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Review

Pancreatic cancer genomics: where can the science take us?

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The incidence of pancreatic ductal adenocarcinoma (PDAC) is steadily increasing and the annual death-to-incidence ratio approaches one. This is a figure that has not changed for several decades. Surgery remains the only chance of cure; however, only less than 20% of patients are amenable to operative resection. Despite successful surgical resection, the majority of the patients still succumb to recurrent metastatic disease. Therefore, there is an urgent need to develop novel therapeutic strategies and to better select patients for current therapies. In this review, we will discuss current management by highlighting the landmark clinical trials that have shaped current care. We will then discuss the challenges of therapeutic development using the current randomized-controlled trial paradigm when confronted with the molecular heterogeneity of PDAC. Finally, we will discuss strategies that may help to shape the management of PDAC in the near future.

Conflict of interest

The authors declare no conflicts of interest.

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer related death in western societies, despite being five times less common than cancers of the breast and bowel. While many cancers are decreasing in incidence as the result of risk factor modification and the advent of screening programs, the incidence of PDAC continues to rise and outcomes are not improving. As a consequence, it is projected to be the second leading cause of cancer death by 2030 (1). The reasons are likely multi-factorial; however, most patients present late with locally advanced or metastatic disease and are not amenable to surgical resection. Despite surgery being the only chance of cure, even for the 20% that are able to undergo potentially curative resection, the prospect of long-term survival is still grim as the majority develop recurrence and succumbs to metastatic disease. Some of the key challenges in treating PDAC are outlined in Table 1 below.

Landmark PDAC clinical trials

Numerous clinical trials have been performed and despite a high attrition rate, particularly for more contemporary targeted agents, several randomized-controlled trials (RCT) have shown modest improvements in overall survival. In advanced PDAC, palliative gemcitabine was superior to 5 flurouracil in overall survival (5.65 *vs* 4.41 months, $p = 0.0025$) and progression-free survival (2.33 *vs* 0.92 months, $p = 0.0002$). Gemcitabine was also associated with significant clinical benefit by alleviating disease-related symptoms (2). This landmark trial in 1997 made gemcitabine the standard of care in advanced PDAC in most countries. It also made gemcitabine the backbone in most RCTs assessing experimental therapeutic regimens. A decade later, the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) in collaboration with Australasian Gastrointestinal Trials Group (AGITG) reported the PA.03 trial, which demonstrated a statistically significant, albeit modest survival benefit of gemcitabine with the tyrosine kinase inhibitor erlotinib compared with gemcitabine alone ($n = 569$, 6.24 *vs* 5.91 months, $p = 0.023$) (3). Erlotinib has remained the only targeted therapy that has demonstrated efficacy in a Phase III trial but has a Quality Adjusted Life Year (QALY) of greater than US $600,000. In 2009, the UK NCRI reported that the combination of gemcitabine and capecitabine was associated with a significantly better progression-free survival [$n = 533$, hazard ratio (HR) = 0.78, 95% confidence interval (CI) = 0.66–0.93] and a trend toward better overall survival (HR = 0.86, 95% CI = 0.72–1.02, $p = 0.08$). The authors then combined two additional trials assessing the same regimen involving 935 patients to demonstrate that GEM-CAP was associated with a significant survival benefit over gemcitabine alone (HR = 0.86, 95% CI = 0.75–0.98, $p = 0.02$) (4). Recently, two trials have significantly changed the management of advanced PDAC. In 2011, the French PRODIGE4/ACCORD 11 trial demonstrated the superiority of a four drug combination FOLFIRINOX (oxaliplatin, irinotecan, flurouracil and leucovorin) over gemcitabine alone on overall survival ($n = 342$, 11.1 *vs* 6.8 months, $p < 0.001$) (5). However, this regimen was associated with significant toxicity and degradation in quality of life. In 2013, the MPACT trial showed that gemcitabine plus albumin-bound paclitaxel (nab-paclitaxel) was superior to gemcitabine alone with a median overall survival of 8.5 *vs* 6.7 months ($n = 861$, $p < 0.001$) with a more tolerable toxicity profile than FOLFIRINOX (6). Numerous other trials assessing various chemotherapeutic and targeted combinations did not demonstrate efficacy (7, 8).

