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Review

The role of molecular testing in pancreatic cancer

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***Abstract*:** Pancreatic ductal adenocarcinoma (PDA) is highly aggressive and has few treatment options. To personalize therapy, it is critical to delineate molecular subtypes and understand inter- and intra-tumoral heterogeneity. Germline testing for hereditary genetic abnormalities is recommended for all patients with PDA and somatic molecular testing is recommended for all patients with locally advanced or metastatic disease. *KRAS* mutations are present in 90% of PDA, while 10% are *KRAS* wild type and are potentially targetable with epidermal growth factor receptor blockade. $KRAS^{G12C}$ inhibitors have shown activity in G12C-mutated cancers, and novel G12D and pan-RAS inhibitors are in clinical trials. DNA damage repair abnormalities, germline or somatic, occur in 5–10% of patients and are likely to benefit from DNA damaging agents and maintenance therapy with poly-ADP ribose polymerase inhibitors. Fewer than 1% of PDA harbor microsatellite instability high status and are susceptible to immune checkpoint blockade. Albeit very rare, occurring in <1% of patients with *KRAS* wild-type PDAs, *BRAF V600E* mutations, *RET* and *NTRK* fusions are targetable with cancer agnostic Food and Drug Administration-approved therapies. Genetic, epigenetic, and tumor microenvironment targets continue to be identified at an unprecedented pace, enabling PDA patients to be matched to targeted and immune therapeutics, including antibody–drug conjugates, and genetically engineered chimeric antigen receptor or T-cell receptor – T-cell therapies. In this review, we highlight clinically relevant molecular alterations and focus on targeted strategies that can improve patient outcomes through precision medicine.

Keywords: biomarkers, molecular testing, pancreatic cancer, precision medicine, targeted therapy

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Introduction

With a 5-year survival of only 11%, pancreatic ductal adenocarcinoma (PDA) is one of the deadliest tumors, highly resistant to chemotherapy, radiotherapy, and immunotherapy.[1–5] Combination chemotherapy regimens, such as FOLFIRINOX (folinic acid, 5-fluorouracil, irinotecan, oxaliplatin) and gemcitabine plus nab-paclitaxel (Gem-nabP), are standard first-line regimens in metastatic disease, with median survival less than 12 months.[6–8] Neither regimen has so far been informed by biomarker status, although recent data suggest that low GATA-binding protein 6 expression by immunohistochemistry (IHC), correlating to a basal subtype PDA, may confer resistance to FOLFIRINOX, whereas no effect on survival was observed with Gem-nabP.[9]

The promise of precision oncology has become a reality for certain malignancies, such as lung cancers and melanomas, and more recently, actionable targets have also been demonstrated in PDA. PDA is largely defined by core driver mutations in genes such as Kirsten ras (*KRAS*, 90%), tumor protein 53 (*TP53*, 64%), cyclin-dependent kinase inhibitor 2A (*CDKN2A*, 17%), and SMA and MAD-related protein 4 (*SMAD4*, 21%), but among them, only *KRAS* p.G12C mutations (1–3% of tumors) and *TP53* p.Y220C mutations (0.64% of tumors) have been clinically targetable

to date.[10–13] Additional mutations in DNA damage repair (DDR) genes and chromatin-modifying genes are present at lower-level frequencies.[14,15] *KRAS* wild-type status, present in 10% of PDA and up to 20% in younger patients, is an entity enriched with targetable alterations including microsatellite instability (MSI-high) and elevated tumor mutational burden (TMB high), *ERBB2* amplification, *BRAF* mutations, as well as *ALK, FGFR1-3, NRG1, NTRK1-3, RET*, and *ROS* fusions.[15,16]

Besides somatic gene alterations, PDA is driven by germline genetic predisposition, with 3–8% of patients harboring deleterious germline variants in *BRCA2/1, PALB2, ATM, CDKN2A, STK11*, or mismatch repair (MMR) genes,[17–20] with higher incidence in patients with a family history of PDA (10–13%),[21–23] those from founder populations (up to 14.2% of Ashkenazi Jewish patients with a family history of breast and pancreatic cancer),[24,25] and those with early onset PDA (28.6% of patients <50 years old at diagnosis).[26]

A main research focus in PDA aims to connect molecular alterations with therapies targeting specific cellular pathways. Germline testing, comprehensive tumor next-generation sequencing, and liquid biopsies testing circulating cell-free DNA (cfDNA) reveal targets for therapeutic intervention.[27] In addition, immune-related biomarkers such as MSI-high, TMB-high,[28] inflamed T-cell signature profiles,[29] and tumor immune microenvironment phenotyping[30] have demonstrated predictive and/or prognostic implications. While PDA has been transcriptionally profiled into 'basal-like' and 'classical' associated with poor *versus* better prognosis, the value of selecting therapies based on these subtypes remains under investigation.[14,31,32]

Preliminary whole exome and RNA sequencing and clinical studies and registries support molecular testing in PDA patients and note the positive impact of personalized therapies on outcomes. Aguirre *et al.* identified therapeutically relevant genomic alterations in 48% of PDA patients, with 18% having pathogenic/likely pathogenic germline alterations.[33] The Pancreatic Cancer Action Network Know Your Tumor registry demonstrated that applying matched targeted therapies to molecular alterations doubled patient survival compared to standard of care chemotherapy.[34] The 2020 American Society of Clinical Oncology (ASCO) Guidelines[35] and National Comprehensive Cancer Network (NCCN) guidelines[6] have incorporated germline mutations testing for all newly diagnosed PDA, and somatic molecular testing from tumor biopsies or cfDNA (when tumor biopsy is not feasible) for all with locally advanced or metastatic disease.

Here we review the most significant actionable molecular alterations in PDA (Table 1, Figure 1) and summarize key clinical studies which evaluated the benefit of targeted therapies, including novel targets and studies in development (Tables 2 and 3).

Table 1. Molecular testing in advanced PDA.

Biomarker	Freq%	Test(s)	Clinical utility	Level of evidence
DDR gene mutation	5–10	Tumor, germline, and paired NGS	Platinum chemo	++++
			PARP inhibitor	++++
			FOLFIRI chemo	++
HRD signature	Unknown	Paired NGS, limited tests and not well validated	Platinum chemo	±
			PARP inhibitor	±
			FOLFIRI chemo	±

(Continued)

Table 1. (Continued)

Biomarker	Freq%	Test(s)	Clinical utility	Level of evidence
MSI/dMMR	~1	MSI, IHC, tumor NGS	Immunotherapy	++
TMB	~1	Tumor NGS large panel	Immunotherapy	++
Tumor immune microenvironment	N/A	Not currently widely available	Immunotherapy	Emerging
KRAS^{G12C}	1–3	Tumor NGS, targeted	*KRAS* G12C inhibitors	++
KRAS$^{Wild\ Type}$	~10	Tumor NGS, targeted	EGFR TKI	+
			EGFR Ab	++
ERBB2 (HER2) overexpression	2-3	IHC, FISH, NGS	HER2 inhibitors (TKI, Ab, ADC)	++
NRG1 fusion	0.5	Tumor NGS, RNA	HER2/3 inhibitors	++
BRAF V600E mutation	2	Tumor NGS, targeted, RNA (for fusions)	BRAF + MEK inhibitors	+++
RET fusion	~1	Tumor NGS, RNA	RET inhibitors	+++
ALK, NTRK1, ROS1 fusions	<1	Tumor NGS, RNA	ALK, ROS1, TRK inhibitors	+++
MET overexpression	>20	Tumor NGS, FISH	MET inhibitor	Emerging
ARID1A mutation	2–8	Tumor NGS	*EZH2* inhibitors	Emerging
TP53^{Y220C}	<1	Tumor NGS, targeted	*TP53* Y220C inhibitors	+
FGFR fusion and mutation	8	Tumor NGS, FISH	FGFR inhibitor	++

Ab, antibody; ADC, antibody–drug conjugate; DDR, DNA damage repair gene (e.g. *BRCA1, BRCA2, PALB2*); dMMR, deficient mismatch repair; HRD, homologous recombination DNA repair; MSI, microsatellite instability; NGS, next-generation sequencing; TKI, tyrosine kinase inhibitor; TMB, tumor mutational burden.

