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Genetic Diversity of Pancreatic Ductal Adenocarcinoma and Opportunities for Precision Medicine

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Abstract

Patients with pancreatic ductal adenocarcinoma (PDA) have a poor prognosis—in spite of new treatments, approximately 7% survive for 5 years. Although there have been advances in systemic, primarily cytotoxic, therapies, it has been a challenge to treat patients with PDA using targeted therapies. Sequence analyses have provided a wealth of information about the genetic features of PDA and identified potential therapeutic targets. Preclinical and early-phase clinical studies have found specific pathways could be rationally targeted; it might also be possible to take advantage of the genetic diversity of PDAs to develop therapeutic agents. The genetic diversity and instability of PDA cells have long been thought of as obstacles to treatment, but now are considered exploitable features. We review the latest findings in pancreatic cancer genetics and the promise of targeted-approaches in pancreatic ductal adenocarcinoma therapy.

Pancreatic ductal adenocarcinoma (PDA) is the most common type of pancreatic cancer[1]. The disease encompasses multiple histological subtypes, which affect patients' prognoses[2]. For example, patients with adenosquamous cancers have particularly poor outcomes, whereas mucinous neoplasms are generally lower grade and are considered to be a less aggressive form of the disease [3, 4]. Irrespective, most cases of PDA are a challenge to treat, with 5 year rates of survival lower than 10% for patients with cancers of all stages[1]. To put

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this into perspective, it has been estimated that by 2020 that PDA will become the 2nd leading cause of cancer-related death in the United States [5].

Most PDA is identified at a late stage, when surgical intervention is not possible. Even with complete resection and negative results from analyses of tumor margins, long-term survival after surgery is poor—tumors recur in virtually all patients [6]. Presumably, this is because micrometastases are present, even in patients whose disease appears confined to the pancreas. These features of the disease have driven the need for systemic treatments to control disseminated disease. Recently approved therapies for treatment of metastatic PDA include combination chemotherapy regimens, such as fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX) or gemcitabine and albumin-bound paclitaxel protocols[7–9]. The only targeted agent approved in treatment of PDA is the epidermal growth factor receptor (EGFR) inhibitor erlotinib, which given in combination with gemcitabine, only slightly increases overall survival time compared with gemcitabine alone[10]. These treatment approaches increase survival times of patients with metastatic PDA; however, the average increase in overall survival is measured in weeks to months[11]. Although many other types of cancer are now treated based on selective features of the disease (*e.g.* HER2-targeted therapies for HER2+ breast cancer, crizotinib for ALK-rearranged lung cancer), there are no validated marker-based therapies for PDA.

Deregulated Pathways

In recognition that a targeted approach could be particularly important for the treatment of PDA there have been extensive genetic analyses of the disease.

Genetics

Initially, genetic characterization of PDA was directed at evaluating known oncogenic and tumor suppressive pathways[12]—essentially searching for genetic variants frequently associated with tumors, such as bi-allelic loss of tumor suppressor genes or activating mutations in oncogenes. Performed before the high-throughput era, these experiments involved classic gene-cloning, single-stranded conformational polymorphism, PCR, and Sanger-sequencing methodologies. In many cases, these efforts explored genes that had been functionally defined in other tumor systems. For example the *KRAS* gene was originally discovered in mouse oncogenic retroviruses and found to be mutated in human bladder cancer cell lines[13–15]. Subsequently, targeted genetic approaches demonstrated that *KRAS* mutations occur in more than 90% of PDA tumors [13–15].

Through functional studies of cultured cells and mouse models, KRAS mutations were found to be required for tumor initiation and maintenance, regulating a range of cell activities, from proliferation to metabolic reprogramming[16–20]. PDAs and other tumors were also found to have frequent mutation of the tumor suppressor TP53[21, 22], which synergizes with KRAS mutations to facilitate tumor development. This synergy was used to develop the long-accepted mouse model of PDA: the K-rasLSL.G12D/+; p53R172H/+; PdxCre (KPC) mouse[23]. Similarly the CDK4/6 inhibitor gene CDKN2A is often deleted, mutated, or epigenetically silenced, in PDA[24]. Individuals with mutations in *CDKN2A* that define

familial melanoma syndrome have a 20-fold increase in risk of pancreatic cancer, compared to individuals without these mutations[12, 25].

The aforementioned combinations of genetic features are detected in many other tumor types, including colon cancer and lung adenocarcinoma. A relatively unique event in gastrointestinal malignancies (e.g. colorectal cancer) is loss of SMAD4, also referred to as DPC4 (deleted in pancreatic cancer), which mediates transforming growth factor (TGF)-b signaling [26, 27]. The frequent loss at of *SMAD4* from 18q in PDAs is a marker of increased metastatic potential and indicates a poor prognosis [28, 29]. Linking genetic events with the histologic features of PDA precursor lesions, such as pancreatic ductal intraepithelial neoplasia (PanIN), has provided an important model of pancreatic cancer progression [30]. For instance, the high-frequency of KRAS mutations in PanIN lesions suggests that this event drives disease initiation with subsequent mutations/genetic events necessary for tumor progression.

Findings from next-generation sequencing analyses

To date approximately 300 PDA genomes/exomes have been sequenced (TABLE 1). This represents a limited collection of cases when compared against lung or breast cancer where greater than 1000 cases have been subjected to exome and whole genome sequencing [31, 32]. PDA is often dominated by desmoplastic stroma, which can constitute up to 90% of the tumor mass[33]; this makes analyses of tumor epithelial cells difficult. This is one reason that the molecular characterization of PDA has lagged behind that of other tumor types.

Researchers have used several approaches to circumvent issues of tumor cellularity. Initially, sequence analyses were performed using tumor xenografts and cell lines, to limit contamination by non-neoplastic human cells, which alter calculations of mutant allele frequencies and copy number alterations[34]. However, this approach potentially selects for specific genetic events required for proliferation of cells in culture or growth of tumors in immune-compromised mice, which might not occur in patients' tumors (or the multiple clones within human tumors). Now that sequencing is more affordable, it is possible to sequence to great depth (e.g. 1000x reads for each nucleotide) in order to computationally enrich for the presence of tumor selective variants. This approach is clearly feasible and yields important insight into PDA genetic features [35, 36], albeit using such an approach could limit the sensitivity of detection and therefore under-represent the mutational burden in relation low frequency alleles to sub-clonal features of disease. The Cancer Genome Atlas and other sequencing efforts require the presence of at least 50% of tumor cells in samples analyzed[37]. Several groups have used microdissection approaches to select tumor cells from tissues [38, 39].

