findings demonstrate a weakness of the current reliance on genetic analysis and suggest that using functional approaches with patient avatars could be particularly important for navigating the diverse therapeutic sensitivity of pancreatic cancer.

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Integrated Patient-Derived Models Delineate Individualized Therapeutic Vulnerabilities of Pancreatic Cancer

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SUMMARY

Pancreatic ductal adenocarcinoma (PDAC) harbors the worst prognosis of any common solid tumor, and multiple failed clinical trials indicate therapeutic recalcitrance. Here, we use exome sequencing of patient tumors and find multiple conserved genetic alterations. However, the majority of tumors exhibit no clearly defined therapeutic target. High-throughput drug screens using patient-derived cell lines found rare examples of sensitivity to monotherapy, with most models requiring combination therapy. Using PDX models, we confirmed the effectiveness and selectivity of the identified treatment responses. Out of more than 500 single and combination drug regimens tested, no single treatment was effective for the majority of PDAC tumors, and each case had unique sensitivity profiles that could not be predicted using genetic analyses. These data indicate a shortcoming of reliance on genetic analysis to predict efficacy of currently available agents against PDAC and suggest that sensitivity profiling of patient-derived models could inform personalized therapy design for PDAC.

INTRODUCTION

A precision approach to cancer medicine is transforming the way in which cancer is treated (Aronson and Rehm, 2015; Blankin et al., 2015). Conventionally, this approach relies on the use of markers to define a treatment strategy for a given disease. There are multiple successes attributed to precision medicine, from the current paradigm for breast cancer treatment stratification based on immunohistochemical markers (e.g., estrogen receptor [ER], progesterone receptor [PR], or human epidermal growth factor receptor 2 [HER2]) to the integrated genetic analysis of lung cancer that reveals multiple targets for therapeutic intervention (e.g., ALK [anaplastic lymphoma kinase] rearrangements or EGFR [epidermal growth factor receptor] mutations) (Deluche et al., 2015; Lindeman et al., 2013). Based on these and other successes, multiple clinical trials utilizing genetic information to guide patient treatment are open.

Pancreatic ductal adenocarcinoma (PDAC) harbors a particularly poor prognosis, and even after resection, long-term survival remains poor due to the frequent recurrence as metastatic disease (Aimhanna and Philip, 2011; Kleger et al., 2014; Paulson et al., 2013; Yeo et al., 1995). Current systemic treatment of PDAC is dependent on chemotherapy, with minimal success of targeted approaches in the clinic. This therapeutic recalcitrance of PDAC is surprising, given substantial pre-clinical investigation and multiple provocative findings that would be expected to yield clinical benefit. This disconnect between preclinical testing and clinical outcomes suggests that more relevant models will be important for making significant inroads into the treatment of PDAC and that some form of patient stratification will be required to yield improved outcome. Notably, exceptional responses to therapy do occur in patients with PDAC (Garrido-Laguna et al., 2015), but these represent a very small segment of the treated population.

To date, pancreatic cancer has largely failed to benefit from the promise of precision therapy. In spite of substantial genetic analyses (Bailey et al., 2016; Collisson et al., 2011; Jones et al., 2008; Waddell et al., 2015; Witkiewicz et al., 2015), the path for the treatment of the majority of PDAC cases remains obscure.
Pancreatic cancer is driven by KRAS, which is currently considered a non-actionable target. Additionally, most pancreatic tumors harbor genetic lesions in multiple oncogenic pathways (e.g., MYC amplification) or tumor-suppressive pathways (e.g., SMAD4 loss) that make it unclear how targeting a single genetic event would yield therapeutic benefit. Thus, the nature and complexity of tumor genetics in pancreatic cancer represent the major impediments to precision treatment.

Here, we use an integrated collection of patient-derived cell lines and xenografts that recapitulate the genetics and biology of PDAC to identify and validate patient-selective therapeutic sensitivities. This approach bypasses the bottleneck of requiring genetic targets for therapeutic intervention and revealed multiple unique combinatorial approaches to target individual tumors.