DNA damage repair

Almost 20% of PDA harbor somatic or germline mutations in DDR genes such as *BRCA1*, *BRCA2*, *PALB2*, *RAD51C*, and *RAD51D*.[36–38] Alterations in some DDR genes, especially biallelic inactivation of *BRCA1* and *BRCA2*, can lead to a homologous recombination repair deficient (HRD) phenotype. HRD imparts susceptibility to irreversible DNA damage upon exposure to DNA damaging agents, including chemotherapy and radiotherapy, as well as poly-ADP ribose polymerase (PARP) inhibitors.[38,39] While several DDR defects other than the core *BRCA1/2/PALB2/RAD51* genes have been thought to impart HRD, recent data in PDA do not demonstrate an HRD phenotype or synthetic lethality with DNA damaging agents from non-core HRD genes.[40–42] Moreover, not all *BRCA1/2/PALB2* gene mutations confer loss of protein function, hence may not uniformly result in therapeutic benefit from targeted therapy with PARP inhibitors. Assessment of functional HRD is critical in identifying patients who benefit from PARP inhibitors.[43]

Several assays such as Myriad's MyChoice HRD CDx assay approved by the Food and Drug Administration (FDA) as a companion diagnostic

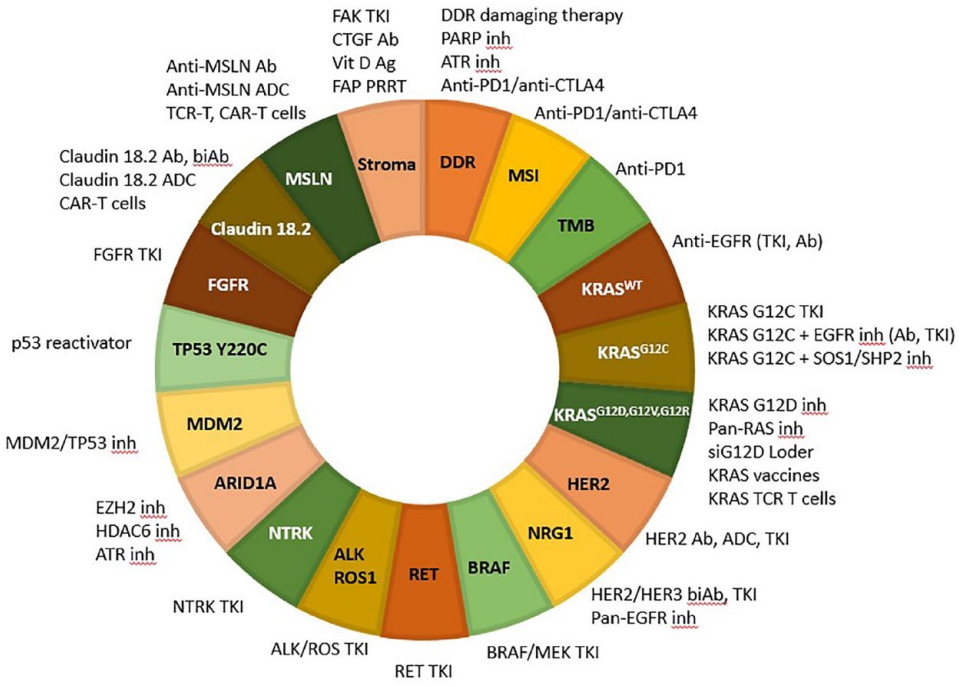

Figure 1. Key molecular targets and targeted therapies in PDA.
Figure 1 shows actionable molecular targets in color-coded sections, and corresponding therapeutic agents are listed outside respective sections.
Ab, antibody; ADC, antibody–drug conjugate; biAb, bispecific antibody; inh, inhibitor; PDA, pancreatic ductal adenocarcinoma; TKI, tyrosine kinase inhibitor.

for niraparib and olaparib in gynecologic malignancies detect *BRCA1/2* mutations and compute a genomic instability score incorporating loss of heterozygosity,[44] large-scale transitions,[45] and telomeric allelic imbalances.[46] In addition, patterns of HRD single base substitution signatures, combined with indel micro-homology, and structural rearrangements created a weighted model called the HRDetect score, able to predict *BRCA1/2* inactivation with a specificity of 98.7%.[47] The applicability of HRD scores to PDA is unknown.

Several clinical studies tested DNA damaging agents such as platinum chemotherapy and topoisomerase inhibitors, and PARP inhibitors in PDA including cancers those harboring *BRCA1/2/PALB2* mutations. Olaparib, rucaparib, and veliparib conferred modest overall response rates (ORR) of 0–22% in previously treated germline *BRCA1/2*-mutated PDA.[48-51] Combination strategies with chemotherapy have also been tested as first- and second-line treatment for metastatic PDA. First-line gemcitabine and cisplatin with or without veliparib in germline *BRCA1/2*-mutated PDA resulted in ORR of 65–74%, median progression-free survival (PFS) and overall survival (OS) of 10 and 19 months, respectively, with no benefit from veliparib.[52] The SWOG S1513 study tested second-line FOLFIRI with and without veliparib in unselected patients, but all underwent genomic sequencing with the BROCA-HR assay.[53] While veliparib did not improve survival among all patients (median OS 5.4 *versus* 6.5 months, and median PFS 2.1 *versus* 2.9 months with veliparib *versus* control), patients with core (*BRCA1/2/PALB2*) and non-core HRD alterations (e.g. *ATM*, *ATR*) *versus* wild-type HRD treated with FOLFIRI had higher median PFS (7.3 *versus* 2.5 months, $p = 0.05$) and OS (10 *versus* 6 months, $p = 0.17$).

Given overlapping myelosuppression and gastrointestinal toxicity between PARP inhibitors and chemotherapy hindering efficacy, maintenance strategies tested PARP inhibitors in patients with platinum-sensitive PDA [after response or stable disease (SD) from platinum chemotherapy]. The phase III POLO trial evaluated maintenance olaparib *versus* placebo in germline

Table 2. Active studies of targeted therapies in PDA.