Combining resultant genetic characterization of PDA with knowledge of the clinical features of the disease has further allowed investigators to study the evolution of cancer from the primary to the metastatic lesion, the genetic features of precursor lesions, and select PDA subtypes (e.g. adenosquamous) [38–42]. In spite of the varied approaches employed, a consensus view on the landscape of pancreatic cancer genetics is emerging, wherein there are a plethora of genetic alterations beyond the canonical KRAS, TP53, CDKN2A and

SMAD4 spectrum. In fact, some of these non-canonical pathways and genes may become the most ‘targetable’ in PDA.

Deregulated pathways

The earliest exome sequence analyses of PDA identified core signaling pathways that were altered at the genetic level [34]. However, due to the relatively limited number of tumor genomes sequenced, the prevalence of these core alterations was not clear. Subsequent genetic analysis has reinforced specific features of the core-signaling concept and characterized important additional features of PDA (FIGURE 1, TABLE 2).

Like many cancers, while the established mutations (e.g. KRAS and TP53) occur at high-frequency many other genetic events occur less frequently (TABLE 2). Only by analyzing many tumor samples can such genes be defined as “significantly mutated in cancer”; in general, it is viewed that more than 1000 different tumors will need to be sequenced to reach saturation[43, 44]. The current definition of significantly mutated is based on the observed frequency of mutations in coding regions of a tumor gene, compared with the chance of random mutations in the gene. Sequence analyses of PDAs have identified significant mutations in *TGFBR2*, *KDM6A*, *AXIN1*, *ACVR1B*, *PIK3CA RNF43*, *GNAS*, *ATM*, *GLI3*, *ARID1A*, *RBM10* [35, 39]. Some of these mutations have been identified in select subtypes of PDA, but could also represent a feature of PDA cases as a whole. For example, mutations in *GNAS*, *RNF43*, and *RBM10* were initially identified in intraductal papillary mucinous neoplasms (IPMNs), but subsequently found in PDAs that did not appear to arise from IPMNs. [45–47]. Additionally, many of these genes are mutated in other cancers, lending credence to their significance in PDA [44].

In addition to mutations within genes, many cancer cells contain copy number alterations, which support the biological significance of a given genetic alteration [48]. For example, approximately 10% of PDAs contain point mutations in *CDKN2A*; however, homozygous deletions are a more common event targeting this gene [35, 39]. Computational approaches can identify regions of significant deletion or amplification in tumor cells [49]. In the case of PDA, this includes many known tumor suppressors (e.g. CDKN2A and SMAD4) and oncogenes (e.g. MYC and CCND1) [35, 39]

In spite of the emerging depth of information, the low frequency of many genetic events identified has called into question their ultimate clinical utility. However, many of the mutations identified are in genes whose products participate in the same pathways. For example, AXIN1, RNF43, APC are all mutated in PDAs and are members of the WNT pathway (TABLE 2)[50]. Similarly CDKN2A, CCND1, and RB function in a single pathway [51]. Therefore, from a therapeutic perspective, although single genetic variants may be too rare to be viable targets, the pathways they alter might be targeted therapeutically. Sequencing studies have identified the KRAS, TGFB, TP53, MYC, chromatin remodeling, DNA repair, cell cycle, WNT–β-catenin, and NOTCH signaling pathways, among others, as those that are disrupted at the genetic level in PDAs and might be targeted (TABLE 2, FIGURE 1). Interactions among these pathways are complex; and most PDAs have genetic alterations that alter distinct subsets of these pathways (FIGURE 1)

Chromosome instability

In addition to gene specific genetic alterations, the overall genetic landscape of PDA could be an important and therapeutically prognostic feature of this disease. Analyses of chromosome architecture and copy number alterations by whole genome and exome sequencing have indicated that there are distinct subtypes of PDA, related to chromosomal instability. Using whole-exome sequencing, it is possible to capture features of amplification and deletion, and it is apparent that some PDA cases have relatively stable chromosome architecture whereas others have many amplifications and deletions [35, 39, 48]. A caveat of exome sequencing is that it cannot be used to identify variants in intragenic regions, which constitute the bulk of translocations and other structural alterations. However, whole-genome sequencing can identify variants in intragenic regions; these types of studies have shown that PDAs contain a wide-spectrum of chromosome alterations[36]. Importantly, there appears to be a correlation between the extent of chromosome instability and mutations in genes involved in DNA break repair by homologous recombination, but not related to p53 [36, 39]. Chromosome instability is a feature of BRCA-deficient cancers and, multiple genes involved in DNA break repair are disrupted in PDAs, including *BRCA1*, *BRCA2*, and *PALB2* (TABLE 2). These genetic variants appear to be directly involved in the etiology of pancreatic cancer, as germline mutations in these genes have been associated with familial predisposition to PDA [52–54].

Additional studies have focused on determining the frequency of the microsatellite instability (MSI) genotype of PDA. Some studies have reported that PDA is more likely to arise in families with Lynch Syndrome [55]. A study performed more than 15 years ago found that fewer than 5% of PDAs could be classified as having MSI [56]. Other studies supported this finding, and correlated MSI genotype with PDA with medullary histology [57]. Nonetheless there has been debate over the frequency of the MSI genotype in all PDAs; one study found MSI frequency to be “irrelevant”, in that it was detected in only 0.3% of 338 consecutive surgically resected sporadic PDA cases [58]. From recent sequencing studies there do appear to be hypermutated cases that harbor a mutation burden consistent with deficiency in mismatch repair occurring in ~2% of cases[36, 39]. Similarly in a computational analysis of PDA mutational spectra, a contribution of mismatch repair deficiency was observed in tumor specimens 59.

Collectively, there appear to be distinct forms of PDA that can be identified based on the extent of mutation burden or chromosomal instability. These factors are likely to be associated with the etiology and/or progression of PDA, as well as patient outcomes and responses to treatment.

Genetic Alterations as Therapeutic Targets

The genetics of PDA could provide a roadmap to targeted therapy. Specifically, multiple pathways that are genetically dysregulated in PDA could serve as targets of therapy (TABLE 2). In general, the genetic features of disease provide the basis for considering two relatively simple approaches for targeted treatment of cancer. Conventionally, it is easy to envision how a specific activating genetic event can be targeted. Classic examples include targeting *HER2* amplification in breast tumors or *BCR–ABL* in chronic myelogenous leukemia with

kinase inhibitors. In these cases, the genetics of the tumor yield the direct target for pharmaceutical inhibition. The alternative approach is to exploit the biological or functional features of the genetic event therapeutically. For example, PARP inhibitors are effective against BRCA1/2-defective tumors due to impact of BRCA loss on DNA repair [60]. In the case of PDA, historically neither approach to targeting the disease has been routinely employed in directing treatment. However, recent findings from genetic studies have identified new targets that can be tested in trials [61]. Below select genetic features that are targetable with agents in clinical development are discussed.

