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Pancreatic cancer genomics

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Pancreatic cancer is one of the most lethal malignancies. The overall median survival even with treatment is only 6–9 months, with almost 90% succumbing to the disease within a year of diagnosis. It is characterised by an intense desmoplastic stroma that may contribute to therapeutic resistance, and poses significant challenges for genomic sequencing studies. It is recalcitrant to almost all therapies and consequently remains the fourth leading cause of cancer death in Western societies. Genomic studies are unveiling a vast heterogeneity of mutated genes, and this diversity may explain why conventional clinical trial designs have mostly failed to demonstrate efficacy in unselected patients. Those that are available offer only marginal benefits overall, but are associated with clinically significant responses in as yet undefined subgroups. This chapter describes our current understanding of the genomics of pancreatic cancer and the potential impact of these findings on our approaches to treatment.

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Introduction

Pancreatic ductal adenocarcinoma (abbreviated as pancreatic cancer for the purpose of this review, PC) is a highly lethal malignancy that is recalcitrant to almost all therapies [1]. It is the fourth commonest cause of cancer-related death in Western societies, with an overall 5-year survival rate that has remained less than 5% for over 45 years [2]. Surgery is the only chance of cure; however, the vast majority of patients have advanced disease at diagnosis and are not amenable to operative resection. Even in patients who undergo resection, 80% eventually recur and succumb to the disease. Systemic therapies are largely ineffective, adding incremental improvements of at best a few months in unselected patients; however, significant responses are sometimes observed in subgroups [3]. Consequently there is an urgent need to develop early detection strategies, methods to better select patients for current therapies and novel therapeutic approaches.

Mutational landscape of PC

Emerging data from large cancer sequencing initiatives [4,5] are unveiling a vast array of molecular aberrations in histologically indistinguishable cancers. In PC, the mutational burden appears to be particularly heterogeneous and this has major implications in therapeutic development and clinical care [6••]. Although significant progress has been made in understanding some of the genomic aberrations in PC, we are only scratching the surface of the complex physical changes in DNA sequence and structure that characterise these tumours. Pioneering work in PC genomics at Johns Hopkins University [7••] used capillary-based exome sequencing and SNP microarrays to define mutations and copy-number alterations in all protein coding genes in cell lines and xenografts derived from primary and metastatic tumours of 24 patients. This study began to reveal the complexity of PC, in that apart from the four highly prevalent genetic aberrations known to be present for many years (*KRAS*, *TP53*, *CDKN2A* and *SMAD4*), the frequency of genes harbouring mutations with potential functional consequences dropped rapidly, with a long tail of genes mutated at a frequency of less than 5%. Nonetheless, these aberrations coalesced into 12 core signalling pathways that contribute to processes described as the hallmarks of cancer [1,7••]. Subsequent studies using next-generation sequencing approaches enabled the characterisation of structural variation in PC in a handful of cases, suggesting a significant contribution of genomic rearrangements to pancreatic carcinogenesis [8•].

More recently, a collaborative effort as part of the International Cancer Genome Consortium (ICGC) performed whole exome sequencing to define mutations and used

DNA microarrays to survey copy-number alterations in 99 primary operable PCs [6]. PC is characterised by an intense desmoplastic stroma, with an average stromal content of ~70% which poses significant challenges for genomic sequencing [9]. To overcome this, methods were developed to enrich for tumour epithelial content by performing full face frozen sectioning and macrodissection. A series of dilution experiments using mixtures of germline and tumour DNA at varying proportions showed that exome capture sequencing approaches detected >50% of mutations in tumours with higher than 20% cellularity. An analysis tool (qpure) [10] was developed and validated using deep mutant *KRAS* sequencing to allow estimation of tumour epithelial content using CNV on SNP arrays and thus predict the relative sensitivity of mutation detection for a given sample before sequencing. These 99 representative samples further uncovered vast heterogeneity of mutated genes in PC. Apart from *KRAS*, *TP53*, *CDKN2A* and *SMAD4*, only a handful of genes were mutated at a frequency of >2% of cases [6]. A total of 2016 genes contained non-silent mutations, with an overlap of 186 of all 998 mutated genes (19%), and a 48% overlap (38 of the 79) in genes that were mutated more than once in 24 samples sequenced by Jones *et al.* [7••] The most frequently mutated genes by combining these datasets are shown in Table 1 (adapted from Biankin *et al.* [6] with permission). Copy-number alterations are common genomic events in PC; however, the degree of instability makes these events difficult to interpret, particularly as tumour cellularity decreases. Further studies of whole genome sequencing and analyses of structural variations are required to better define large genomic alterations and copy-number changes in PC.

