Can we move towards personalised pancreatic cancer therapy?

Expert Rev. Gastroenterol. Hepatol. 8(4), 335–338 (2014)

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Pancreatic ductal adenocarcinoma remains an unyielding adversary, with a 5-year survival of 5%, a figure unchanged in 50 years. Characterised by marked genetic heterogeneity, recent genomic sequencing efforts demonstrate that with the exclusion of a few known mutations, most mutations occur at a prevalence of <5%. Current systemic chemotherapeutics, when used in an all-comer approach, are at best modestly effective, yet are associated with responses in small groups of undefined patients. Defining these subgroups and targeting them with the appropriate therapy in a personalized or stratified approach holds the promise of improved outcomes for this disease.

Pancreatic cancer is genetically heterogeneous

Until recently, genomic analysis of cancer specimens was costly and time consuming. With technological advancement, personalizing cancer treatment according to key genetic aberrations has become possible. Unfortunately, pancreatic ductal adenocarcinoma (PDAC) remains an unyielding adversary, with a 5-year survival of 5%, a figure unchanged in 50 years [1]. Eighty percent will present with unresectable tumors due to invasive or metastatic disease, with few therapeutic options, and with the most effective therapies being the most burdensome. For the 20% who undergo operative resection, despite improved overall survival, most face almost inevitable recurrence. Current systemic chemotherapeutics, when used in an all-comer approach, are at best modestly effective, yet are associated with responses in a small groups of undefined patients. Consequently, there is an urgent need to optimize patient selection for current therapy and identify novel therapeutic strategies.

As we better understand the molecular pathology of cancer, we are discovering substantial complexity, defining it as a composite of multiple diseases rather than the few previously defined morphologically [2]. Large-scale initiatives to map the underlying genomic aberrations by consortia including The Cancer Genome Atlas [3] and the International Cancer Genome Consortium [4] have unveiled significant heterogeneity, even in morphologically indistinguishable cancers. This new understanding poses challenges, but presents opportunities for therapeutic advancement in PDAC.

Accumulating evidence suggests that PDAC is characterized by marked genetic heterogeneity, with recent genomic sequencing efforts demonstrating that with the exclusion of known mutations (*KRAS*, *TP53*, *SMAD4*, *CDKN2A*), most mutations occur at a prevalence of <5% [5,6]. However, these can be grouped into 14 core signaling pathways [6,7]. Whole exome sequence and copy number variation data for 100 PDACs revealed activating mutations of *KRAS* in >90%, and although loss of function events of tumor suppressors predominate, a variety of secondary gain of function events occur in genes that are known drivers of carcinogenesis in other cancer types [5]. This suggests that although *KRAS* is vital early in PDAC evolution, a second gain of function event (e.g., *CSF1R* mutation, amplification of *HER2*, *MET*) may be required for progression, which has substantial implications for therapy as often these are targets of existing pharmacological

Keywords: actionable phenotypes • genomics • pancreatic cancer • sequencing • stratified medicine • translational research

 10.1586/17474124.2014.893820 ISSN 1747-4124

inhibitors used in other cancer types and other diseases (repurposing). Furthermore, identification of mutations in genes involving axon guidance that interact with known mechanisms important in cancer (WNT and MET signaling) may modify therapeutic responsiveness [5]. Such complexity suggests that innovative analytical approaches using large datasets are likely required to understand this complexity and to uncover further, as yet unapparent mechanisms.

Phenotypes of therapeutic responsiveness in PDAC

Improved selection of patients based on predictive biomarkers is the modern paradigm of clinical oncology. Numerous prognostic and predictive biomarkers for PDAC have been explored, yet few have been independently validated [8]. Cohort acquisition in non-uniform ways, limited availability of clinical trial material and a lack of focus on biomarker development are major obstacles hampering advances for molecular phenotype-guided therapeutic strategies.

