Pancreatic Cancer: From Genome Discovery to *PRECISION-Panc*

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Introduction

Pancreatic Cancer (PC) is the third leading cause of cancer mortality in the West, after recently overtaking breast cancer.[1] There has been little improvement in the overall outcomes for PC in the last 50 years. Recently there have been small incremental improvements in overall survival in the metastatic setting based on the PRODIGE-4 / ACCORD-11 and MPACT clinical trials[2,3]; and in the adjuvant setting with Gemcitabine-Capecitabine and FOLFIRINOX combinations[4,5]. Neoadjuvant therapy is gaining popularity in the borderline resectable and locally advanced settings with some exceptional responses leading to long-term survival in undefined subgroups.[6-9] The incremental improvements are likely due to the effects of these small undefined subgroups of responders with the majority of patients gaining minimal or no benefit from systemic chemotherapy such as Gemcitabine combinations and the FOLFIRINOX regimen.[10,11] Therefore, a selection biomarker driven precision oncology approach is urgently needed in PC.

In this editorial we will summarise some of the effort of translating clinically relevant genome discovery into the real world and highlight a few candidate therapeutic segments in development.

The mutational landscape of PC

Recently, there has been a vast expansion in the understanding of the molecular pathology in many cancer types including PC. The ‘omics’ revolution is rapidly generating large-scale datasets including whole genome and whole transcriptome sequencing from a large number of tumours. The Australian Pancreatic Cancer Genome Initiative (APGI, (http://www.pancreaticcancer.net.au)) led the contribution

of ~450 PC to the International Cancer Genome Consortium (ICGC, (https://icgc.org)),[12-14] making significant headway in the understanding of the mutational landscape of the disease, as well as clinically relevant molecular subtypes. Whole exome sequencing and copy number alteration analyses of 142 resected PCs reaffirmed the mutational profile originally described in a landmark study describing 12 core signalling pathways and gained further insights into the molecular pathology of PC.[13,15] The most frequent mutations in PC include *KRAS, TP53, SMAD4 and CDKN2A,* all of which do not confer effective therapeutic options at present.[10,13] Yet, this study from the APGI, revealed therapeutic vulnerabilities in the disease, including mutations in genes such as *BRCA 1 / 2, PALB2, ARID1A* and *ATM*.[13] Similar findings have also been described by large scale studies from Canada and The Cancer Genome Atlas initiative, resulting in ~1000 published genomes of PC to date[16,17]. However, the frequency of actionable mutations in PC remains low and highlights the need to for identify alternative biomarkers of therapeutic response beyond point mutation. Waddell *et al.* performed analysis of the first 100 whole genomes sequenced (WGS) from APGI, and revealed mutational signatures and structural variation patterns associated with defects in DNA damage response (DDR).[12] Four patterns of structural variation were identified and can be associated with underlying mutational processes. The unstable pattern (over 200 structural variations evenly distributed throughout the genome) overlaps with high COSMIC BRCA point mutational signature and mutations in genes involved in DNA maintenance such as *BRCA1, BRCA2* and *PALB2*.[12] Up to 24% of patients with PC exhibit one or more of these putative biomarkers of DDR deficiency, demonstrating the potential responsive subgroup to DNA damaging agents (e.g. platinum) may be higher than previously thought.[12]

Molecular Subtypes of PC

There has been growing interest in molecular 'subtyping' beyond the genome to gain insights into the molecular processes driving tumour progression and therapeutic response. The APGI performed the first large scale multi-omic analyses in PC, using transcriptomes, methylome, mutational data and histopathology from 451 patients and identified clinically relevant differential gene programs that cluster tumours into unique molecular subtypes.[14] The study identified four potentially clinically relevant molecular subtypes of PC termed Squamous, Pancreatic Progenitor, Aberrantly Differentiated Endocrine Exocrine (ADEX) and Immunogenic. The Immunogenic and ADEX subtypes were found to be subclasses of the Pancreatic subtype. The Squamous subtype was so-called as it is enriched for gene programs that have previously been described in squamous cancers of the head and neck, breast, bladder and lung[18]. It is characterised by epigenetic changes such as hypermethylation leading to loss of expression of key genes in endodermal differentiation such as *HNF1B, GATA6, PDX1* and *MNX1*.[14] This subtype is associated with inflammatory pathways, immune evasion and epithelial-to-mesenchymal transition.[14] These molecular features of aggressive disease, in turn, are associated with poor survival.[14]

In contrast, the pancreatic progenitor subtype described by *Bailey et al.* is enriched for genes involved in pancreatic differentiation.[14] The Immunogenic subtype was enriched for immune cell infiltrates including cytotoxic CD8+ T Cells, regulatory T cells and B cells. However, this also included increased expression of CTLA-4 and PD-1 immune checkpoints demonstrating immune-modulation that can potentially be targeted by immune checkpoint inhibition.[14]