Target	Clinical trial Nr (clinicaltrials.gov)	Study design	Study population	Therapy
DNA damage repair	NCT04666740	II	Metastatic PDA with HRD	Olaparib + Pembrolizumab
	NCT04548752	II randomized	*BRCA1/2*+ Metastatic PDA	Olaparib ± Pembrolizumab
	NCT04858334	II randomized	*BRCA1/2/PALB2*+ resected PDA	Olaparib *versus* placebo
	NCT04673448	Ib	*BRCA1/2*+ advanced PDA, breast, ovarian, primary peritoneal	Dostarlimab + niraparib
	NCT04493060	II	*BRCA1/2/PALB2* + advanced PDA	Dostarlimab + niraparib
	NCT02595931	I	Advanced solid tumors	M6620 (ATR inhibitor) + irinotecan
Immune microenvironment	NCT04635995	I	Advanced solid tumors	LVGN7409 (CD40 ag)+ LVGN3616 (anti-PD-1) + LVGN3616 + LVGN6051 (CD137 ag)
	NCT05165433	I/Ib		NG-350A (anti-CD40 adenoviral vector) + pembrolizumab
	NCT04857138	I		RO7300490 (FAP targeted CD40 ag) ± atezolizumab
	NCT04888312	Ib/II	Metastatic PDA	Mitazalimab (CD40 ag) + mFOLFIRINOX
	NCT02600949	I	Advanced PDA or colorectal	Peptide vaccine + imiquimod + pembrolizumab + sotigalimab
Focal adhesion kinase	NCT04331041	II randomized	Borderline or locally advanced PDA	SBRT + defactinib
	NCT03875820	I	Advanced solid tumors	Defactinib + VS-6766 (RAF/MEK inhibitor)
	NCT03727880	II	Resectable PDA	Pembrolizumab ± defactinib
Connective tissue growth gactor	NCT04229004	II/III randomized	Metastatic PDA	Pamrevlumab + gem/nabP *versus* gem/nabP
	NCT03941093	III randomized	Locally advanced PDA	Pamrevlumab or placebo + gem/nabP or FOLFIRINOX
Vitamin D receptor	NCT04524702	II	Advanced PDA	Paracalcitol + HCQ + gem/nabP
ERBB2	NCT04660929	I	HER2+ metastatic solid tumors	CT-0508 (CAR-macrophages)
	NCT04319757	I		ACE1702 (anti-HER2 NK cells)
	NCT04482309	II		Trastuzumab deruxtecan
NRG1	NCT02912949	II	Advanced solid tumors	Zenocutuzumab

(Continued)

Table 2. (Continued)

Target	Clinical trial Nr (clinicaltrials.gov)	Study design	Study population	Therapy
BRAF V600E	NCT04390243	II	Advanced PDA	Encorafenib + binimetinib
ALK/ROS1	NCT03093116	I/II	Advanced solid tumors	TPX-0005 (repotrectinib)
NTRK				
ARID1A	NCT05053971	I/II	Advanced solid tumors	Entinostat + ZEN003694 (BET inh)
SWI/SNF	NCT04104776	I/II		CPI-0209 (EZH2 inh)
	NCT04170153	I		M1774 (ATR inh) ± niraparib
	NCT03682289	II		AZD6738 (ATR inh) ± olaparib
TP53 Y220C	NCT04585750	I/II	Advanced solid tumors	PC14586 (p53 reactivator)
Fibroblast Activating Protein	NCT05432193	I	FAP+ advanced solid tumors	^{177}Lu-PNT6555 (FAP radioligand)
	NCT05098405	I		MP0317 (trispecific FAP × CD40 DARPin® drug candidate)
	NCT05547321	I		OMTX705 (anti-FAP ADC linked to cytolysin) ± pembrolizumab
	NCT04857138	I		RO7300490 (FAP targeted CD40 ag) ± Atezolizumab
	NCT04939610	I/II		^{177}Lu-FAP-2286 (radionuclide therapy targeting FAP)
Claudin 18.2	NCT03816163	II randomized	Metastatic PDA	Zolbetuximab + gem/nabP *versus* gem/nabP
	NCT04404595	Ib/II	Advanced gastric, GEJ, PDA	CT-041 (CAR-T)
	NCT05539430	I	Advanced GE, PDA	LB1908 (CAR-T)
	NCT04856150	I	Advanced solid tumors	Q-1802 (Claudin 18.2 × PD-L1 biAb)
	NCT04805307	I	Advanced solid tumors/ gastric, GEJ, PDA	CMG901 (ADC)
	NCT05009966	I	Advanced solid tumors	SYSA1801 (ADC)
	NCT05043987	I	Advanced PDA, gastric, GEJ	CPO102 (ADC)
	NCT05161390	I/II	Advanced solid tumors	LM-302 (ADC)
Mesothelin	NCT03816358	I randomized	Mesothelin+ advanced PDA	Anetumab ravtansine (ADC) + nivo + nivo/ipi + nivo/gem
	NCT05451849	I/II	Mesothelin+ advanced PDA, ovarian, breast, mesothelioma, CRC	TC-510 (TCR-T)
	NCT04809766	I	Mesothelin+ advanced PDA	FH-TCR T_{MSLN} (TCR-T)

Ab, antibody; ADC, antibody–drug conjugate; ag, agonist; biAb, bispecific antibody; CAR-T, chimeric antigen receptor T cells; CRC, colorectal cancer; FAP, fibroblast activating protein; GEJ, gastroesophageal junction; gem, gemcitabine; HCQ, hydroxychloroquine; HRD, homologous recombination deficiency; inh, inhibitor; ipi, ipilumumab; nabP, nanoliposomal, albumin bound paclitaxel; nivo, nivolumab; NK, natural killer; PDA, pancreatic ductal adenocarcinoma; SBRT, stereotactic body frame radiotherapy; TCR-T, T-cell receptor T cells.

BRCA1/2-mutated PDA after at least 4 months of platinum-based chemotherapy without progression.[54,55] Olaparib improved median PFS [7.4 *versus* 3.8 months; hazard ratio (HR): 0.53, $p = 0.004$], but not OS (19.2 *versus* 19.0 months; HR: 0.83; $p = 0.3487$).[56] The lack of significant OS improvement with olaparib in the POLO trial was likely due to the placebo-treated patients subsequent treatment with PARP inhibitors (27%) and with platinum or irinotecan-based chemotherapy upon progression. A similar benefit was demonstrated with maintenance rucaparib for platinum-sensitive germline or somatic *BRCA1/2* or *PALB2*-mutated PDA: median PFS of 13 months (from starting chemotherapy) and OS of 23.5 months.[56]

DDR defects increase genomic instability, neoantigenic load, and tumor immunogenicity. Furthermore, treatment with PARP inhibitors, by preventing DNA repair, upregulating programmed death-ligand 1 (PD-L1), and activating the stimulator of interferon genes pathway may synergize with immune checkpoint inhibitors (ICI).[57,58] DDR deficiencies predict response to ICI in lung cancers,[59] but data in PDA are anecdotal.[60] In the CCTG PA.7 study,[61] 16 patients harboring germline *ATM* mutations had higher OS with Gem-nabP plus anti-PD-L1/CTLA4 therapy with durvalumab/tremelimumab *versus* Gem-nabP alone (13.9 *versus* 4.9 months).[62] Ongoing clinical trials are testing combinations of maintenance olaparib with pembrolizumab for platinum-sensitive PDA: the POLAR study (NCT04666740) is testing olaparib plus pembrolizumab in cancers with core (*BRCA1/2/PALB2*) and non-core DDR mutations (*ATM, BAP1, BARD1, BLM, BRIP1, CHEK2, FAM175A, FANCA, FANCC, NBN, RAD50, RAD51, RAD51C, RTEL1*) and for platinum-sensitive DDR wild-type tumors, whereas the randomized SWOG S2001 study (NCT04548752) is testing olaparib plus pembrolizumab *versus* olaparib for germline *BRCA1/2*-mutated PDA. In ECOG-ACRIN 2192 (APOLLO) (NCT04858334), adjuvant olaparib *versus* placebo is being studied for germline or somatic *BRCA1/2/PALB2*-mutated PDA, after resection and completion of neoadjuvant/adjuvant chemotherapy. Reiss *et al.* recently reported on maintenance niraparib with the PD-1 inhibitor nivolumab or with the CTLA-4 inhibitor ipilimumab for platinum-sensitive PDA. The combination of PARP/CTLA4 *versus* PARP/PD-1 blockade conferred superior 6-month PFS: 59.6% *versus* 20.6%,[63] and increased ORR, median PFS and OS: 15.4% *versus* 7.7%, 8.1 *versus* 1.9 months, and 17.3 *versus* 13.2 months, respectively. Mutated DDR genes beyond the core *BRCA1/2/PALB2* genes have been associated with benefit from platinum chemotherapy, but small patient numbers preclude definitive conclusions.[64] PARP inhibitors plus ICIs are also investigated for refractory PDA (NCT04673448, NCT04493060).