RESULTS

Pipeline for Genetic and Functional Analysis
A pipeline was developed, wherein patients with resectable PDAC were consented to the collection of tumor tissue, genetic studies, and development of models (Figures 1A and 1B). Primary tumors, patient-derived cell lines, and PDX models were characterized by exome sequencing and exhibited a high level of genetic conservation with the primary (E.S.K., U.B., C.E., C. Moxom, J. Mansour, W.C., E.M.O., and A.K.W., unpublished data). Cell models were used to define therapeutic sensitivities, and selected treatments were subsequently validated in the context of the PDX originating from same primary tumor. From the genetic analysis, more than 1,000 tumor-specific genetic events were identified, many of which impacted on cancer-relevant pathways that are known to be disrupted in PDAC (Figure 1C).

Patient Models Can Inform Genetic-Driven Therapeutic Sensitivity in PDAC
A main precept of precision oncology is that genetic analysis of the tumor will inform therapeutic options. However, from mutational events identified in the 28 cases, only a handful represented targets for therapeutic intervention. Additionally, many of the potentially actionable alleles represented variants of unknown significance. In this setting, the availability of patient-derived models provided a unique opportunity to assess the impact of a given allele on therapeutic sensitivities.

Chromatin remodeling SWI/SNF (switch/sucrose non-fermentable) complex genes (e.g., ARID1A, PBRM1, and SMARCA4) are mutated in up to 20% of PDAC, and recent studies indicated that SWI/SNF deficiency results in sensitivity to EZH2 inhibition (Bilte et al., 2015; Kim et al., 2015). Two cell models exhibited genetic alterations in the ARID1A chromatin remodeling gene, and one harbored a SMARCA2 deletion (Figure 1D). The models were treated with the EZH2 inhibitors GSK126 and GSK343, and survival was determined (Figures 1E and S1). Surprisingly, the EMC7310 model that was wild-type for chromatin modifiers was the most sensitive to EZH2 inhibitors, while models harboring mutations in chromatin remodelers had intermediate sensitivities (Figures 1E and S1). This marked sensitivity could be due to the presence of high-levels of H3 K27-Me3 in the EMC7310 cell line (Figure 1F) and/or other genetic features of the model (e.g., MYC amplification or RB [retinoblastoma] loss). However, these data underscore the challenge of targeting specific genetic events occurring in complex cancer genomes and the need to functionally assess therapeutic sensitivities.

Another case exhibited mutation of the STAG2 gene on the X chromosome in the primary tumor and resultant PDX model. This event can compromise mitotic fidelity and elicit sensitivity to DNA cross-linking agents (Evers et al., 2014; Solomon et al., 2011). Consistent with STAG2 deficiency, the PDX exhibited significant nuclear pleomorphism, mitotic aberrations, and high baseline DNA damage relative to other PDX models (Figure 1G). Consequently, treatment with mitomycin C significantly restricted tumor growth in this model (Figure 1H). This response was marked by extensive DNA damage and evidence of mitotic catastrophe (Figure 1I). Thus, in rare circumstances, the genetics of an individual pancreatic tumor could inform treatment.