Molecular therapeutic targets in PC

Our increasing understanding of the molecular pathology of PC has revealed therapeutic vulnerabilities that extend beyond simple somatic mutations. As discussed above, up to a quarter of patients with PC may harbour genomic evidence of DDR deficiency. This extends beyond germline mutations in *BRCA 1* and *2*, which is currently the selection biomarker for the vast majority of DDR targeted treatment strategies and clinical trials. Using surrogate readouts of DDR deficiency, such as mutational signatures and structural variation patterns, would likely benefit more patients than just point mutations alone. Platinum based chemotherapy regimens have demonstrated benefit in all stages of PC[3,19,20], and response is likely to be related to homologous recombination deficiency (HRD), a dominant subset of DDR deficiency, which occur in 10-20% of patients with PC. Significant responses to platinum-based chemotherapy occurs in up to a fifth of patients and improvements in overall outcomes is likely to due this subgroup of responders to therapy.[10,19] However, platinum-based regimens can be associated with significant adverse effects and even mortality. Thus, it is crucial to identify responsive patient subgroups to prevent unnecessary morbidity in patients who do not benefit from platinum. Surrogate readouts of HRD can be used as a selection biomarker to identify platinum responders. This is one of the key research objectives of *PRECISION-Panc,* a therapeutic development platform that aims to rapidly translate key molecular discoveries into a precision oncology approach for PC (please see accompanying editorial).

Immunotherapy in PC

To date, single agent immunotherapy trials in PC have been disappointing. Immune checkpoint (PD-1 and CTLA4) inhibitors have failed to show significant benefit in PC. The microenvironment of PC varies greatly between tumours, and this is likely due to differential immune signalling between subtypes of tumour epithelium and stroma. The squamous subtype of PC is enriched for immunosuppressive myeloid cell infiltration, with an abundance of neutrophil and macrophage signalling resulting in sparse T and B cell infiltration leading to immune invasion. Recent genetically engineered mouse model studies demonstrated significant benefits in pharmacologically induced myeloid depletion in PC. In an attempt to enhance immune checkpoint inhibition, several pre-clinical studies have targeted the immunosuppressive myeloid cell infiltration seen in PC. Combining CXCR2 inhibitors with a PD-1 checkpoint inhibitor improves survival in mouse models of PC.[21] In another study CSF1R inhibitors reduce immunosuppressive macrophage infiltration and results in an active T cell response.[22] Whether these preclinical findings translate into patient benefit, or if these are subtype specific phenomena remains to be determined in the clinical setting.

The Immunogenic subtype, on the other hand, is enriched for signalling from CD4+ and CD8+ T and B cells.[14] This is coupled with upregulation of PD-1 and CTLA-4 expression. Checkpoint inhibition may benefit this subgroup of patients, whilst is unlikely to demonstrate any response in the Squamous group, as single agent. This indicates that unselected immunotherapy trials, will result in dilution of any therapeutic signals leading to clinical trial failures.

PRIMUS trials: Translating pre-clinical platforms of evidence into the clinic

In an effort to accelerate therapeutic development, a UK wide *PRECISION-Panc* consortium was established in 2016 (please see accompanying editorial). Within the clinical development pillar is the PRIMUS (**P**ancreatic Cance**r** **I**ndividualised **M**ulti-arm **U**mbrella **S**tudy) clinical trial platform (Figure 1). It was designed to rapidly translate preclinical advances into clinical trials for patients with all stages of PC. Clinical trials are traditionally dominated by exclusion criteria, resulting in only around 5% of PC patients currently entering clinical trials. The molecular heterogeneity of PC, and often the prolonged period between translating preclinical advances into clinical trials have severely hampered progress in improving outcomes for PC. A *PRECISION-Panc* Therapeutic Testing Board has been established to regularly review pre-clinical concepts and assess readiness of the concepts to be developed into clinical trial. The Therapeutic Testing Board also meets with the NCRI clinical studies group Pancreatic Cancer workstream twice per year to ensure complementing and aligning therapeutic development. The trials on the PRIMUS platform are designed specifically by the scientific advances described above.

PRIMUS-001 and -002 aims to investigate the efficacy of the platinum containing regimen FOLFOX-Abraxane compared with Gemcitabine-Abraxane in all and biomarker-enriched patients in the first line metastatic and neoadjuvant setting respectively. The initial biomarker being tested is a candidate HRD signature, derived from the specific pattern of genomic structural re-arrangements seen in known HRD cancers from published and unpublished data sets. This is hypothesised to be a biomarker of response to platinum-based therapy. The adaptive design of these studies allows the discovery and development of the predictive signatures and to inform a future molecularly stratified PHASE III trial design if needed. This strategy

is an overarching goal of *PRECISION-Panc* by integrating Discovery, with Pre-clinical development and Clinical trial testing to allow rapid forward and backward translation, thereby accelerating scientific advances into the clinic (see accompanying editorial).

