



Actionable intelligence provided by pancreatic cancer genomic landscape: are targets for curative therapy on the map?

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Comment on: Bailey P, Chang DK, Nones K, *et al.* Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature* 2016;531:47-52.

Submitted Jun 28, 2016. Accepted for publication Jul 04, 2016.

doi: 10.21037/tcr.2016.08.07

View this article at: <http://dx.doi.org/10.21037/tcr.2016.08.07>

Pancreatic Ductal Adenocarcinoma (PDAC) is a devastating disease with severely debilitating comorbidities and dismal prognosis. In 2015, the worldwide incidence of new cases was estimated to be 367,000, and approximately 359,000 were expected to die from this disease (1). In the Western world, it is the 4th leading cause of cancer deaths (1). Overall survival for newly diagnosed PDAC is usually measured in months and while modest improvements have been made in 1-year survival, now estimated at 20% overall; 5-year survival remains less than 10%. Two problems that contribute to this dismal survival are (1) that PDAC is most commonly diagnosed when it is beyond cure by surgical resection and (2) there is a general lack of effective systemic chemotherapy (1).

In a recent tour-de-force integrated genomic, epigenomic and transcriptomic analysis of 456 annotated PDAC tumors, an international group of investigators, reported the most comprehensive and up-to-date PDAC molecular landscape (2) providing actionable intelligence that should spur multiple new approaches to better understand the epidemiologic development of PDAC, potentially lead to earlier diagnostic assays, certainly provide the basis for more rational development of therapeutic agents and approaches and define more precise criteria for stratifying and evaluating clinical trials. Primarily treatment naive PDAC tumors, obtained by the Australian Pancreatic Cancer Genome Initiative as part of the International Cancer Genome Consortium, were analyzed by a combination of whole-genome and deep-exome sequencing, gene copy number analysis, RNA expression profiling and methylation analysis, revealing 23,578 high confidence coding

mutations and another 21,208 high confidence genome rearrangements, from which 32 recurrently mutated genes were identified that aggregated into 10 molecular pathways (*Table 1*). Based on unsupervised RNA expression analysis, these tumors were divided into 4 subtypes termed (I) squamous; (II) pancreatic progenitor; (III) immunogenic and (IV) aberrantly differentiated endocrine exocrine (ADEX). These four subtypes could be differentiated by transcription networks representing distinct biological processes. The genomically determined subtypes had different histopathologic characteristics and they were associated with prognostic differences with the squamous subtype showing the worst median survival, 13.3 months, compared to 23.7 months for pancreatic progenitor, 25.6 months for ADEX and 30 months for immunogenic ($P=0.0302$) (2).

The Squamous Subtype, so named because, although it is a subtype of adenocarcinoma, its upregulated gene pattern is composed of networks involved in squamous differentiation, including Myc activation, TP63 Δ N transcriptional targets, inflammation, hypoxia, metabolic reprogramming, autophagy and also activated EGF signaling, all of which are commonly noted in other squamous subtypes of breast, bladder, lung and head and neck cancers. Many of the genes overexpressed in the squamous subtype were found to be downregulated in the other three subtypes. Of particular interest was the demonstration in the squamous subtype of epigenetic regulation characterized by hypomethylation and downregulation of genes that govern pancreatic endodermal determination (2).

The Pancreatic Progenitor Subtype was primarily defined by transcription factors that determine early

Table 1 Significant recurrent pathway and genetic alterations in pancreatic ductal adenocarcinoma