Table 1

Single nucleotide variations and indels in pancreatic cancer

Gene	ABO + Jones (*n* = 123)	ABO + Jones (%)
KRAS	118	96
TP53	51	41
SMAD4	24	20
TTN	18	15
MLL3	10	8
PCDH15	8	7
MUC16	7	6
TGFBR2	7	6
ARID1A	6	5
CSMD1	6	5
NEB	6	5
SF3B1	6	5
ATM	5	4
DMD	5	4
DNAH5	5	4
LRP1B	5	4
NALCN	5	4
ZIM2	5	4
ABCA12	4	3
ADAMTS20	4	3
AFF2	4	3
CDH10	4	3
CDKN2A	4	3
DOCK2	4	3
DPP6	4	3
FMN2	4	3
HMCN1	4	3
PREX2	4	3
PXDN	4	3
RYR2	4	3
RYR3	4	3
SCN5A	4	3
SYNE1	4	3
XIRP2	4	3

Driver versus passenger mutations

Differentiating candidate driver from passenger mutations is a major challenge in cancer genomics [11]. Numerous computational tools can assist in defining the probability of a given gene and/or mutation as a potential driver of carcinogenesis [12], but these approaches are insensitive and inadequate when confronted with multiple genes that are mutated infrequently [13,14]. The list of genes identified to be Significantly Mutated in PC using these analyses is shown in Table 2, and includes, among others, a strong representation of those involved in DNA maintenance and chromatin remodelling [6]. Integrating data from other systems such as functional screens [15,16] and animal models may be used to enrich for driver events [17,18]. Genetically engineered mouse models of PC are well developed, and more recently, *Sleeping-Beauty* transposon-mediated mutagenesis screens provide a potential rich source of orthogonal data that can be triangulated with human data to refine and prioritise candidate driver events. Such approaches have identified novel candidate genes that are potentially important in PC such as *USP9X* [19] and *MAP2K4* [20], and novel pathways such as Axon Guidance, particularly *SLIT/ROBO* signalling [6••].

Mutational signatures in PC

The accumulation of cohort-based cancer genome sequence data has provided the opportunity to identify common mutational signatures in different cancer types, and in some instances infer the mechanisms underlying mutagenesis (Chapter XX). Rather than being a random assortment of base changes spread across the genome, somatic mutations within a patient's tumour reflect the sum of mutagenic exposures and mutational processes active during the cancer's development and evolution. It has recently become possible to scan whole cancer genome sequence data and define the common mutational patterns. After pioneering work identified five mutational signatures active in a small breast cancer cohort (*n* = 21) [21•] a large collaborative effort led by the Sanger Institute analysed 4 942 984 mutations from 7042 cancers across 30 cancer types and identified more than 20 distinct mutational signatures [21•,22••,23]. Mutational signatures were extracted using nucleic acid base substitutions and included information on the sequence context of each

