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REVIEW

Are targeted therapies or immunotherapies effective in metastatic pancreatic adenocarcinoma?

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Metastatic pancreatic ductal adenocarcinoma (PDAC) is a major health burden due to its increasing incidence and poor prognosis. PDAC is characterized by a low tumor mutational burden, and its molecular pathogenesis is driven by Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations. Response to DNA damage through homologous repair is defective in 15% of tumors. Chemotherapy using FOLFIRINOX (folinic acid, fluorouracil, irinotecan, oxaliplatin) or gemcitabine-nab-paclitaxel significantly improves life expectancy, but the median overall survival remains <1 year. Targeted therapies are not efficient in the overall population of patients with metastatic PDAC. Improvements in overall survival or progression-free survival, however, have been demonstrated in subgroups carrying certain mutations. Maintenance therapy with poly-ADP-ribose polymerase (PARP) inhibitors increases progression-free survival in patients with germline mutations in *BRCA1/2*. Sotorasib shows signs of efficacy against tumors carrying the *KRAS G12C* mutation, and targeted therapies may also benefit patients with *KRAS*-wild-type PDAC. Combining targeted therapies with chemotherapy holds promise because of potential synergistic effects. These associations, however, have not yet demonstrated clinical benefit. Checkpoint inhibitors are not effective against metastatic PDAC. Combined immunotherapies attempt to restore their efficacy but have not succeeded yet. Other immunotherapies are emerging such as therapeutic vaccines or chimeric antigen receptor (CAR) T cells, but these strategies remain to be evaluated in large trials. In the future, treatment personalization based on tumor-derived organoids could potentially further improve treatment efficiency.

Key words: pancreatic adenocarcinoma, targeted therapies, immunotherapy, precision oncology

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is currently the fourth deadliest cancer in Europe, causing 132 000 deaths in 2020.[1] The incidence of PDAC is rising in Europe and Southeast Asia, and to a lesser extent in North America.[2] In Europe, it should become the second cause of death by cancer by 2030.[3]

According to the American SEER database, the overall survival (OS) rate of patients diagnosed with PDAC is 10.8% 5 years after diagnosis. Fewer than 15% of patients have a localized disease at diagnosis, allowing a surgical removal of the tumor. Nevertheless, the 5-year OS does not exceed 41% in localized tumors, due to local or distant recurrence. Some 30% of patients have a locally advanced disease at diagnosis, with a 14.4% OS rate at 5 years.[4] This rate drops to 3.0% in patients with distant metastases at diagnosis.[4] PDAC is one of the most aggressive cancers of the gastrointestinal tract and represents a growing public health concern.

Recently, the rise of genomics allowed clinicians to quantify the mutational burden associated with PDAC. These studies identified the key signaling pathways involved in the disease. They also highlighted the profound genomic heterogeneity of PDAC. This improved understanding of the molecular pathogenesis possibly holds the key for the development of targeted therapies in PDAC. In parallel, immunotherapy has attracted much attention because checkpoint inhibitors have proven efficient against numerous solid tumors.

In the present review, the role of non-targeted therapies is discussed in the era of metastatic PDAC. We will then describe the molecular alterations found in metastatic PDAC, and the corresponding targeted therapies, evaluated alone or in combination with chemotherapy. Finally, we will examine the existing data on immunotherapy in metastatic PDAC, and describe alternative strategies currently tested in clinical trials.

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Non-targeted therapies approved in metastatic PDAC

The first systemic therapy that demonstrated an effect on survival in PDAC was gemcitabine.[5] Gemcitabine is a pyrimidine nucleoside analog: it blocks DNA synthesis, leading to the death of replicating cells. In 1997, a randomized trial evaluated gemcitabine (1000 mg/m^2 once weekly, for 3 consecutive weeks out of every 4 weeks) against 5-fluorouracil (5-FU) in patients with advanced or metastatic PDAC.[5] The median OS was 5.65 and 4.41 months for gemcitabine and 5-FU, respectively ($P = 0.0025$). The survival rate at 12 months was 18% for gemcitabine and 2% for 5-FU.

Since then, two regimens have shown relatively similar outcomes in metastatic PDAC. Nab-paclitaxel is a microtubule inhibitor, bound to an albumin nanoparticle to increase its bioavailability. In metastatic PDAC, nab-paclitaxel (125 mg/m^2) was evaluated in combination with gemcitabine (1000 mg/m^2) on days 1, 8, and 15 every 4 weeks. The median OS was 8.5 months for doublet chemotherapy compared with 6.7 months for gemcitabine.[6] FOLFIRINOX, the other regimen commonly used in first line, is a combination therapy of 5-FU, irinotecan, and oxaliplatin. Irinotecan is a topoisomerase 1 inhibitor whereas oxaliplatin is a platinum-based intercalating agent. Several versions of FOLFIRINOX were approved in various cancers, and even in PDAC in the adjuvant setting. In metastatic PDAC, FOLFIRINOX uses oxaliplatin at 85 mg/m^2, irinotecan 180 mg/m^2, leucovorin (LV) 400 mg/m^2, and 5-FU 400 mg/m^2 given as a bolus followed by 2400 mg/m^2 given as a 46-h infusion, every 2 weeks. The median OS was 11.1 months for triplet chemotherapy compared with 6.8 months for gemcitabine ($P < 0.001$).[7] Altogether, FOLFIRINOX and gemcitabine plus nab-paclitaxel are the two first-line regimens commonly used in patients fit enough to receive treatment.