Patient-Derived Cell Lines Define Selective Therapeutic Vulnerabilities
The relatively low frequency of clear, genetically encoded vulnerabilities, combined with the complexity surrounding many genetic variants, makes predicting therapeutic sensitivities in PDAC difficult. In recognition of this challenge, and to define
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therapeutic vulnerabilities in the majority of tumors that do not harbor actionable genetic events, a drug sensitivity screen with a library of 305 agents (Data S1) was used against the patient-derived cell lines we developed and the established PDAC cell lines. The drug panel included compounds that are in clinical use or advanced development and target multiple aspects of tumor biology (e.g., cell cycle, apoptosis, growth factor signaling pathways). For each cell line, early and late passage cultures were evaluated and showed marked conservation of drug sensitivities (Figure 2A; Figure S2). Cell lines developed directly from the primary tumor or from PDX established from the same primary tumor clustered together, indicating preservation of therapeutic responsiveness across derivative models (Figure 2B). 59 patient-derived cell-line models were screened in total at a dose range of 100 nM–1 μM, and area under the curve (AUC) was calculated per drug per cell line (range = 0.08–4.95) (Data S1). Among the 305 drugs screened, 76 drugs with an AUC <1.5 in at least one model were identified as hits and were clustered based on Euclidian distance (Figure 2C). The data showed that similar targeted agents cluster together, indicating that there are intrinsic sensitivities to specific pathways (e.g., MEK [MAPK/ERK kinase] or EGFR) inhibition or chemotherapy. Unsupervised clustering of models and affinity propagative clustering showed that established cancer cell lines had distinct sensitivities compared to primary models (Figures 2C and S2). In general, established cell line models were significantly more sensitive to chemotherapy agents (i.e., gemcitabine, pemetrexed, and docetaxel), and one targeted agent (mubritinib) was selectively effective in these cell lines (Figure 2D). Across the panel, primary patient-derived cell lines exhibited a highly variable response to treatment with selected models markedly inhibited by targeted therapies. For example, one of the models was exceptionally sensitive to multiple MEK inhibitors (Figures 2E and 2F), while others had selective sensitivity to EGFR or tyrosine kinase inhibitors (Figure S2).

**Patient Models Define Exceptional Responses to Therapeutic Agents**

Identifying exceptional responses to therapy has become one of the critical approaches in defining targeted means to treat PDAC and other therapy-recalcitrant diseases. Although MEK inhibitors had limited single-agent antitumor activity in clinical trials conducted in unselected PDAC patients, there has been evidence for exceptional response in patients with metastatic disease (Garrido-Laguna et al., 2015). Through detailed dose-response analysis, we found that the EMC828 model was sensitive to low-dose MEK inhibition (Figure 3A). In general, resistance to MEK inhibitors is associated with compensatory activation of AKT signaling (Mirzaeva et al., 2009; Pettazzoni et al., 2015). The exceptional sensitivity to MEK inhibitor was associated with a failure to engage the upregulation of AKT, and AKT activity was actually suppressed upon treatment with MEK inhibition in this model (Figure 3B). RNA sequencing revealed that the exceptional response to MEK inhibition was associated with an enrichment of genes involved in cell adhesion, epithelial versus mesenchymal differentiation, and KRAS dependence signature (Singh et al., 2009). This signature was significantly enriched in the EMC828 model versus the less sensitive cell lines (Figure 3C). The sensitivity to MEK inhibition was associated with both inhibition of cell-cycle progression and the induction of apoptosis that translated into overall suppression of viability and clonal assays and 3D organoid cultures (Figures 3D–3F). Since MEK inhibition typically exerts a cytostatic effect, these data reinforced the concept that this select tumor would be highly sensitive to MEK inhibition. The PDX model developed from the same primary tumor was subjected to treatment with the MEK inhibitor AZD6244 (Figures 3G and 3H). Treatment resulted in marked suppression of tumor growth. Importantly, this response was selective to the model predicted to be sensitive, as AZD6244 had minimal effect on tumor growth in another PDX (i.e., EMCT2). The response to AZD6244 in vivo was characterized by suppression of ERK1/2 phosphorylation and Ki67 in the sensitive model EMC828, but not the comparator tumor EMCT2 (Figure 3). Consistent with these data, MEK treatment of xenografts resulted in the suppression of RB phosphorylation and cell-cycle-regulated proteins, as determined by reverse-phase protein arrays (RPPAs) (Figure S3). Less dramatic single-agent responses observed in cell culture with multiple targeted agents (IMD-0354, ABT-737, and CHIR125) were associated with essentially no therapeutic response in corresponding PDX models (Figure S3). These findings suggest that exceptional responses in cell culture are required in order to have therapeutic impact on PDAC tumors in vivo and recapitulate the known therapeutic recalcitrance of the disease.