KRAS–BRAF–MEK

The KRAS pathway is one of the best characterized signaling pathways in cancer [62]. Because most PDAs (~90%) have activating KRAS mutations, the pathway is an obvious choice for targeting. To date, no inhibitor of KRAS has been brought to clinical application, although the National Cancer Institute has a new program specifically directed toward developing KRAS inhibitors [63]. Therefore, whether specific targeting of KRAS in PDA will represent a successful treatment approach remains unknown. PDA cell lines have variable responses to KRAS knockdown[64, 65]. Importantly, in genetically engineered mouse models of PDA, selective deletion of KRAS in established tumors led to a dormant population of cells that could ultimately recover from the ablation of KRAS and were driven by alternative signaling pathways [66, 67]. Thus, even disruption of a key driver of PDA may not produce a durable therapeutic effect.

In recognition of the challenge of targeting KRAS directly, there have been multiple attempts to target effector pathways downstream of KRAS. In particular, MEK signaling is often required for the viability and proliferation of KRAS-driven tumors. Multiple potent MEK inhibitors have been developed, and have activity in models of PDA [68, 69]. In a series of trials, the MEK inhibitors CI-1040A and AZD6244 as single agents were not effective in patients whose disease progressed on prior therapy[70, 71]. AZD6244 did not increase patient survival time, compared with capecitabine therapy, in a randomized phase 2 trial[70]. Trametinib in combination with gemcitabine therapy was not found to be superior to gemcitabine as a single agent in a randomized phase 2 trial [72].

These findings reveal the challenges of targeting a single pathway in PDA. In fact, multiple studies have shown MEK inhibitors to be particularly effective in combination with PI3K inhibitors, due to simultaneous effects of targeting 2 effectors of KRAS signaling [68, 69]. This approach is being tested in a phase 1b trial with the MEK inhibitor MEK162 in combination with the PI3K inhibitor BYL719 in patients with solid tumors, including pancreatic cancer (NCT01449058) (TABLE 5). In addition, the effects of the combination of a MEK and AKT inhibitor, compared to FOLFOX (5-FU, oxaliplatin, leucovorin) as a second-line therapy for PDA, are to be presented in the near future—this strategy is evaluating a combined targeted approach to try to overcome the limitations of single pathway inhibition. A number of mutant KRAS directed trials are underway to test various MEK-targeted combinations in patient with PDAs (TABLE 5)

Although patients with PDA containing KRAS mutations are a challenge to treat, little is known about the behavior of PDA without mutation in KRAS. From recent sequencing

studies several potential oncogenic drivers have emerged for this subset of PDA. Activating mutations in the *GNAS* gene, which encodes a G-protein subunit, were identified in IPMN-derived PDAs [45, 46]. Mutations in BRAF that activate kinase activity (such as V600E) have been identified and are mutually exclusive with KRAS mutations [39, 73]. Cells from a tumor with mutant BRAF had selective sensitivity to vemurafenib, a BRAF inhibitor. Correspondingly, BRAF promotes development of PDA in mice [69]. There is anecdotal evidence that patients with PDAs with the BRAF V600E mutation respond to an approved BRAF inhibitor; patients with metastatic pancreatic cancer may be eligible to receive a BRAF inhibitor based on the genetic profile of the tumor. Thus, simple genetic screening of the conventional KRAS/BRAF pathway could elicit a new therapeutic avenue for a minor subset of patients with PDA. The Individualized Molecular Pancreatic Cancer Therapy (IMPACT) trial is identifying patients with PDAs without mutations in KRAS for testing of specific therapeutic agents [74].

Activating mutations in PIK3CA have been identified in PDAs, but it is not clear how they promote disease progression or whether mutant PIK3CA is a good therapeutic target. In mice, activating mutations in PIK3CA are not sufficient to cause tumor development, and oncogenic mutations in PIK3CA are found in both mutant and non-mutant KRAS tumors [39, 69]. In the context of mouse models, PIK3CA could augment the activity of KRAS in promoting tumor development, and these tumors might be more reliant on PI3K signaling. However, even in the case of breast cancer where PIK3CA mutation contributes to disease initiation/progression, it is unclear whether this event yields selective sensitivity to PI3 kinase inhibitors in the clinic. This is an active area of investigation, as PI3K inhibitors can augment the activity of MEK inhibitors.

DNA repair and chromosome instability

Many PDA cases contain genetic alterations that affect DNA damage repair pathways. Before the advent of next-generation sequencing, a proportion of PDAs were known to contain either germline or somatic mutations in *BRCA1*, *BRCA2*, or Fanconi anemia genes (e.g. *FANCC*, *FANCG*, and *FANCN*/*PALB2*)[75–77]. These genes function in a complex fashion to mediate homologous recombination mediated DNA repair that is required for the maintenance of chromosome stability, and could be hypersensitive to established and new DNA damaging agents[75, 78].

The frequency of BRCA deficiency is estimated to be 5%–8% in unselected patient populations and 12%–15% in certain populations (such as Ashkenazi patients with a family history of breast or ovarian cancer). Recent sequencing studies identified subtypes of PDA characterized by chromosomal instability, probably due to BRCA deficiency or similar deficits in DNA repair[36, 39]. Such deficits in BRCA function have been shown to increase the sensitivity of tumor cells to platinum agents, in multiple models. Consistent with this concept, platinum-based therapy was shown to be effective, in retrospective studies of BRCA-deficient PDAs [79–81]. These observations contradict the concept that BRCA is a biomarker for sensitivity to chemotherapy, as opposed to platinum agents. Trials are underway to evaluate the efficacy of the combination of cisplatin and gemcitabine in patients with locally advanced or untreated BRCA-deficient PDA. A study recently reported that

some patients with chromosomal instability indicative of BRCA deficiency have exceptional responses to platinum-based regimens [36]. Many of these PDAs contained genetic alterations in *BRCA1*, *BRCA2*, or *PALB2*. However, there were cases for which a specific genetic event was not identified.