Liposomal irinotecan (nal-IRI) is a formulation of irinotecan designed to prolong its systemic circulation while minimizing toxicity.[8] In patients progressing after a gemcitabine-based therapy, the combination of nal-IRI + 5-FU (nal-IRI 80 mg/m^2, 5-FU 2400 mg/m^2, and LV 400 mg/m^2 every 2 weeks) demonstrated a survival benefit compared with 5-FU alone (5-FU 2000 mg/m^2 plus LV 200 mg/m^2 weekly for the first 4 weeks of 6-week cycles). Median OS was 6.2 and 4.2 months in the experimental and 5-FU arms, respectively.[9] Consequently, nal-IRI + 5-FU was approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) as a second-line therapy in metastatic PDAC. The choice of the comparator and the dose differences in 5-FU/LV between the two groups, however, prevent a large use of this association in clinical practice.

In a nutshell, chemotherapy has proven some efficacy in metastatic PDAC: it increases median OS by a few months. The survival rate at 5 years, however, remains <5%, demonstrating the urgent need for new therapies.

TARGETED THERAPIES

Molecular alterations in metastatic PDAC

Several studies investigated the genomic complexity of PDAC.[10,11] The tumor mutational burden (TMB) is defined as the number of mutations per megabase of DNA (m/Mb). Among gastrointestinal cancers, PDAC has the lowest mutational load with a median TMB of 1.1 m/Mb, compared with 3.7 for colon cancer or 13 for melanoma.[12] TMB, however, is highly variable between individuals. Values >10 m/Mb are associated with increased survival in patients receiving checkpoint inhibitors, at least in certain tumors.[13,14] In a study on 1021 PDAC patients, 0.5% had a TMB >20 m/Mb, whereas 12.4% had a TMB between 5 and 20 m/Mb.[15]

The genomic landscape of PDAC is dominated by a small number of oncogenes. Only five genes are mutated in >10% of PDACs: Kirsten rat sarcoma viral oncogene homolog (*KRAS*) (88%-92%), *TP53* (75%), *CDKN2A* (44%), *SMAD4* (22%), and *CDKN2B* (21%).[15] These five genes are followed by a long tail of infrequently mutated genes. The most affected intracellular processes are mitogen-activated protein (MAP) kinases (92% of PDACs), cell cycle (>90%), DNA homologous repair (14%-15%), phosphoinositide-3-kinase-AKT (PI3K-AKT) signaling (10%-19%), and chromatin remodeling (15%). Table 1 reports the most common mutations in PDAC. Key signaling pathways involved in PDAC are shown in Figure 1.

KRAS is a GTPase that transduces signal from tyrosine kinase receptors [epidermal growth factor receptor (EGFR),

Table 1. Most prevalent genomic alterations in PDAC. Genes are grouped by signaling pathway or molecular process. Prevalence is reported as % of total PDAC patients. These statistics are adapted from 2 sources: A) an international cohort of 3594 PDAC patients undergoing targeted genomic profiling,[15] and B) a targeted genomic screening of 640 patients treated for pancreatic cancer, of which 591 had an histologically confirmed pancreatic adenocarcinoma.[21]

Pathway	Gene	%
MAP kinases (92%)	KRAS	88-92
	BRAF	2
	ERBB2	2.8-3
	EGFR, FGFR2, MET, MAP2K4, ERBB3, FGFR1, RAF1, ALK, RET, NTRK1	<1 each
PI3K/AKT (10%-19%)	STK11	3-4.7
	PIK3CA	3-3.7
	AKT2	3
DNA homologous recombination (14%-15%)	BRCA2	2.9-4
	ATM	3-4.5
	BRCA1	2
	PALB2, FANCA/C/G	<2 each
Cell cycle control (>90%)	TP53	74-75
	CDKN2A	44-45
	CDKN2B	21
	CCNE1	3
	CHEK1/2	<2 each
Chromatin remodeling (15%)	ARID1A	8
	KDM6A	3
	DNMT3A	3
WNT pathway (5%)	RNF43	3
	APC	2
TGF-ß signaling (22%)	SMAD4	22
Other genes	GATA6	5
	GNAS	3
	MYC	5

MAP, mitogen-activated protein; PDAC, pancreatic ductal adenocarcinoma; PI3K, phosphoinositide-3-kinase; TGF, transforming growth factor; WNT, wingless-related integration site.