**Uncovering Effective Combination Therapies by High-Throughput Screening**

Combination therapies have the potential to increase the frequency and duration of therapeutic response. However, rational design of combination therapies remains difficult, due to...
inadequate understanding of individual targets, drug interactions, and a paucity of biomarkers. Empirical approaches to uncovering combination therapy sensitivities have proved successful in defining new treatment strategies (Chen et al., 2012; Crystal et al., 2014; Vora et al., 2014). To test this approach in PDAC models, 14 clinically relevant drugs were evaluated in all pairwise combinations across 11 different cell models at three doses, yielding a total of 5,880 sensitivity measures (Figure 4A; Data S2). These analyses defined a unique pattern of sensitivity or “barcode” for each tumor model (Figures 4A and S4). In some cases, low-dose responses to combination therapy increased existing monotherapy sensitivity. For example EMC7310 was sensitive to dasatinib, but this sensitivity was augmented by multiple additional agents. However, there were many combinations that could not be predicted from single-agent sensitivities, and, surprisingly, PI3K (phosphatidylinositol 3-kinase) inhibitors (BYL719 and GDC0941) had little positive impact in combination with any other agent (Figure 4B; Data S2). To evaluate combinatorial potency, multi-drug dose-response analyses were conducted and uncovered model-selective synergistic drug interactions (Figures 4C and S4; Data File S2). These data underscore the diversity of therapeutic response and indicate that, in spite of using multiple combinations, there were no universal vulnerabilities in PDAC; however, subsets of cases were sensitive to select combination treatments.

The robust responses to select combinations did not associate with common genomic lesions (e.g., KRAS, TP53, SMAD4) present in models (data not shown), nor did they correspond to previously described predictive markers. For example, the ratio of MCL1/NOXA implicated in resistance to ABT737 could not predict response to ABT737 in combination with chemotherapy (Geserick et al., 2014). Based on these results, gene expression features were explored relative to the response to select combination treatments. Analysis of the top upregulated and downregulated genes revealed a relatively broad spectrum of deregulated biological processes. The elastic-net method was used to more stringently define genes that were positively or inversely correlated with the combinations tested. These combined approaches yielded few genes that were reproducibly associated with combinatorial sensitivities (Figure S4). Unfortunately, data from clinical studies using the tested combinations are not available to validate the performance of the potential biomarkers, which underscores the challenge associated with developing predictive markers for combination therapies.

**Tumor-Selective Trametinib Combinatorial Sensitivity Translates to Disease Control In Vivo**

Among the agents tested, the MEK inhibitor trametinib exhibited potent combination activity with several agents (i.e., dasatinib, docetaxel, and everolimus) and served as the backbone for a trial of cell-line-encoded differential combinatorial sensitivities (Figure 5A). Trametinib, in combination with dasatinib, was effective in several models, including EMC7310 and EMC29 (Figure 5B). In these models, trametinib alone induced compensatory tyrosine phosphorylation events that were blocked by dasatinib and associated with increased apoptosis (Figures 5C–5E). These effects translated into control of tumorigenic growth in the corresponding PDX that, based on cell-line data, would be predicted to be sensitive to this combination (Figure 5F). In contrast, another PDX model that would be predicted to be resistant to this combination failed to respond (Figure S5). Analysis of sensitive PDX tissues confirmed suppression of p-ERK1/2, p-Src, tyrosine phosphorylation, and the induction of apoptosis (Figure 5G).