Challenges moving forward

Clinicians treating PDAC are now presented with several options, a situation that was not the case just a few years ago; however, we do not have an accurate method to predict the optimal treatment for an individual patient. We still select therapies based on performance status, generic adverse effect profile and drug availability. In addition, the advent of FOLFIRINOX, associated with the best overall survival in advanced PDAC RCTs (Fig. 1), has led to major challenges in therapeutic development. First, can we use FOLFIRINOX as the chemotherapy backbone for future trials? Unfortunately, because of the significant adverse effect profile, combination with other chemotherapeutics or targeted therapies is extremely difficult if not impossible. Second, do we have to compare every new approach to FOLFIRINOX? This situation has potentially stalled therapeutic development, particularly for first line therapy. This reduces opportunities for therapeutic development to either maintenance therapy or the second-line setting, substantially reducing the numbers of patients who have the opportunity to receive novel therapies as part of clinical trials. Although gemcitabine + nab-paclitaxel appears to have less toxicity and there is potential for adding an additional targeted agents, many such approaches are also likely to be limited by toxicity. Clearly, the ability to predict which patients will not respond to either of these strategies is vital for advancing therapeutic development.

Molecular heterogeneity and therapeutic responsiveness

Until recently, subtypes of PDAC on the whole were indistinguishable histologically and characterized by early dissemination, cachexia and an associated poor performance status, culminating in rapid disease progression and death. Like triple negative breast cancer, genomic studies are showing significant inter- and intra-tumoral heterogeneity with likely significant impact on prognosis and therapeutic responsiveness. This diversity may explain the overall slow progress of therapeutic development and the high attrition rate of unselected RCTs despite strong pre-clinical evidence. The responsive subtype is likely to fall below the detection threshold of the conventional unselected RCT design despite subgroups of responders for each regimen.

Table 1. Challenges and key questions in the development of therapeutic strategy for PDAC

1	Many patients are cachectic at diagnosis: is this reversible?
2	Operable, borderline resectable, inoperable locally advanced pancreatic cancer, metastatic disease: do they represent the same biological entity?
3	Patient's progress quickly and there is limited opportunities compared to other cancers for 'window of opportunity' studies or second line studies.
4	What are the mechanisms of intrinsic and acquired drug resistance?
5	Can we combine targeted agents or targeted agents and cytotoxics to overcome resistance mechanisms?
6	How do we deliver the drug (or radiotherapy) better so that we can hit the tumor harder while sparing the patient
7	With the more traditional cytotoxics, can we select out the exceptional e.g. gemcitabine, platinum responders?
8	Small molecular sub groups: is it possible to recruit to trials of small sub groups in a rare cancer in a timely fashion?

PDAC, pancreatic ductal adenocarcinoma.

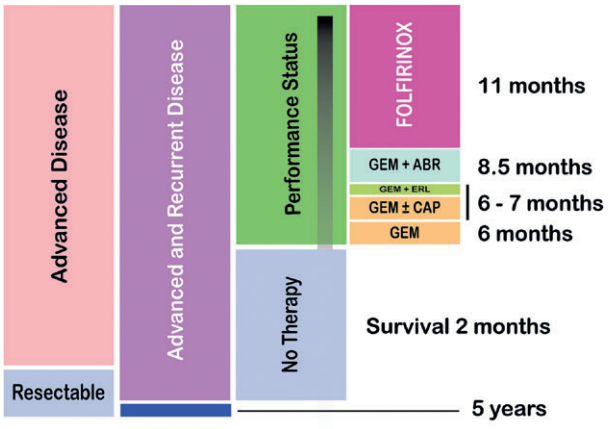

Fig. 1. Current treatment strategies in pancreatic cancer.

Pancreatic cancer genomes

With the rapid advancement next-generation sequencing (NGS), large cancer sequencing initiatives such as International Cancer Genome Consortium (ICGC) (9) and The Cancer Genome Atlas (TCGA) are revealing that histologically indistinguishable cancers are characterized by a high level of molecular heterogeneity. In PDAC, early work from Johns Hopkins using capillary sequencing and single nucleotide polymorphism (SNP) arrays to define mutations in protein coding regions and copy number alterations of 24 cell lines and xenografts derived from primary and metastatic PDACs (10). This pioneering study revealed the heterogeneity of PDAC, where apart from four highly prevalent mutations that have been known for many years (*KRAS*, *TP53*, *CDKN2A* and *SMAD4*), a long tail of infrequently mutated genes at a mutation prevalence of less than 5% predominated. Despite the large number of mutations, the authors were able to classify these genomic aberrations into the 12 core signaling pathways. The authors concluded that therapeutic development by targeting these altered pathways and processes could be more appropriate than targeting individual gene components.