Despite initial benefit, resistance to PARP inhibitors eventually develops.[65] Several mechanisms of resistance lead to restoration of homologous recombination DNA repair and persistent replication forks.[66] Preclinical data suggest that PARP inhibitor-resistant *BRCA1*-deficient cells are increasingly dependent on ATR for survival.[67] Another strategy tackling PARP inhibitor resistance is with the PARP/WEE1 inhibitor combination which induces replication stress.[68]

Data with PARP inhibitors in PDA with non-*BRCA1/2/PALB2* DDR alterations showed low efficacy.[43] Germline and somatic *ATM* defects occur in 5–10% of PDA.[37] ATR inhibitors are active in ATM-deficient cancers,[69–72] and given preclinical synergism with carboplatin and irinotecan, studies are evaluating these combinations (NCT02595931). No studies reported on PARP plus ATR inhibitors in PDA, but the phase II VIOLETTE trial in breast cancer did not show increased efficacy from this combination.[73]

Novel research identified HRD and replication stress as broader targets, beyond *BRCA1/2/PALB2*, encompassing the functional relevance from other DDR alterations.[74] These provocative results highlight that molecular profiling may identify additional therapeutic vulnerabilities.

Microsatellite instability

MSI high/deficient DNA mismatch repair (dMMR) tumors may benefit from ICIs.[75,76] The MMR system recognizes and repairs the erroneous insertion, deletion, and misincorporation of bases that arise during DNA replication and recombination. Tumors harboring MSI-H/dMMR can accumulate thousands of mutations and are characterized by a hypermutated genome, correlating with response to ICI. Testing for MSI-H/dMMR can be done using IHC and

molecular tests including polymerase chain reaction-based MSI testing and novel next-generation sequencing (NGS) approaches.[77]

The frequency of MSI-H/dMMR occurs in approximately 1% of PDA, either in the context of Lynch syndrome[17,18,78] or as somatic mutations.[79–81] In one systematic review, MSI-H/dMMR was strongly associated with medullary and mucinous/colloid histology as well as with wild-type *KRAS* and *TP53* tumors.[82] Moreover, TMB is elevated [defined as ⩾10, and in some studies ⩾20 mutations/megabase (mut/Mb)] in the majority of MSI-H/dMMR PDA, representing another biomarker associated with benefit from anti-programmed cell death protein 1 (PD1)/PD-L1 agents.[83]

The US FDA-approved pembrolizumab based on ORR of 40% among 149 MSI-H/dMMR cancers.[84] In the initial cohort of non-colorectal cancers, five of eight (62%) PDA patients responded.[76] The follow-up KEYNOTE-158 trial noted ORR of 34%, with a median PFS of 4 months and an OS of 23.5 months among pretreated MSI-H/dMMR solid tumors, but 22 PDA patients had ORR of 18.2%, and median PFS and OS of only 2 and 4 months, respectively.[85] These results emphasize the lower benefit from ICI for MSI-H PDA, likely the result of a profoundly immunosuppressive microenvironment.

Tumor mutational burden

Another biomarker with predictive benefit from anti-PD1 therapies is the TMB, commonly reported in comprehensive NGS assays.[58,86,87] TMB is a numeric index of the estimated total number of mutations per coding area of the tumor genome.[88] High-TMB is associated with response to anti-PD-1 therapies.[86,89] TMB is considered high if it exceeds a predetermined threshold, widely variable based on tumor type.[90] In a retrospective analysis of KEYNOTE-158, patients with TMB-high tumors, defined as a TMB ⩾ 10 mut/Mb, demonstrated a significantly higher ORR to pembrolizumab compared to tumors harboring TMB < 10 muts/Mb (29% *versus* 6%).[91] In contrast, Schrock *et al.* identified 37 mut/Mb as the optimal cutoff for colorectal cancers.[92] Little is known regarding the prevalence and the potential predictive role of TMB in PDA. In a systematic review, TMB-high defined as ⩾20 mut/Mb was present in 1.1% of PDA.[28] A significant portion (59.4%) of TMB-high cancers harbor MSI-H/dMMR, while MSS TMB-high tumors have *BRCA2, BRAF*, or *POLE* mutations. In this analysis, eight patients with TMB-high PDA received anti-PD1 therapy: two (also MSI-H/dMMR) had complete response (CR), five partial response (PR), and one SD lasting for 30 months. In a retrospective analysis of 3500 PDA samples, Singhi *et al.* considered TMB-high as ⩾20 mut/Mb, but only 0.5% of tumors met this criterion.[16] Confirming the need for a higher TMB, a recent analysis of 1678 MSS solid tumors including 26 PDA with TMB ⩾ 10 mut/Mb treated with pembrolizumab showed response in only one (4%) PDA.[93]

TMB typically ranges between 1 and 3 mut/Mb in PDA,[93] and it does not appear to be a reliable predictor of benefit from ICI. Nonetheless, in the CCTG PA.7 study of Gem-nabP with and without durvalumab/tremelimumab, cfDNA analysis showed that in a small subset of patients ($n = 8$) with plasma TMB ⩾ 9 mut/Mb, patients had higher median OS with chemoimmunotherapy *versus* chemotherapy alone (14.6 *versus* 1.2 months).[61,94] Recent reports indicate TMB ⩾ 10 mut/Mb to be more common in *KRAS* wild-type (4.5%) compared to *KRAS*-mutated PDA (1%),[15] correlating to higher incidence of MSI-high in this subtype. DDR alterations including biallelic mutations in *BRCA1/2/PALB2, ATM, BARD1, BLM, CHEK2, RAD50*, and *RAD51C* have higher TMB and genomic instability,[95] and these genomic alterations could represent biomarkers for combination ICIs, including anti-PD1, anti-CTLA4, anti-PD1/anti-CTLA4 inhibitors with and without DDR blockade.

NCCN guidelines recommend pembrolizumab for patients whose cancers have TMB ⩾ 10 mut/Mb and have failed prior therapies, or in the first-line setting for patients with poor performance status, not fit for conventional chemotherapy.[6]

Tumor microenvironment

The tumor microenvironment (TME) in PDA consists of immune cells, cytokines, metabolites, fibroblasts, and desmoplastic stroma rich in hyaluronic acid (HA) and collagen. This multifaceted compartment is thought to be, in part, responsible for the resistance to most chemo and

immunotherapies.[95–98] The immunosuppressive TME is characterized by limited infiltration of $CD8^+$ T cells and an abundance of myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), tumor-associated neutrophils, and regulatory T cells. In most patients, the TME prevents anticancer immunity and promotes carcinogenesis. However, a robust host immune response has been identified, with abundant $CD8^+$ T-cell infiltrates and high number of neoantigens in long-term survivors after PDA surgery.[99]