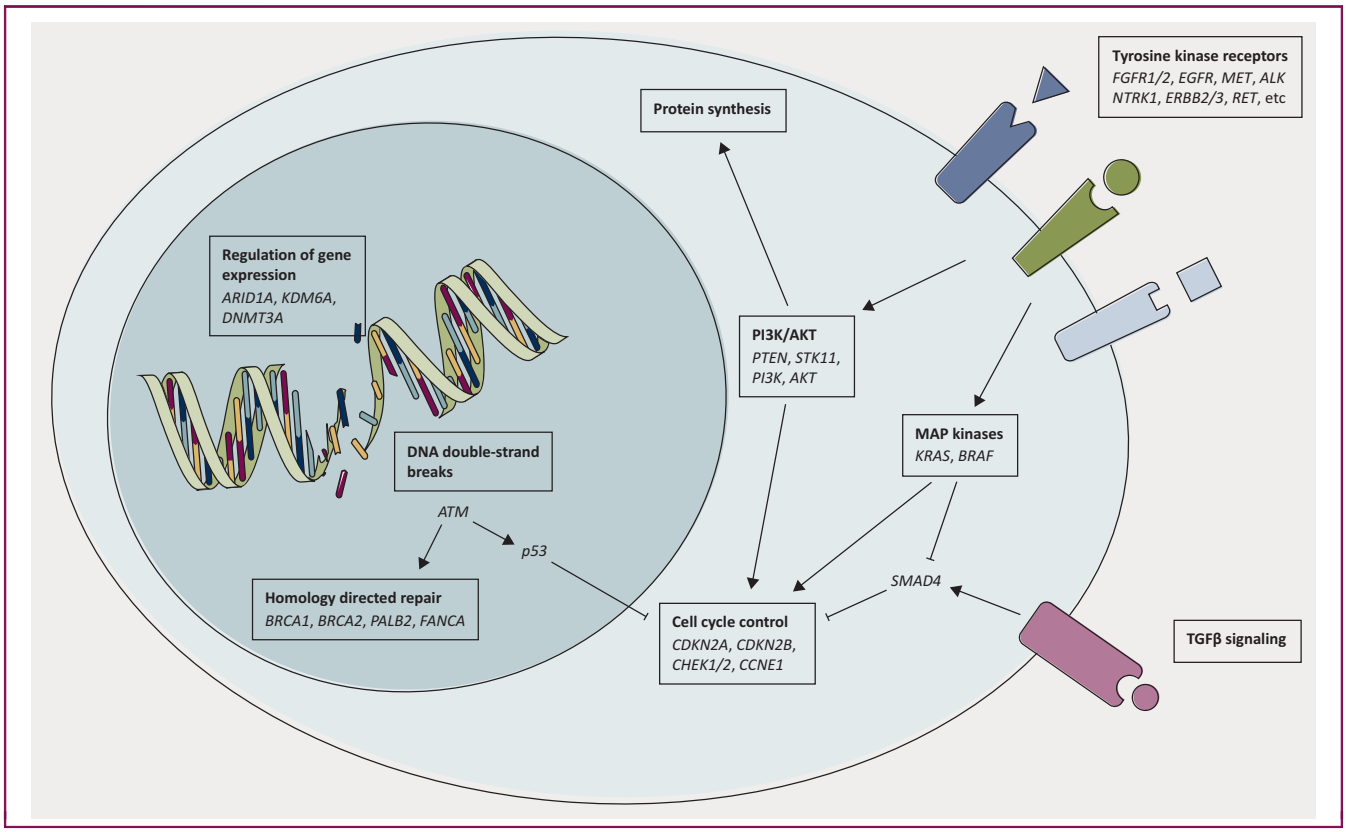

Figure 1. Signaling pathways and cellular processes involved in the pathogenesis of PDAC. For each signaling pathway, genes frequently mutated in PDACs are listed. MAP, mitogen-activated protein; PDAC, pancreatic ductal adenocarcinoma; PI3K, phosphoinositide-3-kinase; TGF-β; transforming growth factor-β.

fibroblast growth factor receptor 2 (FGFR2)...]. The active, guanosine triphosphate (GTP)-bound form of KRAS stimulates cell growth by activating the MAP kinases and PI3K/AKT pathways. Most *KRAS* mutations involve amino acid substitutions at the G12 position, which decrease the intrinsic GTPase activity, resulting in prolonged KRAS activation. *KRAS* mutations are the signature event of PDAC, with a frequency of 88%-92%. They occur at an early stage of the disease. The most common are G12D and G12V, found in 44%-48% and 28%-29% of *KRAS*-mutated PDACs, respectively.[10,16] *KRAS* wild-type PDAC is associated with a better prognosis in most studies. For example, in a retrospective cohort of 235 patients, *KRAS* wild-type status was associated with a 62% decreased hazard of death [hazard ratio ((HR) = 0.38, $P = 0.016$].[16] Similar findings were reported in a retrospective cohort of 741 patients with locally advanced or metastatic disease (HR = 1.26 for mutant versus wild-type *KRAS*).[17]

Mutations affecting the MAP kinases downstream of KRAS are also frequent in PDAC. They are enriched in *KRAS* wild-type PDACs, with a prevalence of 25%-38%.[15] The most frequently affected gene is v-Raf murine sarcoma viral oncogene homolog B (*BRAF*): *BRAF* is mutated in 10% of *KRAS* wild-type and 0.5% of *KRAS*-mutated tumors (20-fold enrichment).[15,18] BRAF is a kinase activated immediately downstream of KRAS. The most frequent mutations involving *BRAF* are: gene fusions (31% of *BRAF*-mutated PDACs), V600E (21%), and a deletion in exon 11 (21% of *BRAF*-mutated PDACs).[19] Other mutations activating MAP kinase signaling in *KRAS* wild-type PDACs include *EGFR* (4.3%), *ERBB2* (3.4%), and *MAP2K1* (2%). Altogether, gene fusions are found in 12% of *KRAS* wild-type tumors. They are mutually exclusive and are absent in *KRAS*-mutated cancers. These fusions predominantly involve *FGFR2*[20] (4.1% of *KRAS* wild-type PDACs), *BRAF* (2.4%), *ALK* (1.7%), *RET*, and neurotrophic tyrosine kinase (*NTRK*).[15]

Wnt is a signaling pathway involved in cell differentiation. In cancers, it stimulates proliferation and facilitates epithelial-to mesenchymal transition. Mutations impairing the Wnt pathway are found in 5% of PDAC. These are mostly loss of function mutations in Wnt inhibitors, such as RNF43 (3%) or adenomatous polyposis coli (APC) (2%).