In addition to platinum agents, work in breast and ovarian cancer have shown that BRCA-deficient cancers are selectively sensitive to poly-ADP-ribose polymerase (PARP) inhibitors[82–84] (Table 3). Ongoing clinical trials are further investigating whether addition of the PARP inhibitor, veliparib, increases patients' response to platinum agents (NCT01585805). Researchers recently presented data from a phase IB trial evaluating the triple combination of cisplatin, gemcitabine, and veliparib in newly diagnosed, untreated patients with PDA and germline mutations in *BRCA* or *PALB2* [85]. These data have defined the safety and tolerability of cisplatin, gemcitabine, and veliparib and indicate the efficacy of the 3-drug combination in these individuals. Significantly higher rates and duration of response and survival were observed in this subgroup, compared in a non-randomized manner to a subgroup of patients with sporadic disease. These observations will be clarified in a prospective randomized phase 2 trial of cisplatin and gemcitabine, with or without veliparib, in patients with newly diagnosed, locally advanced, or metastatic pancreas adenocarcinoma and germline mutations in *BRCA* or *PALB2* (NCT01585805). Results from the first part of phase 1 and 2 trials of 5-FU, oxaliplatin, and veliparib were presented at the American Society for Clinical Oncology-Gastrointestinal Cancers Conference in 2013[86].

PARP inhibitors have activity as single agents and in combination therapies for patients with advanced pancreas adenocarcinoma[87]. Findings have been reported from 2 studies. Kaufmann, et al[88] reported that 5/23 (22%) previously treated patients (23 with gemcitabine and 14 with platinum therapy) with pancreas adenocarcinoma and germline mutations in *BRCA* responded to olaparib as a single agent. Their median survival time was 9.8 months and 41% survived for 1 year. More recently, Lowery et al[89] evaluated velaparib in 16 previously heavily pre-treated patients with PDA and germline mutations in *BRCA*. Although no objective responses were observed, 4 patients (25%) had stable disease ranging from 4 months to 1 year. These studies indicate that a subset of patients with BRCA deficiencies and advanced pancreas cancer can benefit from a PARP-targeted agent. Analogous to the development and registration strategy of olaparib for patients with ovarian cancer, a phase 3 trial (the POLO trial, NCT02184195), is evaluating the maintenance value of olaparib in a 3:2 randomization to placebo following initial treatment with platinum-based therapy in germline BRCA-mutated PDA. This trial has a number of distinctions in that it stands alone as the only phase III trial that is underway in metastatic PDA, and is the first trial to evaluate the role of maintenance therapy in this disease. Table 3 summarizes other related PARP studies underway in patients with pancreatic cancer.

The limits with regard to patient subgroups in targeting tumors with homologous repair defects remain under study. Theoretically, DNA-damaging agents and PARP inhibitors may benefit patients with mutations in *ATM*, *ATR*, *CHEK*, mismatch repair genes, and other genes with similar functions [90]. Targeting the mitotic checkpoint inhibitor WEE1 in cancer cells would ostensibly further sensitize chromosomally unstable PDA cells to

chemotherapeutic agents[91–93]. Trials are underway (NCT01748825 and NCT0182734) to determine the efficacy of this approach in select populations (TABLE 3 and TABLE 5).

Collectively, data support the concept that germline or somatic mutations in BRCA could predict which patients with PDA are most likely to respond to platinum- and PARP-based therapies. Further studies are needed to determine whether mutations in *BRCA* can also be used as prognostic factors for patients with pancreas cancer.

Loss of CDKN2A and CDK4/6 inhibitors

One of the most frequently detected genetic alterations in PDA is disruption or silencing of the tumor suppressor gene *CDKN2A* [94,95]. *CDKN2A* encodes the p16ink4a protein, which inhibits the kinase activity of CDK4 and CDK6[51, 96]. In normal tissue, oncogenic activation of KRAS elicits a stress response that leads to activation of p16ink4a and oncogene-induced senescence [97, 98] Therefore, in many cancers driven by KRAS there is potent selection for the loss of p16ink4a. In PDA this appears to be the preferred mechanism of cell cycle deregulation, consequently PDA may be particularly sensitive to agents that recapitulate the activity of p16ink4a—i.e. the suppression of CDK4/6 activity. Highly potent CDK4/6 inhibitors have been developed, including LEE-011 (ribociclib), PD-0332991 (palbociclib), and LY2835219 (abemaciclib)[51]. These drugs are given orally and are being evaluated in multiple clinical trials.

Preclinical models of PDA have shown mixed responses to these agents. Although a subset of cell lines and patient-derived xenografts appear sensitive to CDK4/6 inhibitors, other PDA cell lines that lack p16ink4a are either intrinsically resistant or rapidly develop resistance in culture [99–101]. These data indicate that loss of CDKN2A/p16ink4a does not, per se, predict response, and that combination approaches will be the most effective means for the use of CDK4/6 inhibitors in PDA. This supposition is consistent with the strong activity of CDK4/6 inhibitors in combination with endocrine therapy in patients with breast cancer [102]. Drug screens have identified mTOR, IGF1R, and MEK inhibitors as effective agents, in combination with CDK4/6 inhibitors, in models of PDA[100, 101].

CDK4/6 inhibitors are not currently in clinical trials for treatment of PDA, specifically. However, there are several ongoing trials of CDK4/6 inhibitors that are germane to PDA (Table 5). Since loss of CDKN2A is common in PDAs, it is likely that patients with this cancer who are enrolled in the Novartis-sponsored SIGNATURE trial will receive the single agent LEE-011 (NCT02187783). Similarly, the trial of palbociclib with the MEK inhibitors PD-0325901 (NCT02022982) or trametinib (NCT02065063) will likely include patients with PDA, given the targeted scope toward tumors with RAS mutations. With the recent Food and Drug Administration approval of palbociclib in combination with letrozole for estrogen receptor-positive breast cancer, it is likely that the number trials investigating the effects of the CDK4/6 combination in patients with PDA will increase.