The concept of *PRECISION-Panc* is further demonstrated by the PRIMUS-003 trial (NCT02583477) which has recently completed recruitment. Based on the preclinical data that CXCR2 inhibition blockade significantly abrogated metastasis, and in combination with PD-1 extended survival in a genetically engineered mouse model,[21] a clinical trial was rationally designed to assess clinical efficacy in a Phase 1b signal seeking fashion.

The PRIMUS-004 umbrella was designed to offer patients a portfolio of 2nd line options, with early phase, signal seeking trial design with clear biomarker-based hypotheses using well tolerated targeted agents. It aims to offer patients a range of options targeting various disease mechanisms and potentially provide therapies for those less fit for further cytotoxic chemotherapy. The first Appendix is investigating if the combination of an ATR inhibitor (AZD6738) with the PARP inhibitor Olaparib is able to overcome acquired resistance to 1st line platinum treatment. Patients who had stable disease or better response to platinum containing regimens are stratified by mutations in key genes in the homologous recombination repair (HRR) pathway to allow comparison between these groups (HRR mutant vs wild-type).

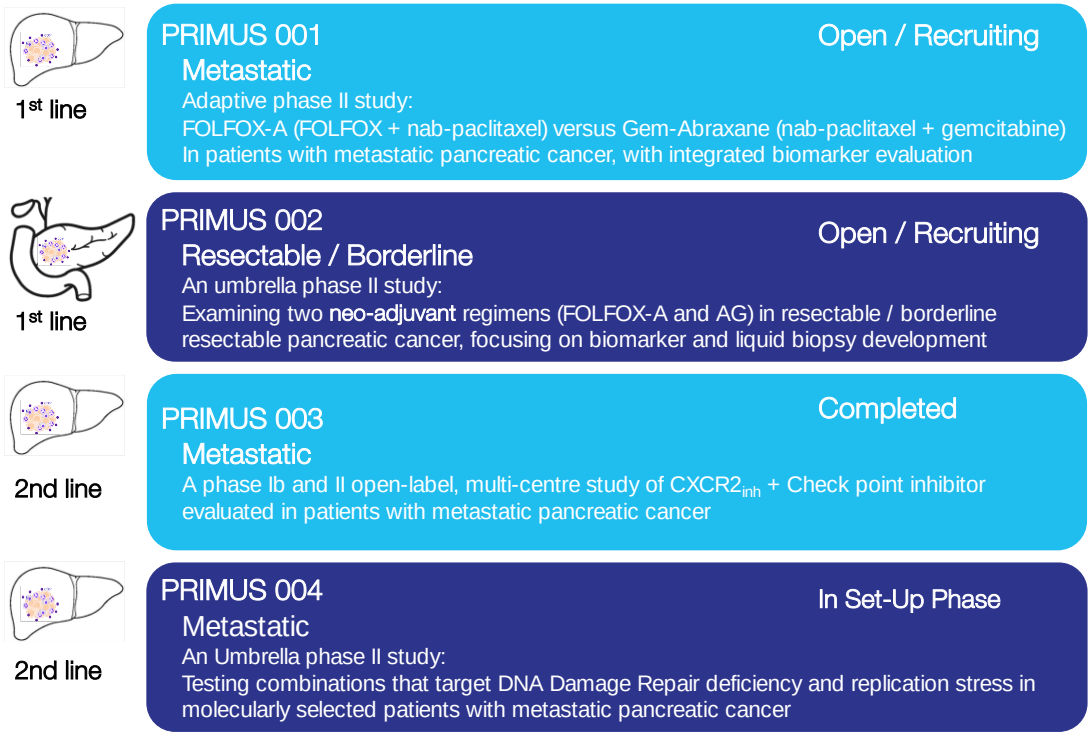

Figure 1: PRIMUS trials currently open or approved on the *PRECISION-Panc* platform

Conclusions

With the growing knowledge base of the molecular pathology of PC, we are revealing novel therapeutic opportunities in this recalcitrant disease. Furthermore, it highlights that underlying inter-patient molecular heterogeneity may have contributed to the significant failure rate associated with unselected clinical trials in PC. The generation and translation of clinically relevant and robust pre-clinical platforms of evidence into well designed clinical trials with parallel molecular profiling of patients' tumours, will ideally lead to improvements in overall survival for this devastating disease.

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