SMAD4 is among the most mutated genes in PDAC, with a prevalence of 22%. SMAD4 is part of the transforming growth factor-β (TGF-β) cascade, a pathway involved in the negative regulation of proliferation. Mutations affecting other genes of the TGFβ pathway are infrequent in PDAC.

Mismatch repair (MMR) is a cellular machinery involved in repairing mismatches or small insertions/deletions in DNA. Deficient MMR (dMMR) results from a mutation or a methylation leading to loss of expression of an MMR protein (MLH1, PMS2, MSH2/6). dMMR is clinically relevant because it predicts sensitivity to checkpoint inhibitors in various tumors. dMMR is uncommon in PDAC, however, affecting only 0.1%-0.8% of patients.[15]

Homologous repair is involved in the repair of DNA double-strand breaks. It is an alternative to non-homologous end joining (NHEJ). Whereas homologous repair replaces damaged DNA *ad integrum*, however, NHEJ introduces mutations and translocations. In homologous repair-deficient tumors, recurrent NHEJ promotes oncogenesis by favoring mutations in oncogenes. Mutations in *BRCA1* or *BRCA2* are the most common cause of homologous repair deficiency, but other genes can be involved (*PALB2, FANCJ, FANCN*). The genomic instability resulting from deficient homologous repair is called BRCAness. Contrary to microsatellite instability (MSI) for MMR-deficient tumors, there is no consensus definition for BRCAness, even though signatures have been proposed.[21] The most widespread approach is to search for mutations in a panel of homologous repair genes. In PDAC, the overall prevalence of these mutations is 14%-15%, and they predominantly affect *BRCA2* (4%), *BRCA1* (2%), and *PALB2*.[15,22] Mutations in *BRCA2/1* are more common in younger patients (<50 years).[15] Ataxia telangiectasia mutated (*ATM*), a homologous repair activator, is mutated in 3-4, 5% of PDACs. In a cohort of 276 patients with metastatic PDAC, mutations in *BRCA* or *ATM* had no impact on 1-year OS.[23]

Among the genomic alterations observed in PDAC, a large majority result from somatic mutational events. Several hereditary syndromes are associated with PDAC, such as: the hereditary breast and ovary cancer syndrome (HBOC) resulting from germline mutations in *BRCA1/2*, the familial atypical multiple mole melanoma (FAMMM) syndrome, caused by mutations in *CDKN2A*, and Peutz-Jeghers syndrome, resulting from mutations in *STK11*. The absolute risk of PDAC of a 70-year-old patient with HBOC reaches 2%-10%, compared with 5%-25% for FAMMM and 36% for Peutz-Jeghers. Other entities, such as Lynch syndrome, are associated with an increased incidence of PDAC, but the absolute risk remains low. A recent study in 250 PDAC patients revealed that 15% carry a pathogenic germline variant; 68% of these variants affect DNA homologous repair.[24] *BRCA2* harbors the highest rate of germline mutations (3% of total PDAC patients), followed by *ATM, CDKN2A, APC*, and *BRCA1*.[15] Among all mutations affecting the homologous repair machinery, 54% are germline.[15]

The dysregulation of the cell cycle is central to PDAC pathogenesis: mutations affecting this process are found in >90% of PDACs. Figure 2 summarizes the actors of cell cycle regulation involved in the pathogenesis of PDAC. *TP53* is the second most frequently mutated gene in PDAC (74%-75% of PDACs). It encodes P53, a transcription factor and tumor suppressor that prevents cells with damaged DNA from proliferating. Upon detection of DNA damage, P53 is phosphorylated, which prevents its degradation. Accumulation of P53 induces cell cycle arrest at the G1/S checkpoint and activates DNA repair. In the absence of efficient DNA repair, persisting activation of P53 induces apoptosis.

Other cell cycle genes mutated in PDAC are *CDKN2A* (44%-45%), *CDKN2B* (21%), and *CCNE1* (3%). *CDKN2A* encodes P14 and P16, two proteins involved in the response to damaged DNA. P16 inhibits cyclin-dependent kinases 4 and 6 (CDK4/6), thereby blocking the transition from G1 to S phase. P14 contributes to cell cycle arrest by promoting the accumulation of P53.

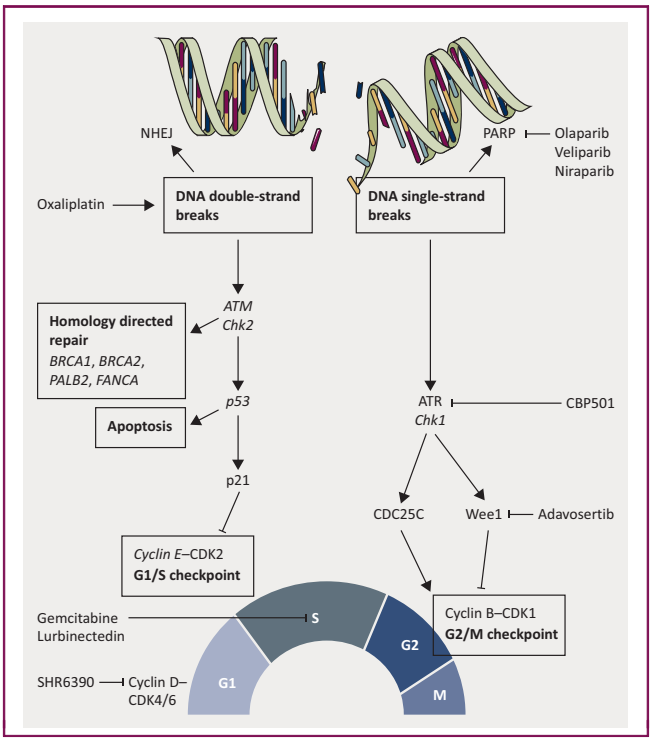

Figure 2. Interplay between DNA damage response and cell cycle control in PDAC. This figure shows the main signaling pathways involved in DNA damage response, and how they connect with cell cycle checkpoints. Genes frequently mutated in PDAC are written in italics. Targeted therapies and chemotherapies currently evaluated in PDAC are depicted, together with their molecular targets. CDK, cyclin-dependent kinase; NHEJ, non-homologous end joining; PARP, poly-ADP-ribose polymerase; PDAC, pancreatic ductal adenocarcinoma.