Despite inherent heterogeneity, it is possible to map putative actionable molecular genotypes/phenotypes in PDAC using numerous tissue- and genomic-based assays, many of which are currently targetable by available therapies. Until recently, gemcitabine was the standard of care for advanced PDAC. Putative responsiveness biomarkers include hENT1, hCNT1/3 and dCK [9], with supportive preclinical evidence; however, clinical utility is not yet established. Retrospective analysis of large Phase III randomized-controlled trials (RTOG 9704, ESPAC 1/3) support a potential role for hENT1 as an adjuvant gemcitabine responsiveness biomarker [10]; however, a recent Phase II randomized-controlled trials stratified by hENT1 expression, comparing gemcitabine and CO-101 in metastatic PDAC failed to demonstrate utility [11]. Such discrepancies may relate to methodological differences or to the fact that hENT1 predictive utility varies in the metastatic versus adjuvant setting.

A hallmark of cancers with BRCA2-PALB2-Fanconi anemia DNA repair pathway defects is hypersensitivity to DNA damaging agents including mitomycin C, platinum or poly (ADP-ribose) polymerase inhibitors [12,13]. Anecdotal evidence for the efficacy of these agents in PDAC harboring these defects is mounting. FOLFIRINOX efficacy, demonstrated by the PRODIGE4/ACCORD 11 study [14] (overall median survival 11.1 vs 6.8 months; $p < 0.001$), suggests activity in patient subgroups. This combination is frequently associated with significant toxicity, and therefore, predicting responders could improve patient management. In addition, some evidence suggests that oxaliplatin is the predominant active agent, implying that definition of tumors harboring DNA damage repair and replication defects may remove the combinatorial requirement, and thus, limit toxicity. Platinum agents and poly (ADP-ribose) polymerase inhibitors are currently being trialed in hereditary breast and ovarian cancer [13], with participants recruited based on germline Fanconi anemia gene variants. A clinical trial assessing the PARP inhibitor Rucaparib in PDAC has recently been opened (CO-338-023 (NCT02042378). Additionally, a recent analysis of 4,942,984 mutations from 7042 cancers [15] revealed four mutational signatures in PDAC: older age, *BRCA*-mediated DNA damage repair defects, DNA mismatch repair deficiency and a signature associated with the APOBEC family of cytidine deaminases. Therefore, mutational signatures associated with specific defects in DNA maintenance may be clinically useful in defining platinum responsive phenotypes and serve to better select patients in the future.

In the NCIC CTG PA.3 study, the addition of erlotinib to gemcitabine demonstrated a modest but significant survival advantage in advanced PDAC [16], but survival was doubled in patients who developed a significant skin rash [16]. A retrospective molecular analysis did not demonstrate either *KRAS* status or *EGFR* gene copy number as predictive biomarkers [17], however, the assay used likely underestimated *KRAS* mutation prevalence, which merits reassessment using contemporary sequencing technologies.

HER2 amplification is a biomarker of trastuzumab responsiveness in breast and gastric cancer. While preclinical PDAC data support anti-HER2 therapy efficacy in *HER2* amplified tumors, trials of trastuzumab have been disappointing [18], possibly as a consequence of non-standardized assays overestimating *HER2* amplification prevalence. Assessment of 469 PDACs using national reference laboratory standardized assays observed only a 2% *HER2* amplification rate, and suggested that *HER2* amplified PDAC has a particular clinical phenotype characterized by a lack of liver and preponderance of lung and brain metastases [19].

PDAC is characterized by an intense stromal component, and is thought to impair drug diffusion and promote resistance. Secreted protein acidic and rich in cysteine (SPARC) regulates extracellular matrix modeling and deposition. High stromal with low epithelial SPARC expression is a poor prognostic factor in PDAC [20], and was developed as a therapeutic target for *nab*-paclitaxel (Abraxane®) to enable 'stromal depletion'. A Phase I/II study of gemcitabine plus *nab*-paclitaxel also suggested that SPARC expression is potentially predictive [21]. The subsequent Phase III Metastatic Pancreatic Adenocarcinoma Clinical Trial study comparing gemcitabine versus gemcitabine plus Abraxane demonstrated a survival benefit with the addition of Abraxane in metastatic PDAC (median overall survival 8.5 vs 6.7 months; $p < 0.001$) [22]; however, the predictive value of SPARC expression requires further evaluation.