Altered pathways	Altered genes	% tumors showing abnormality
KRAS activation	<i>KRAS, MAPK4</i>	92%
Cell cycle, G1/S checkpoint disruption	<i>TP53, CDKN2A, TP53BP2</i>	78%
TGF beta signaling	<i>SMAD3, SMAD4, TGF, TGPBR1, TGFB2, ACVR1B and ACVR2A</i>	47%
Chromatin modification	<i>KDM6A, SETD2, ASCOM complex members MLL2 and MLL3</i>	24%
DNA repair	<i>BRCA1, BRCA2, ATM, PALB2, SATF2</i>	17%*
RNA processing	<i>SF3B1, U2AF1, RBM10</i>	16%
SWI/SNF complex	<i>ARID1A, ARID1B, PBRM1 and SMARCA4</i>	14%
WNT signaling	<i>RNF43, MAPK2, TLE4</i>	5%
ROBO SLIT axonal guidance	<i>ROBO1, ROBO2, SLIT2, MYCBP2</i>	5%
NOTCH signaling	<i>JAG1, NF2, BCORL9, FBXWT</i>	–

Data for *Table 1* abstracted from [Bailey, Chang, Nones (2)]. Percent abnormality ROBO SLIT from [Biankin Waddell, Kassahn (3)]. *17% tumor with abnormal DNA repair genes noted to be 5% Germline, 12% Somatic.

embryonic pancreatic ductal exocrine and endocrine fate and are linked to maturity onset diabetes of the young. This class also notable for increased expression of genes regulating fatty acid oxidation, steroid hormone metabolism and O-linked mucin glycosylation (2).

The ADEX Subtype was identified by transcription networks that characterize later, more mature stages of exocrine and endocrine pancreatic differentiation as well as those associated with maturity onset diabetes. Tumors of the ADEX subtype were also noted to have distinct methylation patterns (2).

The Immunogenic Subtype was reported as being similar to the pancreatic progenitor subtype but uniquely defined by expression of genes characterizing a significant immune cell infiltrate including both B and T cells. Also noted in some of the immunogenic subtype tumors was upregulation of the CTLA4 and PD1 immune suppression pathways which were associated with the poorest survival (2).

This monumental achievement is a testimonial to big team science, to the value of international cooperation in science and a tribute to the multitude of funding agencies that supported this initiative. Since all the specimens were collected in Australia, the possibility exists that the findings and/or their percentage distributions may reflect unique epidemiologic characteristics or exposures of that population. Thus these observations may be modified or confirmed as the cohort is expanded, especially to include populations where differences may exist in ethnic composition or in some of the major risk factors for PDAC including tobacco use, obesity and diabetes (1). While the current results need to be extended to other population

groups, this comprehensive genomic landscape presents a vast trove of data that provides us with unprecedented insights into the operational processes of this tumor. We are now challenged to most effectively use this information in our efforts to defeat PDAC.

Obviously, a major challenge in the war against pancreatic cancer is the identification and optimal application of effective therapies. Extensive studies have been conducted to develop therapeutic approaches to control PDAC, based on anti-metabolic and cytotoxic agents, alone and in combination, by targeted-precision therapy and more recently, by immunotherapy. While many of these approaches were empiric, we now have the unique opportunity to pursue therapeutic strategies based on a more rational and comprehensive understanding of PDAC molecular processes. Before addressing these approaches it is worth noting that since the four different molecular based subtypes appear to have different prognosis, it would initially be useful to identify a genomic signature for each, to prospectively evaluate survival and to stratify patients for clinical trials.

Two antimetabolites, 5 Fluorouracil (5 FU) and Gemcitabine, which as single agents have modest palliative effects, but negligible survival benefits measured in months, serve as the basis for empirically designed combination chemotherapy regimens with statistically significant but still marginally meaningful clinical benefits. Combining Gemcitabine with the Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor, Erlotinib has been reported to improve median survival from 5.91 months with Gemcitabine alone to 6.24 months for the combination (4). Combining 5 FU in the FOLFIRINOX regimen (Folinic