Chromatin remodeling through DNA methylation and histone modifications is a key mechanism for the control of gene expression. Mutations affecting these processes are found in 15% of PDACs: the most frequent involve *ARID1A* (8%), *KDM6A* (3%), and *DNMT3A* (3%).[15] ARID1A is a subunit of SWI/SNF, a chromatin remodeling complex. In a recent retrospective study on 3728 PDAC patients, mutations in the SWI/SNF system were found in 6.1% of tumors and were predictive of a worse prognosis (HR = 0.78, $P < 0.00001$).[25] KDM6A is a histone demethylase, whereas DNMT3A functions as a DNA methyltransferase: these enzymes maintain methylation patterns involved in the control of gene expression.

Targeted therapies as monotherapy. Understanding these molecular events has led clinicians to evaluate targeted therapies in metastatic PDAC. Table 2 presents ongoing trials on targeted therapies as monotherapy in metastatic PDAC.

Cell proliferation and survival. Targeted therapies inhibiting tyrosine kinase receptors or MAP kinases have been developed since the early 2000. They have shown no benefit, however, in PDAC patients not selected for specific

Table 2. Selected ongoing clinical trials evaluating targeted therapies as monotherapy in metastatic PDAC

	Drug	Target	Comparator	Patients	Phase	Status/trial ID
Tumor cell proliferation	Binimetinib HCQ	MEK1/2 Autophagy	No	M, L ≥2, KRAS mutated	I	Recruiting NCT04132505
	Trametinib Ruxolitinib	MEK1/2 JAK1/2	No	Colon or PDAC M or LA, L ≥2 KRAS mutated	I	Recruiting NCT04303403
	Exosomes with KRAS G12D siRNA	KRAS G12D	No	M, L ≥2 somatic KRAS G12D mutation	I	Recruiting NCT03608631
	Vemurafenib Sorafenib	BRAF Multitarget TKI (VEGFR, RAF, PDGFR...)	No	M, L ≥3, KRAS G12D mutation	II	Recruiting NCT05068752
	LY3214996 ± HCQ	ERK Autophagy	No	M, L2-3	II	Recruiting NCT04386057
	BPI-442096	SHP2	No	LA or M, L ≥2, KRAS G12 or BRAF mutation	I	Not yet recruiting NCT05369312
DNA repair	Fluzoparib	PARP inhibitor	Placebo	M, germline BRCA1/2 or PALB2 mutation	III	Recruiting NCT04300114
	Niraparib	PARP inhibitor	No	M, germline or somatic mutation in DNA repair gene	II	Recruiting NCT03553004
	Lurbinectedin	Transcription inhibitor	No	LA or M, germline or somatic mutation in DNA repair gene	II	Recruiting NCT05229588
	Niraparib	PARP inhibitor	No	M, L1, germline or somatic mutation in DNA repair gene	II	Not yet recruiting NCT05442749
Metabolism	SM88	Inhibitor of protein synthesis	GEM ABX or FOLFIRINOX	M, L1-2	III	Recruiting NCT04229004
Antibody-drug Tumor microenvironment	Anetumab-ravtansine* ± GEM ± nivolumab ± ipilimumab	Mesothelin Tubulin inhibitor PD-1 inhibitor CTLA4 inhibitor	No	M, mesothelin expression by IHC	I/II	Recruiting NCT03816358
	Tusamitamab-ravtansine*	CEACAM5 Tubulin inhibitor	No	M, L ≥2, CEACAM5 positive by IHC	II	Recruiting NCT04659603
Personalized treatment	Personalized therapy based on tumor biopsy		Chemotherapy (investigator's choice)	M, L ≥2	II	Active, not recruiting NCT02795650

ABX, nab-paclitaxel; BRAF, v-Raf murine sarcoma viral oncogene homolog B; ERK, extracellular signal-regulated kinases; GEM, gemcitabine; HCQ, hydroxychloroquine; IHC, immunohistochemistry; KRAS, Kirsten rat sarcoma viral oncogene homolog; LA, locally advanced; L, locally advanced; L1, first line; L2, second line; L ≥2, second line or more; M, metastatic; PARP, poly-ADP-ribose polymerase; PD-1, programmed cell death protein 1; PDAC, pancreatic ductal adenocarcinoma; PDGFR, platelet-derived growth factor receptor; siRNA, small interfering RNA; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.
*Stands for antibody-drug conjugate.

molecular alterations. This inefficiency could result from the genomic heterogeneity of PDAC, or from the rapid emergence of resistance. To overcome this limitation, most of the ongoing trials evaluating tyrosine kinases inhibitors in PDAC are using either multitarget inhibitors, or associations of several inhibitors. As an example, a phase I trial evaluates the combined use of trametinib, a MEK1/2 inhibitor, and ruxolitinib, a JAK1/2 inhibitor, in *KRAS*-mutated metastatic PDAC (NCT04303403). A phase II trial testing the association of vemurafenib, a BRAF inhibitor, with sorafenib, a multitarget tyrosine kinase inhibitor known to inhibit mainly vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) is ongoing (NCT05068752). This non-randomized phase II trial enrolls patients with *KRAS G12D*-mutated metastatic PDAC who progressed after at least two lines of systemic therapy.