In order to test the above actionable molecular genotypes/phenotypes, an individualized molecular pancreatic cancer therapy trial has been opened (ACTRN12612000777897). This Phase II study screens the tumors of patients for three predefined actionable genotypes/phenotypes (*HER2* amplification for Trastuzumab therapy, *BRCA1/2* and *PALB2* mutations for Mitomycin C and *KRAS* wild type for Erlotinib treatment) randomizing patients between stratified treatment or standard therapy in first-line metastatic disease. It also has an adaptive feature enabling additional arms beyond the initial three subgroups to be added.

Keys to advancing personalized therapy for PDAC

There are significant challenges in the implementation of a personalized therapeutic strategy for cancer. Issues often overlooked, yet critical for genomic analysis include biospecimen acquisition, methodical processing according to downstream requirements and careful tumor tissue assessment. Biopsy assessment is indispensible for the development of personalized PDAC therapies including baseline pretreatment biopsies (primary and/or metastatic), and if possible, serial biopsies obtained at progression to assess mechanisms of acquired resistance. Owing to the low cellularity of PDAC, development of robust mutation detection and analyses methodologies are imperative for efficient and accurate detection of actionable mutations in clinical diagnostic samples [5].

How best should the research and healthcare community protect patient privacy, but simultaneously share information to advance medical research remains a contentious issue. As discussed earlier, PDAC heterogeneity and the identification of patients with rare molecular subtypes (<5%) requires networking, large consortia and data sharing. The Global Alliance for Genomics and Health, a partnership of over 150 academic and research institutions, funding bodies and industry partners – aims to integrate data that are currently isolated, and thus, tackle cancer heterogeneity [23].

Preclinical genetically engineered mouse models have contributed substantially to the understanding of the molecular pathology of PDAC. Based on mutant $Kras^{G12D}$ targeted to the pancreas using Cre-Lox technology [24], multiple models harboring established and novel oncogenic drivers in PDAC aim to recapitulate their human counterparts, and can be generated to mimic actionable genotypes of PDAC for efficacy assessment of genotype predicted therapy. Increasing numbers of '–omically' characterized patient-derived xenografts and cell lines are accumulating. These models have been successfully used as mouse 'avatars' of human disease, valuable for testing a stratified genotype-guided approach and to inform therapeutic selection in the clinic [25]. These patient-derived xenografts have been successfully used to inform clinical decision-making. Tumor biopsies are engrafted into immunocompromised mice and when established, treated with a number of therapeutics. In a recent report, robust preclinical personalized xenograft activity was used to select DNA-damaging agent used in a patient with gemcitabine refractory metastatic PDAC leading to prolonged (>3 years) clinical response [26]. Global genomic sequencing revealed biallelic loss of *PALB2* offering a mechanistic explanation and a new phenotype of PDAC therapeutic responsiveness. This group have extended utility of personalized xenograft models as a platform to determine molecular drug response mechanisms in other advanced solid cancer types [27]. Since these extensively characterized xenografts are from individual patients, renewable from initial stocks, multiple treatments can be examined, providing the opportunity to model, test and reevaluate proposed personalized medicine strategies currently intractable in the clinic [28].

Advancement of stratified therapeutic approaches for a heterogeneous disease such as PDAC requires integration of discovery efforts, preclinical assessment in appropriate model systems and innovative clinical trials. Accurate delineation of responsive phenotypes, robust assays and approaches targeting small patient subgroups are necessary if improved outcomes are to be realized using a personalized or stratified therapeutic strategy.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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