In 2010, NGS in a handful of PDACs defined structural rearrangements (11) and explored clonal relationships between metastases (12). The authors found significant inter-patient heterogeneity in the pattern of genomic instability, with different prevalence (3–65 per patient) and type of rearrangements. A frequent distinctive pattern of structural rearrangement called 'fold back inversions' was identified, with breakage-fusion-bridge cycles as the most likely mechanism. This form of rearrangement was found to be an early event in the development of PDAC and may play a role in the amplification of cancer genes. Analysis of clonal relationships among metastases in the same patient showed that genomic instability persisted after dissemination, resulting in ongoing evolution in metastases.

More recently in 2012, a collaborative effort as part of the ICGC, whole exome sequencing and copy-number analysis of a cohort of 142 early primary PDACs were reported (13). As PDAC is characterized by an intense desmoplastic stroma with an average stromal content of 70%, the authors developed methods to perform full face frozen sectioning and macro-dissection to improve epithelial cellularity and as a consequence the sensitivity of mutation detection. The cellularity of the tumors was estimated by deep amplicon-based sequencing of exons 2 and 3 of *KRAS* at an average depth of 1000× and SNP array using a novel algorithm (qpure) to inform the sensitivity of mutation detection for each sample (14).

Detailed analysis of a cohort of 99 patients with an epithelial cellularity of >20% identified 2016 genes with non-silent mutations and 1628 copy-number variations. There were on average 26 mutations per patient, which ranged from 1 to 116. Significant Mutated Gene analysis identified 16 genes which included those known to be important in PDAC: *KRAS*, *TP53*, *CDKN2A*, *SMAD4*, *MLL3*, *TGFBR2*, *ARID1A*, *SF3B1*, and identified novel genes involved in chromatin modification (*EPC1* and *ARID2*) and DNA damage repair (*ATM*). In addition, the authors also identified five novel genes that were not previously reported: *ZIM2*, *MAP2K4*, *NALCN*, *SLC16A4* and *MAGEA6*. This study once again demonstrated the significant molecular heterogeneity of PDAC and further affirmed that apart from *KRAS*, *TP53*, *CDKN2A* and *SMAD4*, the majority of mutated genes had a prevalence of less than 2%. This poses significant challenges for differentiating 'driver' from 'passenger' mutations using existing computational analysis. To overcome this, the authors triangulated data from two independent sleeping beauty transposon mutagenesis screens in a *Kras* transgenic model of PDAC (15, 16), and *in vitro* short hairpin RNA (shRNA) of cancer genes in 102 cell

lines (17). Data from these screens confirmed the functional importance of the four most frequently mutated genes and attributed potential functional importance in others. To further the functional analysis, the authors performed a series of pathway analyses and identified mechanisms known to be important in PDAC (18), such as G1/S checkpoint machinery, apoptosis, regulation of angiogenesis and TGF-B signaling, and novel pathways including chromatin modification and axon-guidance, adding two additional core-signaling pathways to the original 12 (19). This was the first study in such scale to have used clinical samples as input material and demonstrated the feasibility of clinical sequencing.

With the dramatic increase in the number of sequenced cancer genomes through the ICGC and TCGA, a large collaborative effort led by the Sanger Institute explored mutational signatures in the genomes of numerous cancer types. Such mutational signatures can inform the underlying mutagenic processes during cancer development and progression (20). This collaborative effort analyzed 4,938,362 mutations from 7042 cancers, across 30 different cancer types and extracted over 20 distinct mutational signatures. Based on 20 whole genome sequencing and 100 whole exome sequencing of PDACs, four mutational signatures were identified. They included older age, APOBEC, *BRCA*-mediated DNA maintenance deficiency, and DNA mismatch repair deficiency.

Clinical application

The recent past has seen a dramatic increase in our understanding of the genomic events that characterize cancer; however, the clinical implementation of this knowledge to inform decision making is a major challenge. Improving patient selection based on predictive biomarkers of therapeutic responsiveness underpins modern medical oncology practice. Although yet to be proven in PDAC, emerging molecular phenotype-guided therapeutic approaches are showing early promise (21). Numerous biomarkers predictive of therapeutic responsiveness have been proposed, but as yet few have been independently validated.