The combination of trametinib with docetaxel was the most potent treatment identified in cell-line screening, with synergistic interaction observed in several models (Figure 6A). In xenograft assays, this combination potently suppressed tumor growth for more than 30 days in two PDX models (Figures 6B and S6). In contrast, single-agent treatments with docetaxel or trametinib were insufficient to prevent rapid disease progression (Figures 6B and S6). Analysis of PDX tissue showed that MEK inhibition generally limited proliferation and that docetaxel elicited cell death, while the combination yielded a dual response (Figure 6C). To determine whether this therapeutic sensitivity was selective, the same treatment was deployed against two PDXs models that would be predicted, based on the cell-line data, to be resistant. In these models, there was veritably no response to the treatment (Figures 6D and 6E). These data indicate that therapeutic sensitivities identified in the primary cell lines translate into responses in PDX derived from the same primary tumor. To further interrogate the idea that sensitivities are model and combination specific, the EMCs19 PDX (resistant to trametinib + docetaxel and trametinib + dasatinib combinations but sensitive...
Figure 4. Combination Treatments

(A) Representation of drug sensitivity to combination treatment. The sensitivity of all treatments was graphed, and the color bar indicates fractional survival relative to DMSO control.

(B) Heatmap clustering the AUC values of effective combinations in at least one of the cell lines. Cell lines and combinations were clustered based on Euclidean distance.

(C) Representative dose-response analysis of the trametinib and dasatinib combination in a model that yielded synergistic toxicity. Value shown in the heatmap is percent survival minus 100 (i.e., 0 indicates no effect on survival; −100 indicates complete lack of survival).
to trametinib + everolimus in cell-line screens) was treated with trametinib and everolimus (Figure 6F). This combination yielded measurable control of the PDX tumor growth (Figure 6F). Together, these data suggest that cell models could be used to infer drug sensitivities to direct the combinatorial treatments.

**Model-Guided Chemotherapy Regimens**

In the analysis of the combination drug screening data, it was apparent that select targeted agents cooperate with chemotherapy. As shown, the BCL2 inhibitor ABT737 and the checkpoint kinase inhibitor AZD7762 resulted in model-specific augmentation of the response to either gemcitabine or docetaxel (Figures 7A–7C). ABT737 was particularly potent when combined with gemcitabine or docetaxel in the EMC29 and EMC3226 lines, respectively (Figures 7B and 7C). In contrast, AZD7762 impacted on a separate collection of cell lines. These data suggest underlying cellular vulnerabilities to apoptotic or cell-cycle checkpoint inhibitors. The response to
**Figure A**

(A) EMC28 and EMC1928 tumor cell lines were treated with multiple concentrations of doxorubicin, trametinib, or a combination of both for 72 hours. The relative tumor volumes were measured over time. 

(B) A time-course analysis of tumor growth for different treatments: trametinib, doxorubicin, and a combination of both. The graph shows a significant reduction in tumor volume for the combination treatment compared to single agents. 

(C) Immunohistochemical staining for pERK, Ki67, and CC3 in tumors treated with different combinations of drugs. 

(D) EMC1229 tumor cell line treated with different concentrations of doxorubicin and trametinib. The relative tumor volume is plotted over time, showing a statistically significant difference (P<0.05) between control and drug-treated groups. 

(E) EMC519 tumor cell line treated similarly to (D). 

(F) EMC519 tumor cell line treated with everolimus and trametinib. The relative tumor volume shows a significant decrease (P<0.01) compared to control groups.
the combination of ABT737 and gemcitabine was synergistic in two cell models (Figure 7D) and, in PDX, translated to stable disease that was associated with a modest effect of therapy on proliferation but a robust induction of apoptosis (Figure 7E). Similarly, the combination of AZD7762 with gemcitabine yielded disease control in the PDX model, and the response was accompanied by extensive DNA damage (Figure 7F). Together, these data similarly illustrate that combinations with chemotherapy can be effective but must be directed.

**DISCUSSION**

PDAC has a particularly poor prognosis, and even with new targeted therapies and chemotherapy, the survival is poor. Here, we show that patient-derived models can be developed and used to investigate therapeutic sensitivities determined by genetic features of the disease and to identify empirical therapeutic vulnerabilities. These data reveal several key points that are of prime relevance to pancreatic cancer and tumor biology in general.