The WNT pathway

The WNT pathway is altered in many types of gastrointestinal malignancies, such as via APC mutation in colorectal tumors [103]. It has come to be recognized that this pathway is deregulated via multiple distinct genetic events in PDA and is functionally important for

disease [104, 105]. Interestingly, unlike colon cancer, in which APC mutations are particularly common, pancreatic tumors contain a wide spectrum of mutations. RNF43 and AXIN1 are more frequently disrupted in PDA, whereas APC is less-frequently lost[36, 39, 73]. RNF43 alterations are observed in IPMN as well as PDAs and associated cell lines [45, 47, 106]. WNT signals to the β-catenin/TCF4 transcription factor that represents the downstream target of the pathway. RNF43 expression is induced by TCF4 in order to attenuate deregulated WNT signaling[50]; therefore, mutation of RNF43 leads to constitutive signaling through the pathway. AXIN and APC participate directly in the degradation of β-catenin. Although WNT signaling has been considered a therapeutic target for many years, only recently have agents specific for the pathway emerged reflecting difficulty in developing therapeutic agents that act on tumor suppressors and transcription factors[50]. The most advanced of these are WNT-974 (also known as LGK974). This agent functions by suppressing porcupine, which is required for secretion of WNT ligands; it has selective activity in pancreatic cancer cell lines deficient in RNF43, as well as in xenograft tumors[106]. Based on these data there is a clinical trial testing LGK974 in patients with tumors with dysregulated WNT signaling (NCT01351103). Criteria for inclusion in the study include the loss of RNF43 or other mediators of WNT signaling that are deregulated in pancreatic cancer.

NOTCH

The NOTCH pathway is also deregulated in multiple tumor types. NOTCH mediates self-renewal and proliferation of cancer stem cells and its activity is associated with chemoresistance and metastasis[107, 108]. Based on genetic analyses, mutations in *NOTCH* are relatively rare, but multiple components of the pathway appear to be amplified, consistent with the overexpression and observed deregulation of the pathway in PDA [39, 109, 110]. Overexpression of NOTCH signaling components in pancreatic tumors has been associated with poor outcomes of patients. NOTCH signaling is required for pancreatic tumor progression and metastasis in mouse models [111], so pancreatic cancer is considered to be relatively dependent on NOTCH signaling—either in parallel with KRAS signaling or independently. Irrespective of the mechanism, studies of cell lines, xenograft tumors, and genetically engineered models have demonstrated that suppression of NOTCH has potential for therapeutic efficacy[112–115].

The NOTCH signaling pathway can be inhibited pharmacologically, with inhibitors of γ-secretase, antibodies, and other mechanisms[108]. The γ-secretase is required to transmit NOTCH signals from the membrane to the nucleus. Inhibitors of γ-secretase have been developed by multiple pharmaceutical companies and have been tested in clinical trials, including those of patients with pancreatic cancer. BMS-906024 and PF-03084014 inhibitors are in clinical development (TABLE 4). There are only a few results from studies of single agents in patients with PDA. The agent RO4929097 was evaluated in a single-arm phase 2 trial of patients with previously treated metastatic PDA, the trial was closed for accrual with discontinuation of the agent by the sponsor.

Monoclonal antibody-based therapies have also been evaluated. Tarextumab (OMP-59R5) is a fully human antibody against NOTCH2 and NOTCH3 that slows growth of xenograft tumors in mice in combination with cytotoxic agents [116]. It is currently being tested in a

randomized, placebo-controlled, phase 2 trial (NCT01647828), in combination with gemcitabine and nab-paclitaxel in untreated patients with metastatic pancreas adenocarcinoma (TABLE 4). In addition to evaluating the treatment signal in all patients, the trial will evaluate specifically the activity of Tarextumab in a biomarker selected subgroup of patients with high levels of NOTCH 3 expression, a particularly unfavorable prognostic subgroup. Preliminary results from the phase 1B trial demonstrated significant activity of a 15 mg/kg dose of tarextumab combined with standard doses of gemcitabine and nab-paclitaxel. The reported median time of progression-free survival was 5.6 months, and median time of overall survival was 11.6 months; 38% of this patient population had a response to the 3-drug combination, and response rate was even higher in the small number of patients whose tumors expressed high levels of NOTCH3 [117].

SWI/SNF chromatin remodeling complex

Members of the family of SWI/SNF chromatin remodeling factors are mutated in many tumor types [118, 119]. All sequence studies of pancreatic tumors have reported disruptions in genes encoding these factors [34, 35, 38, 39]. Loss of ARID1A is the most common single event, but loss of other subunits, including ARID1B and SMARCA4, has been observed (TABLE 2). These molecules function in a large complex to facilitate the fluidity of chromatin between activated and repressed states. The specific effects of loss of these factors are hard to determine, because disruption of this complex affects chromatin stability as well as transcription of many genes. As for many tumor suppressors, it is unclear whether inhibitors of chromatin remodeling factors could be effective, although such agents are under development.

Rather, in response to the loss of ARID1A and other factors that control chromatin remodeling, compensatory pathways could be activated. RNA-intereference screens demonstrated that ARID1A–deficient cells were particularly sensitive to the selective depletion of ARID1B[120]. Although this finding is important for our understanding of the mechanisms of chromatin remodeling, it might not have much clinical application. A complementary drug screen found EZH2 activity to be required for the viability of ARID1A–deficient ovarian cancer cells[121]. Multiple agents have been developed that target EZH2. Several EZH2 inhibitors are being tested in clinical trials. E7438, GSK281612, and CPI-1205 are in phase 1 dose-finding studies, mostly comprising patients with lymphoid malignancies. Given the recent nature of the preclinical findings, no trials are underway to study the efficacy of EZH2 in patients with tumors that have lost ARID1A.

Canonically untargetable pathways

In addition to the pathways described above, there are multiple additional genetic alterations that could in principle be used as the basis for rational treatment. However, these pathways are not routinely targeted pharmaceutically.

Amplifications in *MYC* are frequently observed in PDA (~15% of cases), and overexpression of MYC has been shown to promote tumor development in mice. While generally considered untargetable (as is the case with many oncogenic transcription factors), recent studies have suggested unique vulnerabilities that could be exploited in the context of

MYC-driven disease. These include CDK9 and BET-bromodomain inhibitors which are potently selective for MYC tumors in preclinical models[122, 123]. However, whether this strategy could be effective in MYC-amplified PDA remains unknown.

Approaches to therapeutically target the loss of TGFB signaling in PDA cells could be useful given the frequent loss of SMAD4, TGFBR2, and other elements of the pathway. In spite of the clear importance of targeting loss of this pathway, there has been no definition of synthetic lethal or other approaches that could selectively target this subset of tumors. However, SMAD4 is emerging as a biomarker for a poor prognostic phenotype in PDA [28]. It's utility as a biomarker for clinical decision making is also being prospectively evaluated in a randomized phase II trial in locally advanced pancreas adenocarcinoma where SMAD4 loss and intact status will be evaluated and correlated with an intensively focused loco-regional treatment approach of combination cytotoxic therapy and high dose intensity modulated radiation versus a systemic therapy based approach (NCT01921751).