Biomarkers explored to date

Gemcitabine responsiveness

Until recently, gemcitabine was the standard of care for advanced PDAC (2). Candidate biomarkers of gemcitabine responsiveness include nucleoside transporters such as hENT1, hCNT1/3 and kinases involved in gemcitabine metabolism such as deoxycytadine kinase (dCK). Although there is clear pre-clinical rationale, results from clinical trials have been mixed. Small cohort studies and retrospective analysis of large Phase III RCTs, such as RTOG 9704 and ESPAC 1 and 3, have supported a predictive role for adjuvant gemcitabine responsiveness (22, 23). However, it was not supported by a retrospective analysis of the CONKO-001 trial (24). In addition, a recent Phase II RCT stratified by hENT1 expression, comparing gemcitabine *vs* CO-101 (lipophilic gemcitabine) in metastatic PDAC, also failed to demonstrate utility (25). Its predictive utility was also not demonstrated in the retrospective analysis of the Phase III AIO-PK0104 trial (26). The discrepancy may relate to methodological difference in hENT1 immunohistochemistry, antibodies used, and/or perhaps the prominence of hENT1 as a predictive biomarker varies in the advanced compared with the adjuvant setting.

DNA-damaging agent responsiveness

Pancreatic cancer cells with defects in the *BRCA2-PALB2*-Fanconi DNA repair pathway provide an opportunity for an individualized therapeutic approach. Platinum-based therapies in PDAC have mixed results in clinical trials of unselected patients (27), although a recent meta-analysis of clinical trials (28) and efficacy of the FOLFIRINOX regimen (5) suggest activity in subgroups of patients. The efficacy of FOLFIRINOX on PDAC was demonstrated by the PRODIGE4/ACCORD 11 study (5) (overall median survival 11.1 *vs* 6.8 months, $p < 0.001$). FOLFIRINOX, a combination of 5-fluorouracil, irinotecan and oxaliplatin, can be associated with significant drug-related toxicity. Therefore, predicting responders prior to therapy could significantly improve overall outcomes.

Putative biomarkers of DNA-damaging agent responsiveness have not been well characterized owing to complex interactions between a large number of genes involved in the DNA maintenance machinery. Platinum agents and Poly ADP ribose polymerase inhibitors are currently being assessed in the treatment of hereditary breast and ovarian cancers (29, 30) with recruitment based on germline defects or variants in Fanconi anemia genes. There is a significant opportunity for improvement if we can define a reliable biomarker of platinum therapeutic responsiveness.

Abraxane responsiveness

Secreted protein acid and rich in cysteine (SPARC, also known as osteonectin) regulates extracellular matrix modeling and deposition and may act as a tumor suppressor or an oncogenic driver depending in differential expression in epithelial and stromal components in addition to different cancer types (31). High stromal and low epithelial expression of SPARC is a poor prognostic biomarker in PDAC (32, 33), and because of its role as an albumin 'adhesive', it was developed as a therapeutic target for nab-paclitaxel (Abraxane®) to enable 'stromal depletion' and in turn to improve drug delivery. A Phase I/II study of gemcitabine plus nab-paclitaxel demonstrated that SPARC expression in the stroma, but not in the epithelium, co-segregated with improved survival (34). This lead to a Phase III MPACT (Metastatic Pancreatic Adenocarcinoma Clinical Trial) RCT comparing gemcitabine *vs* gemcitabine plus Abraxane®. It demonstrated the addition of Abraxane® conferred significant survival benefit over gemcitabine alone (median overall

survival 8.5 *vs* 6.7 months; $p < 0.001$) (6); however, a retrospective analysis of SPARC expression in this cohort showed no association with Abraxane® response (35). Furthermore, analysis of the CONKO-001 RCT data revealed that stromal and cytoplasmic SPARC expression is prognostic markers in PDAC patients treated with adjuvant gemcitabine after resection with curative intention but not in patients that received no adjuvant therapy (36).

Erlotinib responsiveness

In the NCIC CTG PA.3 study, the combination of erlotinib and gemcitabine demonstrated a small but statistically significant survival advantage (3). However, for patients who experienced a skin rash, the median survival was doubled (3). A retrospective molecular analysis of the trial did not show any association of *KRAS* mutation status or epidermal growth factor receptor gene copy number with as erlotinib responsiveness (37). There were several limitations to the study as tissue was available in only 32%, and a smaller proportion had *KRAS* and *EGFR* results available (26% and 15%, respectively). The proportion of *KRAS* wild-type tumors (21%) was also higher than expected compared to contemporary studies using using next-generation technology of 7% (13). Despite Food and Drug Administration approval, integration of erlotinib into clinical practice has been slow, owing to the cost, and the mild but noteworthy side effects (primarily rash and diarrhea). Defining the subgroup of patients that are likely to respond may increase the use of erlotinib or other anti-EGFR agents in the future. A recent study of unresectable locally advanced and metastatic pancreatic cancer patients revealed that those with an elevated baseline level of amphiregulin obtained a greater progression free survival and overall survival benefit with erlotinib, suggests some potential value for this biomarker in pancreatic cancer. (38)