As described previously, the PI3K/AKT pathway is a relevant target in PDAC. Idelalisib and buparlisib are two PI3K inhibitors that were evaluated in phase Ib trials in PDAC.[26] The study on idelalisib was ended prematurely, however, due to severe toxicities encountered with this drug in other trials. Buparlisib showed no sign of efficiency in PDAC and was associated with frequent adverse events.[25]

Tumor cell metabolism. PDAC displays specific metabolic features that may unveil therapeutic targets. Because of the fibrosis of their stroma, pancreatic cancer cells grow in an oxygen- and nutrient-deprived environment. Adaptations to this environment comprise an increased expression of glucose uptake systems, a dependence on autophagy to recycle molecular substrates, and a non-canonical use of certain amino acids such as glutamine.[27] Hydroxychloroquine, an autophagy inhibitor, is currently evaluated in several trials, in association with extracellular signal-regulated kinase (ERK) inhibitors (NCT04386057) or with the MEK1/2 inhibitor binimetinib (NCT04132505). D,L-alpha-metyrosine is a tyrosine analogue that blocks protein synthesis in preclinical PDAC models, ultimately leading to apoptosis. In clinical trials, this drug is evaluated within an oral anticancer regimen called SM88. SM88 consists of: a mammalian target of rapamycin (mTOR) inhibitor (sirolimus), a CYP3A4 inducer (phenytoin), an oxidative stress catalyst (methoxsalen), and D,L-alpha-metyrosine, in PDAC. In a phase II trial in 49 patients with heavily pretreated metastatic PDAC, the disease control rate was only 24.3%; there were no partial or complete responses.[28] An ongoing phase III trial will compare SM-88 with chemotherapy

(gemcitabine + nab-paclitaxel or FOLFIRINOX) in first or second line (NCT04229004).

Altogether, targeted therapies in monotherapy failed to improve survival in unselected patients with metastatic PDAC. The genomic heterogeneity of PDAC, however, had led clinicians to evaluate personalized treatments. Precision oncology consists of treatment personalization based on the mutational profile of each patient.

Some 90% of PDACs carry a mutation in *KRAS*. Designing targeted therapies against KRAS represents a challenge, however, due to the absence of a targetable site on the KRAS protein. The main active site of KRAS is the GTP-binding site. A competitive inhibitor cannot be designed, however, due to the extreme affinity for GTP. In 2013, researchers described inhibitors binding to a pocket found specifically on the KRAS G12C-mutant protein.[29]

The *G12C* mutant is infrequent in PDAC, with a prevalence of 1%-2%. Sotorasib, a KRAS G12C inhibitor, was approved by the EMA and FDA for the treatment of advanced lung tumors harboring *KRAS G12C*. In the phase I trial CodeBreaK100, 38 patients with metastatic PDAC received sotorasib. Among them, 8 patients had a partial response, corresponding to an objective response rate of 21%, and 24 had stable disease. The disease control rate (stable disease + tumor response) was 84.2%.[30] The median progression-free survival (PFS) was 4 months and the median OS 6.9 months in these heavily pretreated patients.[30] Another ongoing trial is evaluating sotorasib in combination with chemotherapy in patients with unresectable or metastatic *G12C*-mutated PDAC (NCT04644068).

The phase I/II trial KRYSTAL-1 evaluated adagrasib, another G12C inhibitor, in patients with advanced gastrointestinal tumors. Among the 10 assessable PDAC patients in this trial, 5 experienced a partial response and 5 had stable disease, with a median follow up of 6.3 months.[31] Evidence suggests that recurrence occurs early in patients receiving a G12C inhibitor: progression results either from additional mutations impairing the binding of the inhibitor to KRAS, or from fusions affecting other genes of the MAP kinase pathway.[32]

There is no inhibitor of other KRAS mutants, such as G12D or G12V, that has yet demonstrated clinical effectiveness in PDAC. An alternative strategy currently evaluated in clinical trials consists of inhibiting interaction partners of KRAS, such as SOS1 or SHP2: this may prevent KRAS activation in a mutation-independent manner[33,34] (NCT04111458, NCT05369312). Other methods are being explored, such as small-interfering RNAs (siRNAs) inhibiting KRAS G12D[35] (NCT01676259, NCT03608631). The relevance of this approach is limited, however, since siRNAs allow for a partial and transient inhibition of gene expression. Very recently, small molecule inhibitors of KRAS G12D have been discovered: these molecules will probably soon enter clinical trials.[36]

Mutations in homologous repair genes (*BRCA2, BRCA1, PALB2*) are found in 15% of PDACs. Poly-ADP-ribose polymerase (PARP) is an enzyme involved in the recruitment of the DNA repair machinery to single-strand breaks. PARP inhibition leads to an accumulation of single-strand breaks that give rise to double-strand breaks after DNA replication. Therefore, PARP inhibitors are expected to have maximum efficiency in PDACs with homologous repair defects. POLO, a phase III trial, highlighted the efficiency of olaparib, a PARP inhibitor, as a maintenance treatment of metastatic PDAC in patients carrying a *BRCA 1 or 2* germline mutation. The trial recruited patients whose disease did not progress after 16 weeks of first-line platinum-based chemotherapy. The median PFS was 7.4 and 3.8 months with olaparib and placebo ($P = 0.004$), respectively.[37] Based on these results, the FDA approved olaparib for the maintenance treatment of germline *BRCA*-mutated metastatic PDAC. Final results of the POLO trial showed no difference in median OS between olaparib (19 months) and placebo (19.2 months; $P = 0.35$).[38]