TP53 is mutated in most human tumor types, and multiple drug development programs have been initiated to exploit this event in a targeted manner. Although there have been many promising results from preclinical studies, clinical development of agents designed to reactivate TP53 has been slow. APR-246 is the only agent in this category that is being evaluated in clinical trial (NCT02098343).

Targeting Genetic Diversity in PDAs

PDA contains many genetic alterations that could be targeted therapeutically, based on studies from other tumor types or preclinical investigations. However, there are several important factors to consider in leveraging such information to improve clinical outcomes.

Importance of additional preclinical studies

Preclinical studies are needed to determine the functional effects of the genetic alterations observed in PDA cells. Many agents are known to have strong potency against a select target; at the same time there is an ever-emerging sense that genetic activation of a target does not universally predict response. For example, colon cancer cells with BRAF mutations rarely respond to BRAF inhibitors, due to compensatory EGFR signaling[125].

Preclinical models that recapitulate the genetic diversity of PDA are of paramount importance. Genetically engineered mouse models provide one approach that could be complemented by studies of patient-derived xenografts and/or new, sophisticated, patient-derived in vitro models [126–128].

Pancreatic tumors in genetically engineered mouse models develop along the same pathways as human PDAs (e.g., in the context of KRAS activation) and in an immune-competent host. However, studies from other systems suggested that the genetic factors that contribute to development of tumors in these mice differ from those of humans [129, 130]. Although there have been no formal investigations, it is unlikely that tumors from mouse models have the same level of genetic diversity and pathway activation observed in tumors from patients.

In contrast with genetically engineered mouse models, patient derived xenografts should harbor the genetic features of the parental tumor and therefore better recapitulate the biology of an individual tumor. However, these tumors are grown in a different environment, which could select for additional features distinct from the primary tumor and affect their response to test agents. An emerging model for PDA is that of the organoid culture model system, which has attractive features of providing a model system for interrogation in a proximate time period and providing the opportunity for genetic evaluation, pre-clinical modeling, and other considerations[126].. Importantly, biotechnology companies and academic centers routinely generate models from resected tumors or biopsies in real-time, and the sensitivity of such models to therapeutic agents can be determined and provided to the physician. The extent to which these approaches will translate into patient care remains unclear; however, clinical trials testing this concept are being initiated.

Defining surrogates of response

In contrast with other diseases, it is a challenge to evaluate responses of PDA to drugs via window, neoadjuvant, or serial biopsy analyses. Although there is increasing use of systemic therapy in the neoadjuvant setting, there is no consistent use of pre-operative therapy to determine the ability of agents to impinge on tumors or their target [131, 132]. Additionally tissues are not routinely collected pre and during treatment hampering discovery of potential response markers. In the context of breast cancer, these studies proved that acute suppression of Ki67 to endocrine therapy pre-operatively largely predicted durable response in ER-positive breast cancer. This type of approach and trials which are exploratory in relation to genetics and determinants of response to investigational drugs, are limited in patients with PDA, although several examples are extant (e.g., NCT02241187). Functional imaging or other non-invasive approaches, including evaluation of cell-free DNA and other liquid biopsy approaches to measure tumor response, will be particularly important in evaluating targeted treatment approaches. The only routinely used surrogate in the clinic is the serum marker of tumor burden, CA19–9[133, 134].

Beyond conceived genetic sensitivities

There are a number of promising therapeutic modalities for which there is not a clear concept as to what genetic events could be associated with sensitivity. For example, immunotherapeutic strategies are providing some impressive, landmark outcomes in other tumor systems (e.g., melanoma) [135, 136] To date, such successes have not been matched in PDA, yet still there is hope that development of a vaccine or checkpoint-based approaches will work in some patients[137]. However, it has recently emerged that mismatch repair deficiency, as is observed in a small set of PDA cancers likely represent a predictive marker[138]. Similarly, approaches to target the PDA microenvironment, such as hyaluronic acid, are being tested in early-phase trials[139].

The NCI Match trial is a large-scale, phase 2, disease-agnostic trial design that proposes to evaluate a series of targeted agents in parallel arms[140]. Eligibility will be determined based on results of next-generation sequence analyses; it is anticipated that approximately 3000 patients will be screened and approximately 1000 patients will be selected based on targetable genomic features of their tumors. The study will evaluate approved and

investigational agents. Tumor response will be the primary endpoint, along with progression-free survival. This trial will present a broad opportunity for a percentage of patients with PDA to participate in a precision medicine-based approach.

Doublet and Combination Therapies

With a few exceptions, it seems unlikely that targeting a single genetic feature of pancreatic cancer will produce durable or transformative effects. This concept is reinforced when reviewing the pathway landscape of PDA (FIGURE 1), wherein most case exhibit a range of deregulated pathways. Studies of MEK inhibitors in PDA have provided insight into lack of efficacy—most PDAs have genetic deregulation of KRAS, so MEK should be an ideal target. Therefore, it seems to be necessary to target multiple pathways to improve treatment outcomes. This concept has been particularly well developed for estrogen receptor-positive breast tumors, in that addition of an active agent to endocrine therapy improved the durability of response to therapy (as shown with mTOR and CDK4/6 inhibitors). Against this backdrop, trials such as the NCI MATCH and Novartis SIGNATURE are largely matching single agents with single genetic features of tumors. Given that most pancreatic tumors have multiple genetic features that promote their progression, it will be important to move beyond single agent approaches, based on genetics, and consider strategies to target two or more key signaling pathways in parallel.

How can we perform multi-genetic and combination studies? Ostensibly, patients with tumors that contain two genetic variants frequently detected in pancreatic cancer could be treated with a particular drug combination. For example, patients whose tumors have a combination of KRAS mutation and CDKN2A loss could receive a combination of MEK and CDK4/6 inhibitors. Although there have already been a number of combination trials directed specifically against tumors with mutant KRAS (TABLE 5), specification of the drug combination has not been rationally directed. An alternative approach would be to use a standardized backbone therapy, which would be combined with a pathway selective inhibitor (e.g. *NOTCH* amplification specifies a g-secretase inhibitor, or an *RNF43* mutation specifies a porcupine inhibitor). Such a trial design would be completely dependent on a better understanding of the effects of drug combinations, not only in relation to their potential benefit but the toxicity profile and optimized dosing schedules.