**The Challenges of Using Genetic Analysis to Inform Treatment in PDAC**

Precision oncology is dependent on the existence of known vulnerabilities encoded by high-potency genetic events and drugs capable of exploiting these vulnerabilities. At present, the repertoire of actionable genetic events in PDAC is limited. Rare BRAF V600E mutations are identified in PDAC and could represent the majority of models. Specific combinations were effective across the majority of models. Specific combinations were effective across several models, indicating that, by potentially screening more models, therapeutic sensitivity clades of PDAC will emerge. In the pharmacological screens performed in this study, MEK inhibition, coupled with MTOR, docetaxel, or tyrosine kinase inhibitors, was effective in $\approx$30% of models tested. Resistance to MEK inhibitors occurs through several mechanisms, including upregulation of oncogenic bypass signaling pathways such as AKT, tyrosine kinase, or MTOR (mammalian target of rapamycin) signaling. In the clinic, the MEK and MTOR inhibitors (e.g., NCT02583542) are being tested. An intriguing finding from the drug screen was sensitivity of a subset of models to combined MEK and docetaxel inhibition. This combination has been observed to synergistically enhance apoptosis and inhibit tumor growth in human xenograft tumor models (Balko et al., 2012; McDaid et al., 2005) and is currently being tested in a phase III study in patients with KRAS-mutated, advanced non-small-cell lung adenocarcinoma (Jänne et al., 2016). Interestingly, in the models tested herein, there was limited sensitivity imparted through the combination of gemcitabine and MEK inhibition. This potentially explains why the combination of MEK inhibitor and gemcitabine tested in the clinic did not show improved clinical success, most probably, due to the diverse therapeutic sensitivity of individual PDAC cases, suggesting that, with an unselected patient population, it will be veritably impossible to demonstrate clinical benefit. Additionally, very few models exhibited an exceptional response to single agents across the breadth of a library encompassing 305 agents. We could identify only one tumor that was particularly sensitive to MEK inhibition and another model that was sensitive to EGFR and tyrosine kinase inhibitors.

In contrast to the limited activity of single agents, combination screens yielded responses at low-dose concentrations in the majority of models. Specific combinations were effective across several models, indicating that, by potentially screening more models, therapeutic sensitivity classes of PDAC will emerge. In the pharmacological screens performed in this study, MEK inhibition, coupled with MTOR, docetaxel, or tyrosine kinase inhibitors, was effective in $\approx$30% of models tested. Resistance to MEK inhibitors occurs through several mechanisms, including upregulation of oncogenic bypass signaling pathways such as AKT, tyrosine kinase, or MTOR (mammalian target of rapamycin) signaling. In the clinic, the MEK and MTOR inhibitors (e.g., NCT02583542) are being tested. An intriguing finding from the drug screen was sensitivity of a subset of models to combined MEK and docetaxel inhibition. This combination has been observed to synergistically enhance apoptosis and inhibit tumor growth in human xenograft tumor models (Balko et al., 2012; McDaid et al., 2005) and is currently being tested in a phase III study in patients with KRAS-mutated, advanced non-small-cell lung adenocarcinoma (Jänne et al., 2016). Interestingly, in the models tested herein, there was limited sensitivity imparted through the combination of gemcitabine and MEK inhibition. This potentially explains why the combination of MEK inhibitor and gemcitabine tested in the clinic did not show improved clinical success, most probably, due to the diverse therapeutic sensitivity of individual PDAC cases, suggesting that, with an unselected patient population, it will be veritably impossible to demonstrate clinical benefit. Additionally, very few models exhibited an exceptional response to single agents across the breadth of a library encompassing 305 agents. We could identify only one tumor that was particularly sensitive to MEK inhibition and another model that was sensitive to EGFR and tyrosine kinase inhibitors.