Table 2

Significantly mutated genes in pancreatic ductal adenocarcinoma

Gene symbol	Gene name; function	SB mut	shRNA
KRAS	Oncogene; GTPase; Activation of MAPK activity	×	×
TP53	Tumour Suppressor p53; DNA damage response		×
CDKN2A	Cyclin-dependent kinase inhibitor 2A; G1/S transition of mitotic cell cycle; Tumour Suppressor	×	
SMAD4	Mothers against decapentaplegic homolog 4; BMP signalling pathway	×	×
MLL3	Myeloid/lymphoid or mixed-lineage leukemia protein 3; DNA binding; regulation of transcription	×	×
TGFBR2	Transforming growth factor-beta receptor type II; Regulation of growth	×	
ARID1A	AT-rich interactive domain-containing protein 1A; SWI/SNF complex; Chromatin modification	×	×
ARID2	AT-rich interactive domain-containing protein 2; Chromatin modification	×	
EPC1	Enhancer of polycomb homolog 1; Histone acetylation	×	
ATM	Ataxia telangiectasia mutated; DNA damage response		×
SF3B1	Splicing factor 3B subunit 1; Nuclear mRNA splicing		×
ZIM2	Zinc finger imprinted 2; Regulation of transcription		×
MAP2K4	Dual specificity mitogen-activated protein kinase kinase 4; Toll-like receptor signalling pathway	×	×
NALCN	Sodium leak channel non-selective protein; Sodium channel activity		×
SLC16A4	Solute carrier family 16 member 4; Monocarboxylate transporter		×
MAGEA6	Melanoma-associated antigen 6; Protein binding		ND

SB mut, significant insertion sites in 2 independent Sleeping-Beauty mutagenesis screens [19,20]; *shRNA*, in vitro shRNA screens in 102 cancer cell lines with effect on cell survival [16]; ND, not determined.

mutation. There are six classes of base substitution (C > A, C > G, C > T, T > A, T > C, T > G). Incorporating information on the bases immediately 5′ and 3′ to each mutated base results in 96 possible mutations. Several signatures were associated with age of the patient at diagnosis, known mutagenic exposures, or defects in DNA maintenance, but approximately half were of unknown origin. While some signatures are present in many cancer types, some are more specific.

Knowledge of the biological mechanisms that underpin mutational processes that are important in cancer evolution has significant potential clinical relevance and utility in diagnosis, early detection, prevention and treatment. At this stage (based on 20 whole genome and 100 whole exome sequences), there are four mutational signatures that infer known biological processes in PC. They include, older age, *BRCA*-mediated defects in DNA damage repair, DNA mismatch repair deficiency and a signature associated with the APOBEC family of cytidine deaminases [22••].

Intra-tumoural heterogeneity

In-depth analysis of subclones in individual PCs and associated metastases suggests that in addition to inter-tumoural heterogeneity, there is significant intra-tumoural heterogeneity, and between metastases in the same patient. Although the impact on therapy is unclear at this stage, it has an immediate potential impact on biomarker-driven therapeutic strategies with regard to tumour sampling [24•]. In addition, studies of tumour evolution through the analysis of subclonal mutations suggest that the lead-time before clinical detection is substantially longer than anticipated, at well over a decade, suggesting that there is a significant window of opportunity for early intervention [24•].

Pancreatic cancer predisposition genes

Up to 10% of PC is thought to be the result of inherited susceptibility loci [25]. About 20% of hereditary PC (defined as a pair of first degree relatives with PC) occurs as part of cancer syndromes (Hereditary Breast Ovarian Cancer (HBOC), Peutz-Jegher Syndrome (PJS), Familial Atypical Multiple Mole Melanoma (FAMMM), Li-Fraumeni syndrome, Hereditary Non-Polyposis Colorectal Cancer (HNPCC) and Familial Adenomatous Polyposis (FAP)) or as a consequence of hereditary pancreatitis which is predominantly due to *PRSS1* or *SPINK1* mutations [25]. Of the remaining 80% of familial PC, some PC susceptibility genes have been identified: *BRCA2* [26,27], *PALB2* [28] and *ATM* [29], however, a significant proportion is as yet unexplained.

Personalised medicine for PC

Clinical implications of cancer heterogeneity

The inter-tumoural heterogeneity of PC poses a significant clinical challenge for patient management, especially when trying to identify the most effective chemotherapeutic for an individual. PC is traditionally known to be refractory to many forms of treatment, and very few chemotherapeutics have shown 'efficacy' in clinical trials. This phenomenon may be due to genomic heterogeneity, as an overall benefit is unlikely to be detected with non-selective clinical trial designs assessing overall efficacy of a chemotherapeutic regimen. This is likely to be the case even if specific molecular aberrations are effectively targeted as each of the responsive phenotypes are small, unless combinations that capture a greater number of responsive phenotypes such as FOLFIRINOX [30,31], are used. Such combinations are often associated with increased toxicity, which is a particular challenge in patients with advanced PC who often suffer from cancer cachexia. Identifying the most effective treatment first is