Several ongoing trials attempt to extend the use of PARP inhibitors to somatic *BRCA* variants or to patients displaying mutations in other genes. In a single-arm phase II trial, the PARP inhibitor rucaparib showed signs of efficiency as a maintenance therapy in patients with either somatic or germline mutations in *BRCA1/2* or *PALB2*. The overall response rate was 41.7%, and median PFS and OS were 13.2 months and 23.5 months, respectively.[39] Only 2 of the 42 patients, however, had somatic mutations. The ongoing MAZEPPA trial (NCT04348045) will evaluate olaparib in patients harboring a BRCAness phenotype resulting from somatic mutations.

Patients carrying germline mutations in *BRCA1/2* or *PALB2* also display increased responses to platinum-based chemotherapy.[11] Indeed, cross-linking of platinum to DNA results in double-strand breaks. In a retrospective cohort of 26 patients carrying germline mutations, the overall response rate was doubled compared with matched controls (59% versus 28%, $P = 0.002$) with no difference depending on the platinum derivate used.[40]

Mutations in *BRAF* are found mostly in *KRAS* wild-type PDACs, with a prevalence of 10%. Retrospective cohorts provide indirect evidence for the use of targeted therapies in these patients. Among 17 patients with *BRAF*-mutated PDACs treated with BRAF, MEK, or ERK inhibitors, the objective response rate was 53% and 36% of patients presented a partial response.[19]

Larotrectininb, an NTRK1/2/3 inhibitor, was first tested in pediatric tumors where *NTRK* fusions are the main genetic driver. In a pooled analysis of trials, larotrectinib demonstrated tumor-agnostic activity, with 73% of adult patients experiencing a partial response and a median PFS of 28.3 months. This study, however, included only two PDACs. Entrectinib is another NTRK1/2/3 inhibitor with a broader inhibition spectrum also encompassing ALK and ROS1. In a phase II basket trial, three patients with metastatic PDAC carrying *NTRK* or *ROS1* fusions received entrectinib: all experienced partial responses.[41] Larotrectinib and entrectinib obtained FDA approval for the treatment of solid tumors displaying *NTRK* gene fusion.[42]

Gene fusions involving neuregulin-1 (*NGR1*) result in aberrant expression of the EGF-like domain of NRG1 on the

cell surface, which serves as a ligand for human epidermal growth factor receptor 3 (HER3). This leads to pathologic activation of PI3K/AKT and MAP kinase pathways. NRG1 fusions are rare in PDAC (<1%). A phase II basket trial evaluated zenocutuzumab, a bispecific antibody targeting the HER3 pathway, in tumors harboring *NRG1* fusions. Among 10 PDAC patients treated with zenocutuzumab, the overall response rate was 40% and the disease control rate reached 90%.[43]

To standardize the evaluation of precision oncology strategies, the European Society of Medical Oncology (ESMO) defined the ESCAT scale (ESMO Scale for Clinical Actionability of Molecular Targets). An ESCAT score of I, corresponding to the best level of evidence, was attributed to three genomic alterations in PDAC: germline mutations in *BRCA1/2* (IA), MSI-H status (IC), and *NTRK* fusions (IC).[44]

Combination of targeted therapies and chemotherapy. Combining targeted therapies with chemotherapy is a promising approach that could help overcome tumor resistance to targeted therapies. Table 3 presents ongoing trials testing this strategy in metastatic PDAC.

Tumor cell proliferation. Several trials combined chemotherapy with targeted therapies inhibiting MAP kinases. Erlotinib, an EGFR inhibitor, was evaluated with gemcitabine versus gemcitabine alone in locally advanced or metastatic PDAC. The median OS was improved by 10 days with combined therapy (6.24 versus 5.91 months, $P = 0.038$).[45] This benefit is statistically significant but was not considered clinically relevant by health authorities. Trametinib, a MEK inhibitor, combined with gemcitabine was not superior to gemcitabine alone.[46] Overexpression of HER2 is identified in 10%-15% of PDACs. Trastuzumab in combination with capecitabine, however, showed no efficacy in metastatic PDAC.[47] Anlotinib is a multitarget inhibitor targeting FGFRs, VEGFRs, and PDGFRs. In advanced PDAC, an ongoing trial is evaluating anlotinib in association with gemcitabine, nab-paclitaxel and a programmed cell death protein 1 (PD-1) inhibitor (NCT04718701). Similarly, a phase II trial is testing batiraxcept, a fusion protein that neutralizes GAS6, in combination with gemcitabine and nab-paclitaxel in advanced PDAC (NCT04983407).

Cell cycle genes guard cellular integrity by halting proliferation at various checkpoints (G1/S, G2/M), allowing repair of damaged DNA. Figure 2 presents cell cycle regulation in PDAC and the therapies directed against these pathways. CDK4/6 are involved in progression through the G1/S checkpoint by phosphorylating the retinoblastoma protein (Rb). CDK4/6 inhibitors are approved in metastatic breast cancer with substantial toxicities resulting from inhibition of other CDKs. SHR6390, a more specific CDK4/6 inhibitor, is currently evaluated in association with gemcitabine and nab-paclitaxel in advanced PDAC (NCT05185869).