Future Directions

Progress in the treatment of PDA has been incremental. Arguably, combination cytotoxic therapies such as FOLFIRINOX, along with gemcitabine and albumin-bound paclitaxel, have provided meaningful gains, but there is room for improvement. Our understanding of the PDA genome has increased and provides insight into focused therapeutic approaches; there is emerging consensus that subsets of patients with PDA may benefit from targeted approaches. Agents designed to exploit DNA repair pathways and NOTCH signaling are in late stages of clinical development. It will be important to identify subgroups of patients with tumors most likely to benefit from agents designed to target specific pathways or genomic features.

The next generation of clinical trials needs to be thoughtfully designed and based on optimal preclinical results. It is important to select rationally tailored approaches for each study participant, and produce detailed results that provide insight into mechanisms of sensitivity and resistance. Yielding a transformative impact on survival rates for PDA will require a multi-fold approach. Fundamental research that provides a better understanding of the pathways/genes driving PDA singly and in the complex patterns observed in human disease will be required to define key drug targets and therapeutic vulnerabilities that can be exploited in the clinic. Well-designed biomarker-driven clinical trials that acknowledge the genetic complexity and challenges of treating PDA will be seminal for a targeted approach to treatment of PDA. Iterative learning from mis-steps, exceptional responses, and selected subgroup analyses will support the ultimate development of guided treatment for progressively more patients with PDA. Hopefully, such a concerted effort will yield the critical advances that have long proved elusive in this therapy recalcitrant disease.

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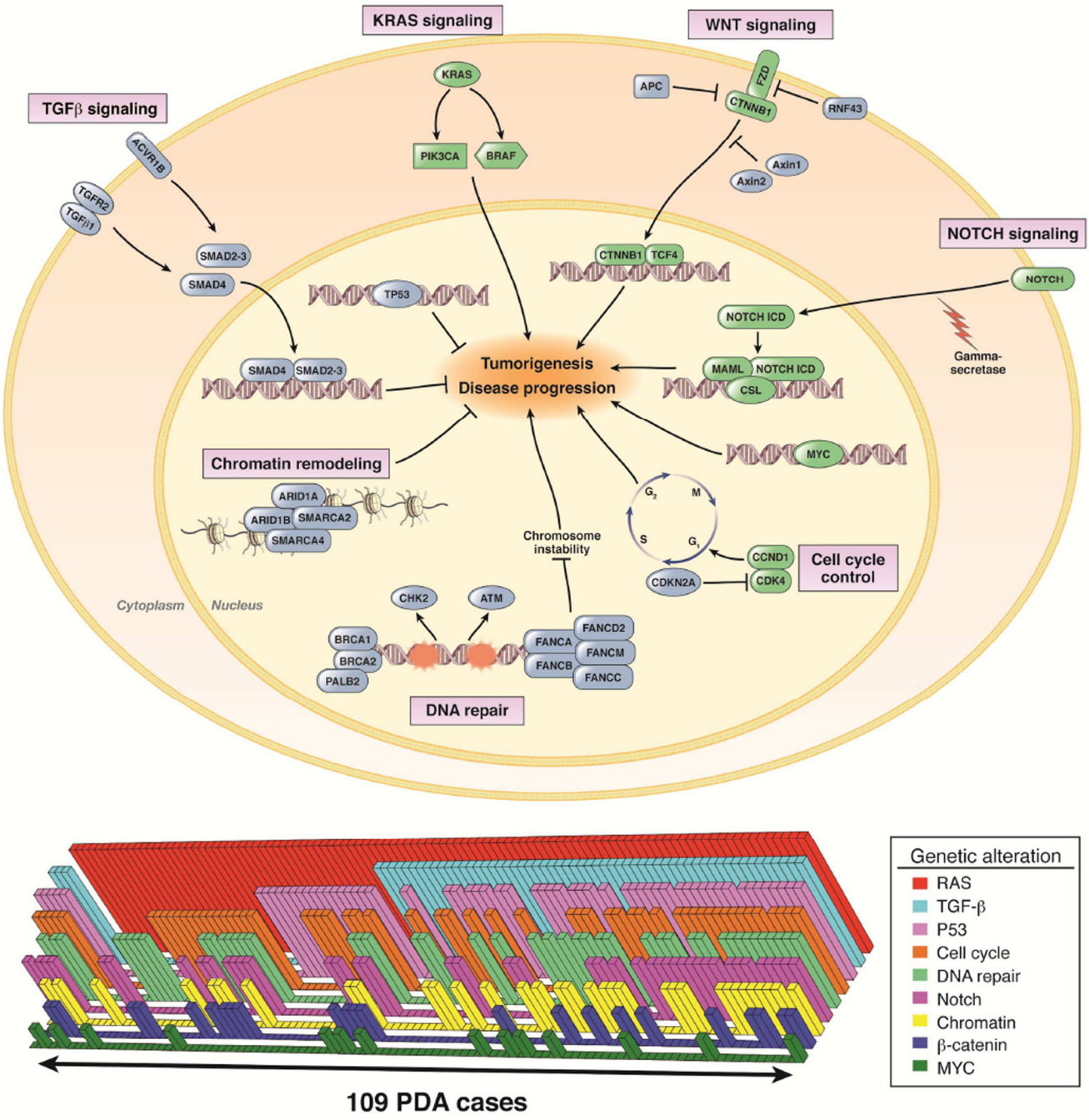

Figure 1. Diversity of oncogenic and tumor suppressor pathways in PDA

A diagramatic representation of pathways that contribute to the etiology or progression of pancreatic cancer. Green denotes oncogenic and blue denotes tumor suppressive activities that are genetically altered in PDA. The lower bars represent alterations in the indicated pathway across 109 cases from Wiktiewicz et al., 2015. This graph illustrates the multi-fold complexity of pathway alterations between individual cases.

Table 1

Summary of whole exome and genome sequencing studies in PDA.

Study	Clinical Cases	Xenograft Cell Lines	Method
Jones et al., 2008		24	Exome
Yachida et al., 2010[2]		7	Exome
Campbell et al., 2010[2]	3	10	Genome
Liang et al., 2012	3		Genome
Biankin et al., 2012	99		Exome
Wang et al., 2012		15	Exome
Murphy et al., 2014[1,3]	10		Exome
Waddell et al., 2015	75	25	Genome
Witkiewicz et al., 2015[1]	109		Exome
Dal Molin et al., 2015	8		Exome

[1]Denotes microdissected cases.

[2]Denotes models from matched primary metastatic cases.

[3]Denotes studies inclusive of PANIN lesions

Table 2

Approximate frequency of selected genetic alterations in PDA.