Table 3

Actionable molecular phenotypes in pancreatic cancer

Actionable molecular phenotype	Therapeutic	Rationale	Proposed biomarkers	Estimated prevalence (%)
Gemcitabine responsive	Gemcitabine	Phase 3 clinical trial data	High *hENT1* expression	15
DDR deficient	Platinums	Clinical Trials; Case reports	*BRCA2*/*PALB2* mutations	7
nab-paclitaxel responsive	*nab*-Paclitaxel	Clinical Trial; preclinical models	*SPARC* expression	10
Fluoropyrimidine responsive	5-Fluorouracil; Capecitabine	Phase 3 Clinical Trials	Unknown	3
Anti-EGFR responsive	Erlotinib	Phase 3 clinical trial data (PA3)	*KRAS* wild type	7
Irinotecan responsive	Irinotecan	FOLFIRINOX trial	Topoisomerase 1 overexpression	2
HER2 amplified	Trastuzumab	Rescuing	*HER2* amplification	2
Hedgehog	SMO inhibitors	Rescuing	PTCH mutations	2
CSF1R mutated	Sunitinib	Repurposing	*CSF1R* mutation	1
STK11/*LKB1* null	mTOR inhibitors	Emerging	*STK11*/*LKB1* mutation/loss	1

therefore of particular importance, as relatively few patients with advanced PC survive long enough to receive second line therapy. This issue has become more relevant recently with an increasing number of therapeutic options, all of which are associated with incremental overall benefit but meaningful responses in undefined subgroups, and can be associated with significant toxicity.

Rationale of genotype-guided medicine

Over the last two decades, a deeper understanding of the genetic and molecular basis of cancer has led to new classes of therapies that selectively target the molecular mechanisms that are important for the survival and proliferation of cancer cells. Clinical benefit from these therapies is dependent on the presence of specific cellular targets and is optimally directed by the presence of a companion biomarker of therapeutic responsiveness [3]. Although yet to be proven, for a cancer with vast genomic heterogeneity such as PC, a genotype-guided stratified approach may be beneficial, especially since overall outcomes have changed little for decades.

Successful translation of findings from large-scale genomics and other '-omic' approaches into improvements in patient care and outcome is likely to necessitate a fundamental shift in clinical oncology. These include a new molecular taxonomy, where in addition to an organ-based and morphology-based classification, individual cancers are grouped and selected for optimal therapy based on their molecular signature or 'biotype', particularly for molecularly diverse and less common cancers such as PC [32]. Trial designs such as 'basket studies' [33] may be a way forward to address the efficacy of companion biomarker (therapeutic target)-directed approaches across cancers of different organs where the prevalence of the actionable phenotype is low. Genotyping individual cancers presents the opportunity to test the efficacy of targeted agents used in other cancer types in PC patients where the tumour harbours the same molecular target ('repurpose').

Although the prevalence of individual actionable molecular phenotypes for currently available therapies for PC is low, if other phenotypes where matched therapeutics already exist and can be 'repurposed' or 'rescued', then cumulatively they begin to add up to a potentially significant opportunity to improve outcomes in a shorter timeframe than novel therapeutic discovery and development. Anecdotal reports of exceptional responders to chemotherapy in PC are accumulating, particularly in the case of defects in DNA maintenance, especially DNA damage repair mechanisms in patients with mutations in genes such as *BRCA2* and *PALB2* [34]. Analysis of genomic data is identifying candidate actionable molecular phenotypes with existing therapeutics already approved for human use in other cancers and/or other diseases (Table 3).

Actionable molecular phenotypes in PC

In general, actionable molecular phenotypes for cancer therapy can be classified into five groups based on supporting evidence:

1. Clinical trial evidence that a therapeutic is effective when the actionable molecular phenotype is present (e.g. *HER2* amplification and trastuzumab therapy in breast and gastric cancer, *BRAF* V600E mutation and vemurafenib therapy in melanoma, but not yet the case for PC).
2. Those where clinical trials have shown incremental overall benefit, but often with significant responses in subgroups that are not well defined (e.g. platinum-based therapy in PC).
3. Those that have failed in clinical trials, but with subgroups of responders and supportive preclinical data (e.g. trastuzumab and hedgehog inhibitors in PC).
4. Opportunities for repurposing therapies used in other cancers or other diseases (e.g. mTOR inhibitors).
5. Emerging novel compounds that target specific molecular aberrations.