Single agent and combinations of ICI have been ineffective in advanced PDA, but many clinical trials testing new combinations are underway.[100–102] The randomized phase Ib PRINCE trial evaluated Gem-nabP with nivolumab, with the CD40 agonistic monoclonal antibody sotigalimab (aimed to activate dendritic cells and repurpose immunosuppressive M2 to proinflammatory M1-TAMs), or with nivolumab plus sotigalimab.[103] While only the nivolumab/Gem-nabP arm improved 1-year OS (57.7% *versus* historical 35% control, $p = 0.006$), distinct immune signatures were associated with survival in each arm. A less suppressive TME and higher numbers of activated antigen-experienced circulating T cells at baseline predicted benefit from nivolumab/Gem-nabP, while greater intratumoral $CD4^+$ T cells and circulating differentiated $CD4^+$ T cells and antigen presenting cells predicted benefit from sotigalimab/Gem-nabP.[104]

It has been well described that *KRAS* mutations negatively impact the TME in PDA.[105] Recently, differences were noted in the immune microenvironment of *KRAS* wild-type and *KRAS*-mutated PDA, with a larger proportion of infiltrating, active effector T cells, and fewer MDSCs in *KRAS* wild-type tumors, suggesting that this subtype may be more susceptible to targeting by ICI, and possibly accounting for improved survival.[15]

The extracellular matrix promotes the immunosuppressive TME and impedes perfusion by compressing the tumor vasculature. Targeting stroma intends to improve systemic drug delivery and allow effector immune cell infiltration. Several stromal targeting strategies have been tested, most without the guide of a predictive biomarker. HA content has been thought to predict benefit from pegvorhyaluronidase alfa (PEGPH20) based on preclinical data, and due to encouraging results in combination with gemcitabine or with Gem-nabP for PDA with high HA content.[106,107] Nevertheless, the phase III HALO-301 study in HA-high PDA demonstrated an equivalent median OS of 11 months with Gem-nabP with or without PEGPH20.[108] A possible explanation for the lack of benefit from PEGPH20 was that biomarker selection of the HA-high threshold as 50% HA expression of any intensity by an IHC assay was not adequate. Other stroma targeting strategies, albeit without biomarker selection, include the vitamin D receptor agonist paricalcitol with chemo- and immunotherapy,[109] focal adhesion kinase inhibition with defactinib, VS-6766 or IN10018 with chemotherapy, radiotherapy and/or ICI (NCT02758587, NCT02546531, NCT04331041),[110] and connective tissue growth factor (CTGF/CCN2) blockade with pamrevlumab (FG-3019) with chemotherapy and/or ICI (NCT04449004, NCT03941093, NCT03727880, NCT04331041) (Table 2).[111]

Fibroblast activating protein (FAP) is a type II membrane bound glycoprotein which activates cancer-associated fibroblasts.[112] FAP is expressed on activated fibroblasts in tumors stroma. FAP targeting has previously been unsuccessful, but FAP imaging may select patients for FAP-targeted therapies. More recently, FAP has been identified as a potential target for peptide receptor radionuclide therapy.[113] Results with ^{90}Y-labeled FAPI-46 radioligand therapy for refractory solid tumors with high FAP expression by PET/CT have been recently reported.[114] Among 119 screened tumors, 21 were eligible (3 PDA). ORR and disease control rate (DCR = CR + PR + SD) were 6% and 38%, respectively, but no response/SD occurred in PDA. Another phase I study is exploring [Lu-177]-PNT6555 in FAP-avid solid tumors as determined by the [Ga-68]-PNT6555 PET/CT (NCT05432193).

KRAS

Oncogenic *KRAS* mutations occur in >90% of PDA and are the hallmark of this disease. *KRAS* encodes a small GTPase, which oscillates between active (GTP-bound) and inactive (GDP-bound) state, and when active, signals to major downstream pathways: RAF/MAPK/ERK

and PI3K/AKT/mTOR.[115–118] *KRAS* mutations in codons G12, G13, and Q61 prevent GTPase-activating proteins from hydrolyzing GTP to inactive GDP. Signals from receptor tyrosine kinases (RTK) flow through adapter proteins to son of sevenless homolog 1 (SOS1), a key guanine exchange factor which promotes GDP exchange to GTP, and Src homology region 2-containing protein tyrosine phosphatase (SHP2).[117] PDA are enriched in $KRAS^{G12D}$ (35.5%), $KRAS^{G12V}$ (28.2%), and $KRAS^{G12R}$ (15.9%) point mutations.[12,15] Due to lack of accessible binding pockets, most efforts to target *KRAS* directly have been difficult. However, $KRAS^{G12C}$ (1–3% of PDA) has recently emerged as an actionable target.[12] In addition, novel $KRAS^{G12D}$ and pan-RAS inhibitors, as well as SOS1 and SHP2 inhibitors as monotherapy and in combinations, entered clinical trials (Table 3).

KRAS G12C. The mutant cysteine-12 is located next to a cryptic pocket of the switch II region in the inactive GDP-bound conformation of *KRAS*. Several small-molecule covalent inhibitors bind specifically and irreversibly to mutant cysteine and disrupt both switch I and switch II exchange factors, trapping mutant *KRAS* in an inactive GDP state. Sotorasib (AMG510) and adagrasib (MRTX849), among others, have been optimized for favorable pharmacokinetic (PK) properties including long half-life, extensive tissue distribution, dose-dependent PK, as well as central nervous system penetration. In the phase I/II KRSYTAL-1 study, adagrasib was evaluated in patients with advanced solid tumors harboring $KRAS^{G12C}$ mutations, including 12 heavily pretreated PDA.[119] Among 10 evaluable patients, 5 responded (50%), DCR was 100%, and a median PFS was 6.6 months. Sotorasib was evaluated in the phase I/II CodeBreaK100 trial in advanced solid tumors harboring a $KRAS^{G12C}$ mutation, including 38 PDA.[120] Most patients (79%) had $\geqslant 2$ prior lines of therapy. The ORR was 21.1%, DCR was 84.3%, and median PFS and OS were 4 months and 6.9 months, respectively.

While $KRAS^{G12C}$ inhibitors are well-tolerated, the benefit is transient. Mechanisms of resistance include secondary *KRAS* mutations, alterations in cell cycle regulation, activating mutations in other RTK and downstream RAS-MAPK pathways, and emergence of new gene fusions.[121] The diversity of resistance mechanisms supports the development of combination regimens, including with agents targeting EGFR, SHP2, SOS1, MEK, CDK, mTOR, targetable fusions, and PD-1 inhibitors (Table 3).

KRAS non-G12C. Inhibitors targeting other *KRAS* variants, such as $KRAS^{G12D}$ (MTRX1133, siG12D Loder), pan-*RAS* inhibitors (RMC6236, BI1701963), novel *KRAS* vaccines, and adoptive immunotherapies targeting various *KRAS* alleles are in development and expected to broaden efficacy in PDA patients (Table 3). Adoptive chimeric T-cell receptor (TCR) T cells therapy has been successfully used in a previously treated PDA patient with lung metastases who obtained a PR and a PFS of 6 months+.[122] This patient received autologous T cells genetically engineered to clonally express two allogeneic HLA-C*08:02-restricted TCRs targeting mutant *KRAS G12D*. Several TCR cell therapies targeting *KRAS* variants are being explored. Future trials are likely to include combination immunotherapy approaches and next-generation TCR-T cells with chimeric co-stimulatory molecules, such as activating receptors or ligands.

Table 3. Targeting *KRAS*: active clinical trials.