Biomarker-driven therapeutic development strategies

PDAC is relatively uncommon, and there appears to be a significant number of molecular phenotypes that are at low prevalence, such that running individual trials to investigate each separately is slow, expensive and inefficient. The concept of umbrella trials is gaining momentum, with studies such as FOCUS 4 (39) and lung-MAP recruiting well. These studies run effectively as a group of parallel Phase II studies (often randomized), such that patients are profiled using a molecular test, then recruited into one of the arms, depending on their molecular profile – or an 'all comer arm' if the test does not define that they would likely benefit from a targeted approach. In addition, the randomized Phase II designs provide an ability to test both the prognostic and predictive benefit of biomarkers in one study. STAMPEDE in prostate cancer was one of the first to have a multi-arm approach (although not determined by molecular profile) and this paved way for future studies (40). In more sophisticated designs, it is possible to move directly from Phase II to III in arms which show promise, and equally appealing to drop arms which are underperforming. As arms are not being directly compared to each other, they are also appealing for the pharmaceutical industry and provide an excellent platform which can respond to scientific advances in a timely and efficient way.

Emerging clinical trials

Individualized Molecular Pancreatic Cancer Therapy Trial

In order to test some of the actionable molecular phenotypes present in PDAC, an Individualized Molecular Pancreatic Cancer Therapy (IMPaCT) trial has been established (Australian New Zealand Clinical Trial Registry ID: ACTRN12612000777897). The IMPaCT Trial is a Phase II prospective trial that randomized patients to standard therapy or stratified treatment in a first line metastatic setting after patients have been screened for actionable molecular phenotypes. The initial three subgroups of interest are HER2 positive (*HER2/neu* amplified), DNA-damaging agent responsive (*BRCA1/2* or *PALB2* mutations), and anti-EGFR responsive (*KRAS* wild-type or *KRAS* codon 13 mutation). This trial was designed to be 'adaptive', enabling additional arms to be included as emerging and novel actionable molecular phenotypes become better defined.

In addition, other pilot studies are emerging: a prospective Phase II trial of molecular profiling to guide neoadjuvant therapy in patients with operable disease using immunohistochemistry at the University of Wisconsin (NCT01726582), and a Phase II trial of chemotherapy selection based on therapeutic targets (*K-RAS, EGFR, ERCC-1,* thymidylate synthase) in advanced disease (Madrid NCT01726582) that refines the chemotherapy regimen but does not include molecularly-targeted agents. A further Phase II trial currently recruiting (NCT02042378) aims to test the PARP inhibitor Rucaparib in PDAC patients with germline or deleterious somatic *BRCA1* or *BRCA2* mutations.

Another novel application of early genomic technology is its role in the development of non-invasive methods for the detection of tumors and to monitor therapeutic responsiveness by detecting circulating tumor DNA (ctDNA) or circulating tumor cells (CTC's). One recent large study reported by Bettegowda et al., used digital polymerase chain reaction technologies to evaluate the feasibility of ctDNA in the detection of tumors in 640 patients with various cancer types (41). The authors found that ctDNA was detectable in more than 75% of advanced cancers including PDAC, ovarian, colorectal, bladder, gastro-oesophageal, breast, melanoma, hepatocellular, and head and neck cancers. Furthermore, the authors were able to detect ctDNA in 73%, 57%, 48% and 50% of the localized colorectal, gastro-oesophageal, pancreatic and breast cancers, respectively. Using a separate cohort of 206 patients with colorectal cancer, authors were able to detect *KRAS* mutation using ctDNA with a sensitivity of 87.2% and specificity of 99.2%. For proof of concept, the authors also demonstrated the EGFR

inhibition resistance mechanisms in 24 patients by detecting mutations in genes in the mitogen-activated protein kinase pathway using ctDNA.

Summary

We are faced with enormous challenges in the management of PDAC; however, as discussed in this review, we are also presented with a unique opportunity if we can harness these applications for clinical use. Both the molecular phenotype-driven therapeutic strategy and each molecular phenotype of therapeutic responsiveness need to be prospectively tested. There are significant foreseeable challenges in doing so as it is paradigm changing, and there are several inherent challenges in the approach, such as small responsive phenotypes in a relatively low incidence cancer where many patients are too cachectic to receive therapy. However, we believe that PDAC is one of the most appropriate diseases to be testing such a personalized cancer treatment approach, owing to its significant molecular heterogeneity, and the fact that overall survival rate has changed so little over the decades, despite research efforts to date.

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