In addition to this approach, computational modelling can identify candidate signatures of susceptibility to specific therapies in the absence of a specific target or biomarker.

In PC, even with the limited knowledge we have currently, we can identify opportunities to rationalise the selection of patients for existing therapies that are approved for use in PC, and 'rescue' or 'repurpose' existing therapeutics. Until recently, gemcitabine was widely considered the standard of care for advanced disease, and forms the backbone of many non-platinum-based combination therapies. Expression of *hENT1*, a key membrane transporter of gemcitabine, can modulate sensitivity and resistance to gemcitabine therapy in preclinical models [35–37]. However, its clinical applicability is yet to be established [38–41]. Further refinement of such biomarkers using genomic approaches may yield supportive evidence to better define subgroups of responders. A feature of tumours with mutations in the Fanconi Anemia/BRCA homologous recombination pathway is their hypersensitivity to DNA damaging (cross-linking) agents, such as mitomycin C, platinum and PARP inhibitors [42,43]. Platinum based therapies in PC have mixed results in clinical trials of unselected patients [44], although a recent meta-analysis of available clinical trials [45] and the efficacy of FOLFIRINOX [31] regimen demonstrated in the PRODIGE 4/ACCORD 11 trial, suggest activity in subgroups. Moreover, case reports of exceptional responses in patients who harbour defects in DNA damage repair and replication are increasingly being reported [34,46–48]. Other therapies with incremental overall survival benefit, but with subgroups of responders include *nab*-paclitaxel in combination with gemcitabine [49]. Although high protein expression of SPARC has been proposed as a biomarker of responsiveness, its clinical utility is yet to be defined. Similarly, erlotinib therapy is associated with subgroups of responders [50]. Although activating mutations of *EGFR* are associated with sensitivity to anti-EGFR therapy in lung cancer, such mutations have not been identified in PC. Activating mutations in *KRAS* occur in 93% of PC [6], however it is still not clear if *KRAS* wildtype tumours confer sensitivity to anti-EGFR therapy as seen in colon cancer. Biomarkers predictive of fluorouracil-based therapies, the only other approved existing therapy for PC, are yet to be adequately defined.

A multitude of other agents have been tested in patients with PC in an unselected fashion, and the vast majority have failed to demonstrate efficacy in late phase clinical trials. Technological advancements leading to a better understanding of the molecular basis of carcinogenesis have provided us the opportunity to reassess therapeutic strategies using a more selective approach. Preclinical data support the efficacy of anti-HER2 therapies in PC [51], and although clinical trials of trastuzumab have not demonstrated efficacy, these trials did not select patients using standardised assays, and as a consequence may be significantly underpowered [52,53]. *HER2* amplification occurs in 2% of PC based on standardised diagnostic assays [54] compared to a reported range of 0–80% when various assays and cut-points are used. If patients are selected appropriately, this may present a significant opportunity to 'rescue' trastuzumab as a therapy for a subgroup of patients with PC.