Agent(s)	Mechanism of action	Clinical trial Nr (clinicaltrials.gov)	Remarks
Adagrasib ± Pembrolizumab Afatinib Cetuximab	KRAS G12C inhibitor Anti-PD1 Ab Anti-EGFR/ERBB2 TKI Anti-EGFR Ab	NCT03785249	Phase I/II KRYSTAL 1 trial; expansion cohorts with combinations for tumors with G12C mutations
Adagrasib + BI1701963	KRAS G12C inhibitor SOS1 inhibitor	NCT04975256	Phase I KRYSTAL 14 trial for tumors with G12C mutations

(Continued)

Table 3. (Continued)

Agent(s)	Mechanism of action	Clinical trial Nr (clinicaltrials.gov)	Remarks
Adagrasib + TNO155	KRAS G12C inhibitor SHP2 inhibitor	NCT04330664	Phase I/II KRYSTAL 2 trial for tumors with G12C mutations
RMC-6236	Tri-complex RAS(ON) inhibitor	NCT05379985	Phase I trial for tumors with G12D, G12V, G12R mutations
RMC-6291	Tri-complex KRAS G12C(ON) inhibitor	NCT05462717	Phase I trial for tumors with G12C mutations
Sotorasib ± combinations	KRAS G12C inhibitor	NCT04185883	Phase I/II CodeBreak 101 trial for tumors with G12C mutations
Sotorasib + Nal-Iri/5FU or Gem/nabP	KRAS G12C inhibitor Chemotherapy	NCT05251038	Phase II trial for second-line advanced G12C-mutated PDA
HBI-2438	KRAS G12C inhibitor	NCT05485974	Phase I trial for tumors with G12C mutations
LY3537982 ± combinations	KRAS G12C inhibitor	NCT04956640	Phase 1 trial with multiple arms
D-1553	KRAS G12C inhibitor	NCT04585035	Phase I/II trial for tumors with G12C mutations
GDC-6036 ± combinations	KRAS G12C inhibitor	NCT04449874	Phase I trial for tumors with G12C mutations
HBI-2376	SHP2 inhibitor	NCT05163028	Phase I trial for tumors with KRAS or EGFR mutations
JAB-3312	SHP2 inhibitor	NCT04045496	Phase I trial for solid tumors
RMC-4630 + LY3214996	SHP2 inhibitor ERK inhibitor	NCT04916236	Phase I SHERPA trial for KRAS-mutated tumors
ERAS-601 ± cetuximab	SHP2 inhibitor Anti-EGFR Ab	NCT04670679	Phase I FLAGSHP-1 trial
BBP-398	SHP2 inhibitor	NCT04528836	Phase I trial for solid tumors
Sotorasib + BBP-398	KRAS G12C inhibitor SHP2 inhibitor	NCT05480865	Phase I trial for tumors with G12C mutations
GDC-1971 + atezolizumab	SHP2 inhibitor Anti-PD-L1 Ab	NCT05487235	Phase I trial for solid tumors
mDC3/8-KRAS vaccine	Dendritic cell vaccine	NCT03592888	Phase I trial for KRAS-mutated resected PDA
KRAS peptide vaccine + nivolumab/ ipilumumab	Mutant KRAS peptide vaccine Anti-PD1 Ab Anti-CTLA4 Ab	NCT04117087	Phase I trial for resected CRC and PDA

KRAS wild type. Approximately 10% of PDA and up to 20% of young-onset PDA (age <50 years) are KRAS wild type ($KRAS^{WT}$), with better prognosis and more therapeutic opportunities.[15] $KRAS^{WT}$ PDAs are enriched in *BRAF*, DDR, chromatin remodeling, cell cycle control gene mutations, *FGFR2, ALK, RET, NTRK* and *NRG1* fusions, as well as *FGF3*, *ERBB2*, *FGFR3*, and *MET* amplifications, and are more likely to exhibit MSI-high and TMB-high status. In all, almost 30% of $KRAS^{WT}$ PDA have targetable alterations.

Several reports in the past decade suggested that KRAS^{WT} PDA have a better prognosis overall, including when treated with anti-epidermal growth factor receptor (EGFR) therapies. In 2005, based on the phase III study NCIC CTG PA.3, the FDA approved the anti-EGFR tyrosine kinase inhibitor (TKI) erlotinib combined with gemcitabine in metastatic PDA due to a 18% improvement in median OS *versus* gemcitabine and placebo (6.2 *versus* 5.9 months, HR: 0.82; $p = 0.03$).[123] This study did not observe significant correlations with *KRAS* status or EGFR expression among 26% of cancers with available tumor samples for analysis, but OS was higher for KRAS^{WT} PDA treated with gemcitabine/erlotinib *versus* gemcitabine/placebo (6.1 *versus* 4.5 months, HR: 0.66, $p = 0.34$), whereas KRAS-mutated (KRAS^{MUT}) PDA derived no benefit (6.0 *versus* 7.4 months, HR: 1.07, $p = 0.74$).[124] Kim *et al.* noted a 4-month survival advantage for KRAS^{WT} *versus* KRAS^{MUT} PDA treated with gemcitabine/erlotinib (OS: 9.7 *versus* 5.2 months, $p = 0.002$).[125] Similarly, a retrospective biomarker analysis in the phase III study AIO-PK0104 identified *KRAS^{WT} versus KRAS^{MUT}* to confer superior OS (7.9 *versus* 5.7 months, HR: 1.68, $p = 0.005$) for patients treated with chemotherapy plus erlotinib.[126] Increased OS was also noted with the anti-EGFR antibody cetuximab plus gemcitabine/oxaliplatin among KRAS^{WT} *versus* KRAS^{MUT} PDA (8.7 *versus* 5.4 months).[127]

No prospective randomized phase III study evaluated anti-EGFR therapies *versus* placebo in KRAS^{WT} PDA until the NOTABLE study was reported at ASCO 2022.[128] Nimotuzumab, a humanized IgG1 monoclonal antibody against the extracellular domain of EGFR,[129] showed encouraging efficacy with gemcitabine *versus* gemcitabine alone in a randomized phase II study (OS: 8.6 *versus* 6 months), and OS was significantly higher (11.6 *versus* 5.6 months) in KRAS^{WT} PDA.[130] Based on these results, the phase III study NOTABLE evaluated nimotuzumab plus gemcitabine *versus* placebo plus gemcitabine for first-line treatment of KRAS^{WT} advanced PDA.[128] Nimotuzumab significantly increased median OS (10.9 *versus* 8.5 months, HR 0.50, $p = 0.024$). This study should provide impetus for further exploration of anti-EGFR therapies in KRAS^{WT} PDA in combination with contemporary multiagent chemotherapy.

ERBB2/HER2

Human epidermal growth factor receptor 2 (HER2) overexpression occurs in 2–3% of PDA.[131,132] HER2 and HER3 are obligate partners and together are implicated in the progression of multiple cancers.[133] Monoclonal antibodies that bind either directly to the extracellular domain of HER2 (e.g. trastuzumab), block the interaction of HER2 and HER3 (e.g. pertuzumab), or the antibody–drug conjugate (ADC) trastuzumab deruxtecan carrying a topoisomerase I inhibitor payload, are currently in clinical practice for breast and gastroesophageal cancers.[134,135]

Several studies tested anti-EGFR/HER2 TKIs afatinib or lapatinib in biomarker unselected PDA, without significant benefit.[136–139] The MyPathway phase II basket study tested trastuzumab plus pertuzumab in 258 refractory cancers, including 10 PDA with HER2 overexpression by IHC, fluorescence *in situ* hybridization (FISH), or NGS.[140] ORR was 23.3%, higher in KRAS^{WT} (26%) *versus* KRAS^{MUT} tumors (4%). ORR was 33% in KRAS^{WT} PDA.