Acid, 5 FU, Irinotecan and Oxaloplatin), has been reported to improve median overall survival from 4.4 months for 5FU alone to 24 months with the combination (5). More recent studies suggest that Gemcitabine in combination with nanoparticle albumin bound paclitaxel may increase median overall survival to 8.5 months (6). In addition, studies with Gemcitabine plus Capecitabine have shown improved overall response rates with combination 19.1 *vs.* 12.4% but only slight increase in median overall survival, 7.1 months for combination *vs.* 6.2 months for Gemcitabine alone (7). In another study, patients with good Karnofsky performance scores, receiving the Gemcitabine-Capecitabine combination showed median overall survival 10.1 *vs.* 7.4 months compared to Gemcitabine alone (8). While each of these combinations provide a statistically significant increase in survival, the overall prognosis remains grim. Moreover, given the recent demonstration, in malignancies such as lung cancer, of the apparent impact of genomic subtypes on survival, it must be considered that molecular based stratification might significantly alter survival statistics.

Beyond empiric approaches to single agents and their combinations, rational-precision approaches have targeted some of the major mutated oncogene and tumor suppressor drivers in PDAC, including K-RAS, TP53, CDKN2A and SMAD4 (1,5,9,10). However, despite their high frequency of mutation in PDAC (*Table 1*), and numerous attempts at drug development, no agents have been identified to interfere with these oncogenes and tumor suppressors in a clinically useful manner, causing them to be generally regarded as “undruggable” targets. Nonetheless, the expanded array of recurrent pathways and genetic aberrations identified in this new study (*Table 1*) should provide an abundance of novel targets for development of strategic agents (2).

We have previously noted, tumors frequently have multiple growth promoting pathways that interact at numerous levels (11). As a result, attempts to block any single pathway with a specific inhibitor may be bypassed by extensive networks of crosstalk, feedback loops and collateral signaling through alternative pathways. Such pathways have been proposed to contribute to the overall PDAC resistance to targeted therapies (1). Moreover, the newly reported multitude of pathways indicated by RNA expression analysis (2) provides strong support that this phenomenon of collateral escape routes is operational in these PDAC tumors and is likely to contribute to their therapeutic resistance. At the same time, documentation of

these genomic aberrations and expression pathways should provide the basis for rational development of strategic approaches to simultaneously blockade multiple pathways.

Because of the multiple collateral growth, metabolic and resistance pathways, new agents may be identified that will interfere with a single pathway but not stop tumor growth, whereas rational development of combinations to block collateral pathways may be required to halt progression of PDAC tumors. For example, simultaneous targeting of mutant K-RAS along with multiple parallel and downstream pathways such as Insulin Growth Factor 1, MAPK, AKT, Hepatocyte Growth Factor Receptor, NOTCH and Hypoxia Inducible Factor 1 α may provide effective strategies to alter metabolism, and disrupt alternate bypass and resistance pathways (1,6,12). Similar approaches have been effectively applied to sensitize B-RAF mutant tumors with intrinsic or acquired resistance to the tyrosine kinase inhibitor Vemurafenib (13-15). This approach has the potential to develop agents that interfere with targets at the molecular level but whose ability to halt tumor growth may not be evident until collateral pathways are simultaneously inhibited. Thus, this approach may require simultaneous testing in patients of combinations that have been shown to work together in model systems but whose efficacy cannot be shown in traditional single agent clinical trials.

The differences in the chromatin methylation and gene expression patterns identified in the PDAC subtypes suggest differences in epigenetic regulation, that may be subtype specific, contribute to overall resistance, and may be exploitable from a therapeutic viewpoint. This possibility indicates the importance of (I) more fully characterizing the epigenetic landmarks of PDAC; (II) identifying the PDAC expression pathways regulated by epigenetic control; (III) screening epigenetic modifiers (16) to identify those that control growth promoting, chemotherapy resistance and other metabolic pathways; (IV) identify epigenetic signatures to predict subtypes and patients likely to respond and (V) conduct clinical trials of epigenetic modifiers alone and in combination with cytotoxic or targeted agents in patients stratified according to genomic and epigenomic signatures (17,18). Recent studies have in fact shown antitumor efficacy in model PDAC systems for epigenetic targeted agents, including the histone deacetylase (HDAC) inhibitor SAHA, and JQ1, an inhibitor of bromodomain and extraterminal (BET) protein binding to chromatin (19).