Pathway	Gene	Approximate % Altered	Predominant Genetic Events	Potential Therapeutic Targets
KRAS	KRAS	90	Mutation	MEK, PI3K
	BRAF	3	Mutation	BRAF
	PIK3CA	3	Mutation	PI3K
TGF-β	SMAD4	30–40	Mutation/Deletion	NA
	TGFBR2	5–10	Mutation	
	ACVR1B	6	Mutation	
P53	TP53	50–70	Mutation/Deletion	P53 reactivation
MYC	MYC	10	Amplification	CDK9, BET domain
CELL CYCLE	CDKN2A	40–60	Deletion/Mutation	CDK4/6
	CCND1	10	Amplification	CDK4/6
	CDK4	10	Amplification	CDK4/6
WNT	RNF43	10–15	Mutation/Deletion	WNT, Tankyrase, Porcupine
	AXIN1	5–10	Mutation/Deletion	WNT, Tankyrase, Porcupine
	APC	2	Mutation/Deletion	WNT, Tankyrase, Porcupine
NOTCH	NOTCH1	10	Amplification	Gamma Secretase, Notch
	NOTCH2	6	Amplification	Gamma Secretase, Notch
	NOTCH3	6	Amplification	Gamma Secretase, Notch
CHROMATIN	ARID1A	10–25	Mutation/Deletion	EZH2, PI3K
	ARID1B	5–20	Mutation/Deletion	
	SMARCA4	5	Mutation/Deletion	
	SMARCA2	5–20	Mutation/Deletion	
DNA Damage	BRCA1	5	Mutation	DNA cross link, PARP
	BRCA2	7	Mutation	DNA cross link, PARP
	PALB2	3	Mutation	DNA cross link, PARP
	ATM	6	Mutation	
	FANC genes (A-M)	10–15	Mutation	DNA cross link

Table 3

Selected trials evaluating PARP inhibitors in advanced pancreatic adenocarcinoma

NCT	Trial Description	N	Sponsor
01489865	FOLFOX + Veliparib Wild-type + germline BRCA Untreated, previously treated Phase I-II	48	AbbVie
01585805	Cisplatin, Gemcitabine +/− Veliparib Germline BRCA, PALB2 Randomized phase II	50	MSKCC/NCI Lustgarten
01585805	Veliparib Germline BRCA, PALB2 (previously treated) Phase II	15	MSKCC/NCI Lustgarten
01296763	Irinotecan, Cisplatin, Mitomycin C +/− Olaparib Wild-type + germline BRCA Phase I-II Results awaited	18	John Hopkin's Cancer Center
01482715	Rucaparib Germline, somatic BRCA (previously treated) Phase II	100	Clovis
01286987	BMN-673 Germline BRCA (any solid tumor) Phase I		BioMarin
02184195	Platinum therapy followed by Olaparib/Placebo Germline BRCA Phase III maintenance	145	Astra-Zeneca POLO Trial

Table 4

Selected novel agents in development in pancreatic adenocarcinoma in the metastatic setting

NCT	Trial Design	N	Target	Sponsor
02428270	A Study of GSK2256098 and Trametinib in Advanced Pancreatic Cancer	24	FAK MEK	University Health Network, Toronto
01839487	Gem + nab-P ± PEGPH20 Rand phase II	132	Hyaluronan	Halozyme
01959139	mFOLFIRINOX ± PEGPH20 Rand phase II	172	Hyaluronan	SWOG/ S1313
01621243	Gem + nab-P ± Necuparanib Rand phase II	148	Anti-stromal Heparin mimetic	Momenta
01647828	Gem + nab-P ± Tarextumab (Alpine) Rand phase II	140	Notch, stem cell	OncoMed
01844817	Gem + nab-P ± OGX-427 (Rainier) Rand phase II	132	HSP27	OncoGenix
02101021	Gem + nab-P ± Momelotinib Phase IB - rand ph II	336	JAK 1/JAK2	Gilead
02289898	Gem + nab-P ± Demcizumab (Yosemite) Rand phase II	201	Anti-DLL4, stem cell	OncoMed
02077881	Gem + nab-P ± Indoximod Rand phase II	80	IDO	NewLink Genetics
02109445	Gem + nab-P ± PF-03084014 Rand phase II	193	γ-Secretase inhibitor, Notch	Pfizer
02194829	Gem + nab-P ± MK-1775 Phase IB - rand ph II	133	Wee-1 inhibitor	Merck
02050178	Gem + nab-P + OMP-54F28 Phase IB	20	Frizzled	OncoMed
02101580	Gem + nab-P + ADIPEG 20 Phase IB	21	Arginine depletion	Polaris

Table 5

Selected marker targeted studies that could enroll PDA patients for targeted interventions

NCT	Trial Design	N	Target	Sponsor
02079740	Trametinib and Navitoclax in Treating Patients With Advanced or Metastatic Solid Tumors (KRAS mutant tumors)	130	MEK BCL2	GSK
02230553	Lapatinib Plus Trametinib in KRAS Mutant Malignancies (M14LTK)	30	MEK EGFR	Netherlands Cancer Institute
02039336	Dacomitinib Plus PD-0325901 in Advanced KRAS Mutant Malignancies	35	MEK EGFR	Netherlands Cancer Institute
01986166	A Study of MEHD7945A and Cobimetinib (GDC-0973) in Patients With Locally Advanced or Metastatic Cancers With Mutant KRAS	50	MEK EGFR	Genentech
01449058	A Phase Ib Study of MEK162 Plus BYL719 in Adult Patients With Selected Advanced Solid Tumors	138	MEK PI3K	Novartis
02187783	LEE-011 for tumors with pathway defects (loss of CDKN2A amplification of CCND1/3 or CDK4/6) SIGNATURE	90	CDK4/6	Novartis
02065063	Palbociclib in combination with trametinib for solid tumors (Phase 1/2)	100	CDK4/6 MEK	GSK
02022982	Palbociclib in combination with PD-0325901 for KRAS mutant tumors (Phase 1)	30	CDK4/6 MEK	Pfizer
01959139	LGK974 in Patients With Malignancies Dependent on Wnt Ligands	100	WNT	novartis
02152254	IMPACT 2: Randomized Study Evaluating Molecular Profiling and Targeted Agents in Metastatic Cancer	1362	Multiple	MD Anderson
01827384	NCI-MPACT: Molecular Profiling-Based Assignment of Cancer Therapy for Patients With Advanced Solid Tumors	700	DNA repair KRAS PI3K/MTOR	NCI
Pending	NCI-MATCH: Molecular Analysis for Therapy Choice	1,000	20–25 Agents	NCI