**Empirical Definition of Therapeutic Sensitivities and Clinical Relevance**

Cell lines offer the advantage of the ability to conduct high-throughput approaches to interrogate many therapeutic agents. A large number of failed clinical trials have demonstrated the difficulty in treating PDAC. Based on the data herein, the paucity of clinical success is, most probably, due to the diverse therapeutic sensitivity of individual PDAC cases, suggesting that, with an unselected patient population, it will be veritably impossible to demonstrate clinical benefit. Additionally, very few models exhibited an exceptional response to single agents across the breadth of a library encompassing 305 agents. We could identify only one tumor that was particularly sensitive to MEK inhibition and another model that was sensitive to EGFR and tyrosine kinase inhibitors.

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**Figure 6. Patient-Selective Targeting of Trametinib Combinations**

(A) Dose-response analysis of combination treatment, indicating the synergistic interaction of trametinib and docetaxel in two models. Value shown in the heatmap is percent survival minus 100 (i.e., 0 indicates no effect on survival; −100 indicates complete lack of survival).

(B) The PDX was randomized to treatment with single-agent trametinib (n = 4) or docetaxel (n = 4), or the combination (n = 5). Tumor volume was measured, and the mean and SEM are shown. Mice were sacrificed when the single-agent mice became moribund or at the end of 33 days of treatment.

(C) Representative immunohistochemistry and quantification. The average and SD are shown from at least three tumors. Statistical analysis was by unpaired t test. \(p < 0.01; \{p < 0.001, \text{relative to the vehicle control. Scale bars, 100 \mu m.}

(D and E) Two independent PDX models that were predicted to be resistant to trametinib + docetaxel failed to respond to treatment. \(p > 0.05\). Error bars indicate SEM.

(F) Dose-response analysis of combination treatment, indicating the synergistic interaction of trametinib and everolimus from a model resistant to trametinib and docetaxel. Response of the PDX to trametinib and everolimus treatment (n = 5 per arm). Tumor volume was measured, and the mean and SEM is shown.
Figure 7. Differential Cooperation of Apoptotic and Cell-Cycle Checkpoint Inhibitors with Chemotherapy

(A) Heatmap showing the AUC of single and combination treatments with ABT737 or AZD7762 and chemotherapy. Cell lines and drugs were clustered based on Euclidian distance.

(B) Relative survival of the indicated cell lines treated with increasing concentrations (25, 84, and 250 nM) of gemcitabine or docetaxel, as measured in triplicate from two independent experiments.

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efficacy over gemcitabine alone (Infante et al., 2014). Another promising strategy that emerged from this study involves using CHK or BCL2 inhibitors as agents that drive enhanced sensitivity to chemotherapy. Together, the data suggest that the majority of PDAC tumors have intrinsic therapeutic sensitivities, but the challenge is to prospectively identify effective treatment.

Patient-Derived Model-Based Approach to Precision Medicine

This study supports a path for guiding patient treatment based on the integration of genetic and empirically determined sensitivities of the patient’s tumor (Figure S7). In reference to defined genetic susceptibilities, the models provide a means to interrogate the voracity of specific drug targets. Parallel unbiased screening enables the discovery of sensitivities that could be exploited in the clinic. The model-guided treatment must be optimized, allowing for the generation of data in a time frame compatible with clinical decision making and appropriate validation. In the present study, the majority of models were developed, cell lines were drug screened, and select hits were validated in PDX models within a 10- to 12-month window (Figure S7). This chronology would allow time to inform frontline therapy for recurrent disease for most patients who were surgically resected and treated with a standard of care where the median time to recurrence is approximately 14 months (Saif, 2013). Although most models were generated from surgically resected specimens, two of the models (EMC3226 and EMC62) were established from primary tumor biopsies, indicating that this approach could be used with only a limited amount of tumor tissue available. In the context of inoperable pancreatic cancer, application of data from a cell-line screen without in vivo validation in PDX would permit the generation of sensitivity data in the time frame compatible with treatment. We acknowledge that model-guided treatment is also not without significant logistical hurdles, including the availability of drugs for patient treatment, clinically relevant time frames, patient-performance status, toxicity of combination regimens, and quality metrics related to model development and therapeutic response evaluation. Additionally, it will be very important to monitor ex vivo genetic and phenotypic divergence with passage and try to understand the features of tumor heterogeneity that could undermine the efficacy of using models to direct treatment. As shown here, drug sensitivities remained stable with passage in cell culture and, importantly, were confirmed in PDX models, suggesting that the dominant genetic drivers and related therapeutic sensitivities are conserved.