Neuregulin-1

Neuregulin-1 (NRG-1) is a ligand which binds primarily to ERBB3/HER3 and ERBB4/HER4, leading to hetero- or oligomerization with other ERBB members, and activation of the PI3K/AKT/mTOR pathway. Cancers with *NRG1* gene fusions have constitutive activation of NRG1 and HER2-HER3 pathways, and are sensitive to HER2/HER3 targeted therapies.[141] *NRG1* gene fusions (with *CD74, ATP1B1*, and *SDC4*) are detected mostly in invasive mucinous lung adenocarcinomas and PDA (0.5%) and are enriched in *KRAS^{WT}* tumors.[142,143]

Zenocutuzumab is a common light chain immunoglobulin G1 bispecific antibody with enhanced antibody-dependent cellular cytotoxicity (ADCC) that docks on HER2 and blocks the interaction between *NRG1* and HER3. In a phase I/II trial for patients with refractory solid tumors harboring *NRG1* fusions detected by RNA sequencing (77%), DNA sequencing (22%), or Nanostring (1%), zenocutuzumab conferred ORR of 34%, with median duration of response (DoR) of 9 months.[144] Among 19 PDA patients, ORR and DCR were 42% and 74%, respectively. Given meaningful efficacy with HER2/HER3-targeted

therapies in cancers with *NRG1* fusions, RNA sequencing should be included in molecular testing platforms.

BRAF

PDA has constitutive activation of the MAP kinase pathway due to gain-of-function mutations in *KRAS*,[145] but KRAS^{WT} tumors can harbor RAS-independent *RAF* alterations: *BRAF* p.V600E in exon 15, *BRAF* p.N486_P490del in exon 11, and *SND1-BRAF* fusions, each present in 0.4–0.7% of pancreatic tumors.[15] In all, *BRAF* alterations are observed in 2% of PDA.[146]

Evidence regarding benefit from BRAF-targeted therapy in PDA is expanding. In the phase II MyPathway basket study, four PDA patients with activating *BRAF p.V600E* mutations or other BRAF alterations were treated with the BRAF inhibitor vemurafenib, and one patient with a *CUX1-BRAF* fusion achieved a PR.[147] The Know Your Tumor registry included one patient with a BRAF p.V600E-mutated PDA who received matched therapy with BRAF/MEK inhibitors dabrafenib/trametinib and had a response for 11 months.[34] A retrospective case series of 81 PDA patients with *RAF* alterations including *BRAF* p.V600E (exon 15), *BRAF* ΔNVTAP (exon 11), and *SND1-BRAF* fusions showed variable benefit from BRAF/MEK-targeted therapies, with best responses (100%, 3/3 patients) observed for *BRAF* p.V600E-mutated PDA.[148] Atypical variants and multiple oncogenic drivers predicted lower/no response. The NCI-MATCH basket trial treated 35 solid tumors (3 PDA) harboring *BRAF* p.V600E mutations with dabrafenib/trametinib. One PDA patient had SD as best response. ORR was 35% among all patients, and median PFS and OS rates were 11.4 and 28.6 months, respectively, leading to the cancer agnostic FDA approval of this combination in pretreated cancers with *BRAF* p.V600E mutations.[149] A single-arm phase II trial is examining combined BRAF/MEK inhibition with encorafenib/binimetinib for pretreated advanced *BRAF* V600E-mutated PDA (NCT04390243).

RET

Rearranged during transfection (*RET*) proto-oncogene activates the downstream RAS/MAPK/ERK and PI3K/AKT pathways.[150] *RET* fusions can be detected with FISH, IHC, NGS, or RNA sequencing, and are most prevalent in papillary thyroid carcinomas (10–20%) and non-small-cell lung cancer (1–2%),[151] where RET inhibitors selpercatinib and pralsetinib gained FDA approval. *RET* fusions have been identified in 1% of PDA.[152] The phase I/II LIBRETTO-001 basket study with selpercatinib in advanced *RET*-altered (fusions or mutations) solid tumors included 11 PDA patients with ORR of 55%,[153] whereas in the phase I/II ARROW trial with pralsetinib for *RET* mutant/fusion positive tumors all four enrolled PDA patients responded (ORR: 100%), including one lasting 24 months+.[154] Selpercatinib was FDA approved for all solid tumors harboring *RET* fusions.

ALK, NTRK, and ROS-1

Anaplastic lymphoma kinase (*ALK*), neurotrophic tyrosine receptor kinase (*NTRK*), and c-ros oncogene 1 (*ROS-1*) fusions each have a prevalence of less than 1% and occur in KRAS^{WT} PDA.[155] *ALK* fusions predict benefit from ALK inhibitors crizotinib, ceritinib, and alectinib. In a cohort of five patients with *ALK*-fusion positive advanced PDA (EML4-ALK and STRN-ALK), all of whom were *KRAS^{WT}* and younger than 50 years, four patients received an ALK inhibitor, and three demonstrated SD, radiographic response, and/or normalization of serum CA 19-9.[155] Screening for *ALK* rearrangements should be considered in young patients with KRAS^{WT} PDA.

TRK inhibitors, larotrectinib and entrectinib, have been FDA approved for tumors that harbor *ROS1, NTRK1, NTRK2*, and *NTRK3* gene fusions. Among 159 patients with *TRK* fusion-positive cancers (2 PDA) treated with larotrectinib, ORR was 79% with a median DoR of 35.2 months and median PFS of 28.3 months.[156] In a pooled analysis of 121 patients with 14 tumor types (4 PDA) treated with entrectinib in the STARTRK-2, STARTRK-1, and ALKA-372-001 trials, the ORR was 61%, with a median DoR of 20 months and median PFS 13.8 months.[157] The NCCN guidelines recommend larotrectinib and entrectinib in PDA patients with *NTRK* gene fusions who have either failed prior therapies or in the first line setting if they have poor performance status and unfit to received conventional chemotherapy.[6] Like other targeted therapies, acquired

resistance to these inhibitors develops. Second-generation pan-TRK inhibitors are being investigated, including selitrectinib (LOXO-195) and repotrectinib (TPX-0005, NCT03093116).

MET

Upregulation of the hepatocyte growth factor (HGF)/c-MET pathway occurs in more than 20% of PDA. HGF is produced by pancreatic stellate cells and its receptor, c-MET is expressed on epithelial PDA and endothelial cells. Elevated serum HGF levels have been reported to correlate with disease progression,[158] and high tumor c-MET expression is associated with poor survival.[159] Recently, a phase Ib study tested ficlatuzumab, a recombinant humanized anti-HGF antibody in combination with Gem-nabP as first-line treatment of 26 metastatic PDA patients, and showed acceptable tolerability (16% grade 3 hypoalbuminemia and 8% grade 3 edema), ORR of 29%, and median PFS and OS of 11 and 16.2 months.[160] Correlative biomarkers noted that responders had significantly higher baseline tumor pMET expression by IHC than non-responders (histoscore 80 *versus* 10, $p = 0.047$). While these data are encouraging, it is likely that combined ligand and receptor targeting would be needed for adequate targeting in future studies.[161]

ARID1A

Mutations in epigenetic modifiers, such as the SWItch/sucrose non-fermentable component AT-rich interactive domain-containing protein 1A (*ARID1A*) promote the mesenchymal phenotype during pancreatic carcinogenesis.[162] *ARID1A* is a tumor suppressor harboring mutations in 2–8% of PDA.[163] *ARID1A* has also been implicated in double-stranded DNA repair *via* both homologous recombination and non-homologous end-joining, thought to confer platinum sensitivity when mutated. Loss of *ARID1A* leads to increased expression of the PI3K-interacting protein 1 gene (PIK3IP1), which downregulates PI3K-AKT signaling.[164] EZH2 inhibits *PIK3IP1* gene transcription, and EZH2 blockade can upregulate PIK3IP1 upon *ARID1A* loss.[164] Other studies suggest that *ARID1A*-mutated cancers depend on HDAC activity and HDAC6 inhibition triggers cellular apoptosis. These mechanisms explain why epigenetic targeting of EZH2 methyltransferase with EZH2 inhibitors, and histone deacetylases with HDAC inhibitors for *ARID1A*-mutated cancers[165,166] and are being investigated in PDA (NCT05053971). Lastly, there is a synthetically lethal interaction between *ATR* and *ARID1A*, and pre-clinical models have shown that ATR inhibition exploits a pre-existing DNA decatenation defect in *ARID1A* mutant tumor cells which causes premature mitotic progression.[167] M1774, an ATR inhibitor, is being tested in advanced solid tumors with loss of function in *ARID1A* (NCT04170153).