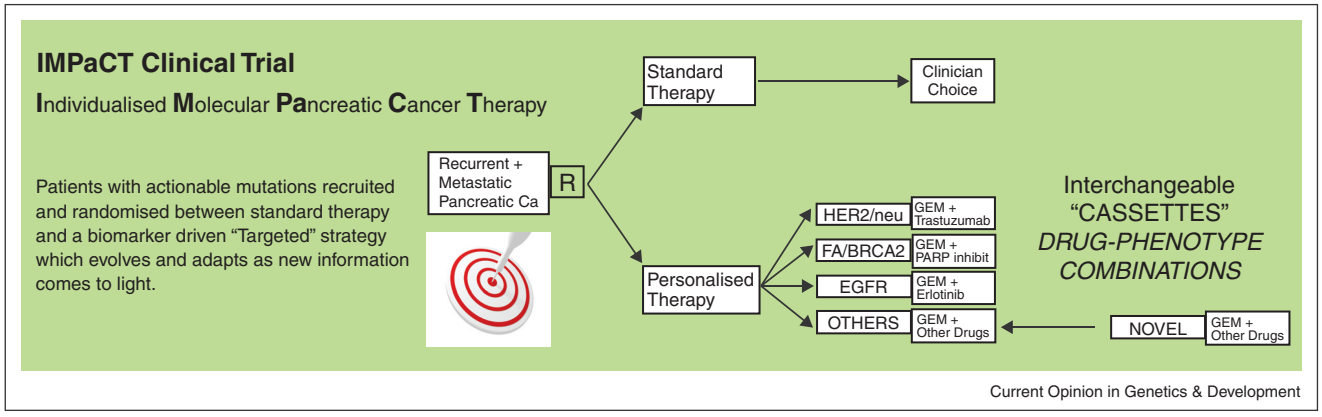
A recent clinical trial of the smoothened inhibitor saridegib in patients with PC was stopped before the recruitment target was reached due to poor survival in the experimental arm, despite promising efficacy in preclinical models. Activity of the hedgehog pathway was not used as a biomarker for patient selection, and patients with tumors that harbor mutations in genes involved in hedgehog signaling may represent an appropriate target population. Mutations in *PTCH* are known to activate hedgehog signaling in experimental models, and are detected in 2% of PC [6]. If patients are selected appropriately, this may present another opportunity to 'rescue' a therapeutic option.

mTOR inhibitors are emerging as therapies in other cancer types, and case reports of responses to these agents in PC are emerging [55,56]. Somatic mutations, copy-number alterations, and loss of expression of genes involved in the mTOR signaling pathway including *STK11/LKB1*, *TSC1* and *PTEN* are present in PC, and may represent an appropriate population to target when testing these agents. Mutations of other druggable targets with existing therapeutics also occur at low frequency, for example, CSF1R mutations, that may be targeted with sunitinib.

Prospective testing

Prospective testing of these actionable molecular phenotypes in the clinic is challenging and novel, nimble approaches are required. A collaborative effort including the Australian Pancreatic Cancer Genome Initiative has commenced a pilot study of an umbrella trial design to test the feasibility of assessing a more stratified approach in the management of PC using predefined actionable molecular phenotypes. Initially, patients are screened for 3 molecular phenotypes, and those who are positive are recruited to the trial called IMPaCT (*I*ndividualised *M*olecular *Pa*ncreatic *C*ancer *T*herapy), a randomised Phase II first-line study comparing gemcitabine to a stratified approach (Figure 1). The initial molecular phenotypes and their corresponding therapeutic agents are: firstly, *HER2* amplified — trastuzumab therapy, secondly, germline pathogenic *BRCA1/2* or *PALB2* mutations — DNA damaging agent or PARP inhibitor therapy, and thirdly, *KRAS* wildtype or *KRAS* codon 13 mutant — erlotinib therapy.

Figure 1



Schema of the IMPaCT trial: a Phase II clinical trial where patient with pre-defined actionable molecular genotypes/phenotypes (three initially) are randomised to standard or stratified/personalised therapy. The trial is designed to be 'adaptive'.

Exceptional responders

In addition to the above-mentioned strategies for the development of a stratified approach to the management of PC, advances in genomics have presented us the opportunity to better understand some of the phenomena that could not be adequately explored in the past. By studying 'exceptional responders' to specific therapies, particularly those in clinical trials, including those that 'failed' to detect an overall benefit may provide valuable insights. The NCI of the USA has launched such an 'exceptional responders' programme to commence in 2014.

Conclusion

Genomic studies of PC are revealing marked inter-tumoural and intra-tumoural heterogeneity and complexity, and may explain the lack of success of conventional approaches to therapeutic development. Although a better understanding of the underlying molecular pathology will undoubtedly lead to novel therapeutic development, there are existing opportunities for more rapid improvements in outcomes by adopting a more stratified, or personalised approach using genomic biomarkers. Such advances will require adjustments in current systems and approaches to therapeutic development, clinical care and regulatory approval.

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