In spite of these challenges, progressively more effort is going into the development of patient-derived models for guidance of disease treatment (Aparicio et al., 2015; Boj et al., 2015; Crystal et al., 2014; van de Wetering et al., 2015). Several ongoing trials use PDX models to direct a limited repertoire of agents (e.g., NCT02312245, NCT02720796, and ERCAVATAR2015). Given the experience here, PDAC cell lines would provide the opportunity to rapidly interrogate a larger portfolio of combinations that could be used to guide patient care and provide a novel approach to precision medicine. Validation of this approach would require the establishment of challenging multi-arm or N-of-1 clinical trials. However, considering the dire outcome for PDAC patients and the long-lasting difficulty in developing effective treatments, this non-canonical approach might be particularly impactful in pancreatic cancer.

EXPERIMENTAL PROCEDURES

Cell Culture and PDX

Tumor tissue acquisition was performed under an Institutional Review Board (IRB) protocol approved at the University of Texas Southwestern Medical Center and Ochsner Clinic. Informed consent was obtained from all patients. PDX models were developed and utilized in accordance with Institutional Animal Care and Use Committee approved protocols at UT Southwestern Medical Center. Primary cell models were established and cultured on collagen-coated tissue culture plates in supplemented KSF media. Cells were passaged by trypsinization and used at early passage (p < 5) and late passage (p > 20) for the analysis of drug sensitivity. Established cell lines were from the ATCC and cultured using published methodology. The detailed description of these models will be published elsewhere, but the description of the derivation approach is provided in the Supplemental Experimental Procedures.

Drug Treatments

Cell models were subjected to drug screening with libraries, combination treatments, and single agents, as summarized in the Supplemental Experimental Procedures. The treatment of PDX models was in accordance with institutional animal care and use committee (IACUC) protocols at the University of Texas Southwestern Medical Center and is summarized in the Supplemental Experimental Procedures.

Immunohistochemistry and Model Analysis

Immunohistochemistry was performed on a DAKO stainer using conditions as described in the Supplemental Experimental Procedures. Immunoblotting, immunofluorescence, RPPA analysis, flow cytometry, and other methods were performed using standard procedures. The specific features of the experimentation are provided in the Supplemental Experimental Procedures. RNA sequencing was performed on an Illumina instrument with paired-end reads.

(C) Relative survival of the indicated cell lines treated with increasing concentrations (25, 84, and 250 nM) of the indicated drug combinations as measured in triplicate from two independent experiments.
(D) Summary of the cooperation between ABT737 and gemcitabine in a sensitive model. Dose-response analysis of combination treatments, indicating selective synergistic interaction in the EMC29 model and related cooperative index. Value shown in the heatmap is percent survival minus 100 (% indicates no effect on survival; –100 indicates complete lack of survival).
(E) The PDX was randomized to treatment with vehicle control (n = 3) or gemcitabine and ABT737 (n = 8), and tumor volume was determined. The average tumor volume and SEM are plotted. Mice were sacrificed when the control arm became moribund. Statistical significance was determined by ANOVA. Representative staining and quantitation of the indicated markers by immunohistochemistry. The average and SD are shown. Scale bars, 100 µm. Statistical analysis was by unpaired t test. *p < 0.05; **p < 0.01.
(F) A PDX model sensitive to AZD7762 and gemcitabine was treated singly or in combination (n = 5 per arm). Tumor volume was measured as a function of time, and statistical significance was determined by ANOVA. Representative γH2AX staining is shown (scale bars, 50 µm). Error bars indicate SD in (B) and (C) and SEM in (F).
The accession number for the expression values reported in this paper is GEO: GSE84023.

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and two data files and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.07.023.


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