MDM2

The oncogene murine double minute 2 (*MDM2*) is overexpressed in up to 10% of PDA[168] and exerts its oncogenic activity *via* both p53-dependent and -independent pathways, promoting cancer cell growth and invasion and inducing resistance to chemotherapy.[169] *MDM2* overexpression is a negative prognostic factor in PDA.[170] As a critical negative regulator of p53, MDM2 inhibition has growth inhibitory effects in p53 wild-type tumors. BI 907828, a highly potent MDM2-p53 antagonist is being studied in *TP53* wild type, *MDM2* amplified solid tumors, and preliminary data demonstrated clinical efficacy in sarcomas, pancreatic and biliary cancers (NCT03449381).[171]

TP53 p.Y220C

Inactivating mutations in the tumor suppressor gene *TP53* are common in pancreatic carcinogenesis and occur in 60% of PDA. Specific 'hotspot mutations', notably *TP53 p.Y220C*, affect the DNA binding domain and influence protein function.[172] Selective inhibitors designed against *TP53 p.Y220C* can stabilize p53 in the wild-type conformation, restoring transcription and tumor-suppressor function.[173] A phase I/II first-in-human study of PC14586 demonstrated an ORR of 24.2% in patients with advanced solid tumors, which included four patients with PDA (two SD, one PR).[174]

FGFR

FGFR alterations occur in 8% of PDA, particularly in $KRAS^{WT}$ cancers.[15] In the phase I/II FIGHT-101 study evaluating the FGFR1–3 inhibitor pemigatinib in solid tumors harboring

FGFR alterations, one of four PDA patients responded.[175] The RAGNAR phase II study recently reported on erdafitinib, a selective pan-FGFR TKI in 178 patients with advanced solid tumors with FGFR alterations. In all, 13 PDA patients had encouraging an ORR of 31%, a DCR of 85%, and a median DoR of 7.1 months.[176]

Claudin 18.2

Claudin 18.2 is a tight junction protein expressed on normal gastric epithelial cells and overexpressed in several cancers including 16% of PDA.[177] While its role in cancer progression is poorly defined, because of its differential expression on cancer cells during carcinogenesis, Claudin 18.2 poses as a unique epitope to target. Zolbetuximab, a chimeric IgG1 monoclonal antibody which binds to Claudin 18.2 and mediates tumor cell death through ADCC, and complement-dependent cytotoxicity has shown promising activity in advanced gastroesophageal cancers in combination with chemotherapy.[178] A randomized phase II study is ongoing with Gem-nabP with and without zolbetuximab in patients with advanced PDA and high Claudin 18.2 expression (NCT03816163).

Claudin 18.2 also serves as a promising target for cellular immunotherapies, specifically chimeric antigen receptor (CAR) T cells. A phase I study evaluated a Claudin 18.2-directed CAR-T cell (CT041) in patients with pretreated gastroesophageal and other adenocarcinomas, including five PDA. An interim analysis showed an ORR of 49%, a DCR of 73%, and a 6-month OS of 80%.[179] Additional trials are being conducted among patients with gastroesophageal, PDA, and other gastrointestinal cancers (NCT04404595, NCT05539430). CD3 bispecific antibodies and ADCs against Claudin 18.2 have preliminary activity in gastrointestinal cancers, including PDA.[180] Multiple trials in advanced solid tumors, including PDA, are ongoing with Claudin 18.2-targeting bispecific antibodies (NCT04856150) and ADC (NCT04805307, NCT05009966, NCT05043987, NCT05161390).

Mesothelin

Mesothelin is highly expressed in many cancers, including PDA (⩾75–85%), with low levels expressed in healthy tissues.[181] Mesothelin activates the NF-κβ pathway, induces IL-6-mediated cancer cells proliferation, inhibits apoptosis, and stimulate invasion and migration *via* the p38 MAPK pathway.[182] These key roles highlight its importance in PDA progression, and the potential benefit of targeting this pathway.

A first-in-human clinical trial with anetumab ravtansine, an ADC of anti-mesothelin antibody linked to maytansinoid DM4, was conducted in patients with mesothelin expressing advanced solid tumors.[183] Among 148 patients enrolled, three of nine PDA (30%) patients had SD as best response. Durability of responses appeared to correlate with degree of mesothelin expression, with ⩾60% expression by IHC associated with the greatest benefit.

LMB-100, a recombinant immunotoxin (iTox) consisting of a mesothelin-binding fragment antigen-binding antibody region (Fab) for targeting and a modified Pseudomonas exotoxin A payload, in combination with nabP was tested in 20 refractory PDA patients.[184] While clinical activity was observed, the combination treatment was not tolerated due to capillary leak syndrome.

Cellular immunotherapy targeting mesothelin may be a promising approach, but has modest results to date.[185] A phase I study with mesothelin-specific CAR-T cells in six patients with chemotherapy-refractory metastatic PDA resulted in two patients achieving SD with PFS of 3.8 and 5.4 months, respectively.[186] Another phase I study of a single infusion of lentiviral-transduced mesothelin CAR-T cells in 15 subjects, including five with PDA, showed limited clinical activity with SD (11/15) as best response and only transient persistence of CAR T-mesothelin cells.[187] A limitation of CAR-T-cell therapy is its dependence on antigen expression on the cell surface. However, the majority (85%) of tumor-associated antigens and neoantigens are intracellular and are solely expressed in the context of an major histocompatibility molecule.[188] To sidestep this barrier, tumor antigen-specific T cells expressing a TCR can target intracellular proteins.[189] Preclinical studies in PDA noted benefit from TCR T cells targeting mesothelin,[190] and a phase I first-in-human study of autologous T cells expressing a high-affinity mesothelin-specific TCR is ongoing in refractory PDA

(NCT04809766). Nevertheless, we recognize that T-cell exhaustion within the TME is likely to cause transient and suboptimal benefits and expect that further engineering of adoptive T cells to enable survival and effector function, while concurrent targeting of the immunosuppressive TME may lead to increased efficacy.[191]

Conclusions

Large-scale genomic and transcriptomic analyses have provided unprecedented insight into the biology of PDA and promoted precision oncology by identifying novel targets and designing new drugs and combinations for targeted therapy (Figure 1). In all, 20–25% of PDA patients harbor targetable molecular alterations. Although PDA remains a devastating disease, by identifying subgroups of patients with actionable molecular alterations and applying biomarker-driven therapies, meaningful survival gains have been accomplished.

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Consent for publication

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Author contribution(s)

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