Immunotherapy provides an important alternative to chemotherapy and radiation since it attempts to harness a different set of antitumor mechanisms including

antibody and cell mediated cytotoxicity (20). In addition, immunotherapy usually has a different set of host toxicities. Recent studies of immunotherapy for solid tumors have focused on the development of strategies to recruit host immune cells to recognize and destroy tumor cells bearing tumor specific antigens and also to use anti checkpoint blockade agents to reduce tumor immunosuppressive effects (21,22). These agents have been shown to be successful in immune active tumors, characterized by infiltration of increased CD8 + T Cells but not in immune quiescent tumors which lack these cells (23). Melanoma, non small cell lung cancer, colorectal cancer and renal cell cancer fall into the category of immune active tumors showing promising responses to immune checkpoint blockade inhibitors (24). Unfortunately, pancreatic cancer appears to be an immune quiescent tumor, limiting its response to such agents (23). However, the new genomic classification of PDAC (2) suggests that the immunogenic subtype may be an appropriate group on which to focus these therapies since it already has an immune cell infiltrate. Moreover, since this tumor subtype is reported to show worse survival when it overexpresses CTLA-4 and/or PD-1 (2), patients with these expression patterns may constitute a unique PDAC subgroup for clinical trials with anti-CTLA4, anti PD-1 and anti PD-L1 antibodies. In terms of focusing this immune approach on the immunogenic subtype or PDAC, it is interesting to note that the anti PD-1 agent Pembrolizumab has shown significant antitumor activity in colorectal cancer (CRC) with mismatch—repair deficiency, but not in CRC that is mismatch—repair proficient (24). This study has important relevance for several reasons. First, it clearly shows that highly specific targeted therapies are likely to have their efficacy manifest in subgroups of tumors with selected markers and pathways. Second, these studies showed that efficacy occurred in mismatch repair deficient tumors which contain a mean of 1,782 somatic mutations compared to 73 in the mismatch repair proficient tumors, providing the basis for generation of multiple immunogenic neo-antigens. Third, the sensitive tumors showed an abundance of CB8+ lymphocyte infiltrates supporting the proposal that immune therapies are most likely to work in tumors already showing a major lymphocytic infiltrate.

Vaccine based strategies are being implemented to increase many immune effector cells to improve tumor infiltration and focus their immunotoxic effects on tumor destruction. Mesothelin has emerged as a tumor antigen with early promise for PDAC targeted vaccines (22,25). The repertoire of mutated oncogenes and tumor suppressors now identified

in PDAC (*Table 1*) should provide an expanded array of neo antigens, unique to tumors and not present in normal tissues, to serve as targets for vaccine development [(2) Bailey *et al.* 2016]. Since many of these mutated genes are present at early, premalignant stages of pancreatic tumor development and before immunosuppressive mechanisms develop (26), it is possible, that once shown to be effective, these vaccines may even be useful to treat PDAC precursors on a preventive basis.

In conclusion, it is worth noting that since single modality therapies such as chemotherapy, radiation therapy and immunotherapy have shown minimal efficacy, it will most likely require combinations of these approaches to improve responsiveness. Focused on its newly defined genomic landscape, we now have a host of actionable intelligence to understand the command and control mechanisms of PDAC, the pathways it uses to escape our therapeutic interventions and the basis to begin its systematic eradication.

Acknowledgments

Funding: NAB was supported in part by NIH, National Cancer Institute GI SPORE P50 CA150964.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Xiaotian Sun (Department of Internal Medicine, Clinic of August First Film Studio, Beijing, China).

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2016.08.07>). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Berger NA. Actionable intelligence provided by pancreatic cancer genomic landscape: are targets for curative therapy on the map? *Transl Cancer Res* 2016;5(S2):S243-S247. doi: 10.21037/tcr.2016.08.07