When the G1/S checkpoint is impaired, as in p53-deficient cancers, cells rely on the G2/M checkpoint to prevent entry of damaged DNA into mitosis. Wee1 is a regulator of the G2/M checkpoint. An ongoing trial is evaluating adavosertib, a Wee1 inhibitor, with gemcitabine plus nab-paclitaxel in advanced PDAC (NCT02194829). CBP501 is an inhibitor of checkpoint kinase 1 (Chk1), another regulator of the G2/M checkpoint.[48] In addition, CBP501 enhances the cytotoxic effect of cisplatin by increasing platinum influx into tumor cells.[49] A phase II trial is evaluating CBP501 in combination with cisplatin and nivolumab, versus cisplatin and nivolumab alone, in metastatic PDAC (NCT04953962).

Tumor cell metabolism. Polyamine metabolism is a metabolic process that controls protein and nucleic acid synthesis. In metastatic PDAC, a phase I trial evaluated SBP-101, an inhibitor of polyamine metabolism, with gemcitabine and nab-paclitaxel. Preliminary results show partial responses in 12 of 28 assessable patients (43%) and 11 additional patients had stable disease at 8 weeks.[50] Another trial will evaluate SBP-101 in combination with gemcitabine and nab-paclitaxel versus chemotherapy alone in treatment-naive metastatic PDAC (NCT05254171).

Tumor microenvironment

PDAC is characterized by a dense fibrotic stroma, called a desmoplastic reaction. Hyaluronan is a glycosaminoglycan that accumulates in the extracellular matrix in PDAC. A phase III trial evaluated the hyaluronidase PEGPH20 plus gemcitabine and nab-paclitaxel versus chemotherapy alone, in hyaluronan-high PDAC: there was no difference in OS or PFS between the two arms.[51] Another candidate drug is pamrevlumab, an antibody directed against CTGF, a glycoprotein that plays a central role in fibrosis. An ongoing phase III trial is evaluating pamrevlumab in association with gemcitabine and nab-paclitaxel in metastatic PDAC (NCT04229004).

TGF-beta plays an ambiguous role in PDAC.[52] On one hand, it exerts a tumor suppressive role by promoting cell cycle arrest. Consequently, TGF-beta signaling is impaired in 22% of PDACs through mutations in SMAD4. By contrast, TGF-beta has a pro-oncogenic effect on cancer-associated fibroblasts. An ongoing phase II study is evaluating the anti-TGF-beta monoclonal antibody NIS793 plus gemcitabine and nab-paclitaxel, versus chemotherapy alone, in metastatic PDAC (NCT04390763). A phase III trial with a similar experimental design started recruiting (NCT04935359).

Hedgehog is another signaling pathway that may promote pancreatic carcinogenesis.[53] Hedgehog signaling in cancer-associated fibroblasts stimulates the synthesis of the desmoplastic matrix. An ongoing phase I/II study will evaluate NLM-001, a Hedgehog inhibitor, in combination with gemcitabine, nab paclitaxel, and a CTLA4-inhibitor in metastatic PDAC (NCT04827953).

Nuclear factor-κB (NF-κB) is a signaling pathway involved in tumor neovascularization. Glycogen synthase kinase-3β (GSK-3β), besides its role in glycogen synthesis, is a positive regulator of NF-κB. In metastatic PDAC, an ongoing phase II study is evaluating 9-ING-41, an inhibitor of GSK-3β, in association with FOLFIRINOX and losartan (NCT05077800).

Table 3. Selected clinical trials evaluating targeted therapies in combination with chemotherapy in metastatic PDAC

	Drug	Target	Comparator	Patients	Phase	Status/trial ID
Cell proliferation/ survival	APG-1387 GEM ABX	IAP	No	M or LA, L ≥2	I/II	Recruiting NCT04643405
	Selinexor GEM	XPO1	No	M, L ≥2	II	Suspended NCT02178436
	Anlotinib Toripalimab ABX	Multitarget TKI (FGFR2, VEGFR...) Anti-PD-1	No	M or LA, L2	II	Recruiting NCT04718701
	Fruquintinib GEM ABX	VEGFR1/2/3	No	M, L1	II	Recruiting NCT05168527
	Adavosertib GEM ABX	WEE1	GEM ABX	M or LA, L ≥1	I/II	Active, not recruiting NCT02194829
	SHR6390 GEM ABX	CDK4/6 inhibitor	No	M or LA, L1	II	Not yet recruiting NCT05185869
	9-ING-41 Losartan FOLFIRINOX	GSK-3β TGF-β signaling	FOLFIRINOX	M, L1	II	Recruiting NCT05077800
	CBP501 Cisplatin ± nivolumab	Chk1 (G2 checkpoint) Anti-PD-1	Cisplatin Nivolumab	M, L ≥3 WBC <10G/L	II	Active, not recruiting NCT04953962
	Batiraxcept GEM ABX	GAS6-AXL signaling	GEM ABX	M or LA	I/II	Recruiting NCT04983407
	Sotorasib (GEM ABX or Nal-IRI + 5-FU)	KRAS G12C	No	M or LA, L ≥2, KRAS G12C mutated	I/II	Recruiting NCT05251038
	SGT-53 GEM ABX	P53 gene therapy	No	M, L1-2	II	Recruiting NCT02340117
Tumor cell metabolism	Galeterone ± GEM	Androgen receptor inhibitor	No	M, L ≥3	II	Recruiting NCT04098081
	Paricalcitol HCQ GEM ABX	Vitamin D analog Autophagy inhibitor	No	M or LA, L1	II	Recruiting NCT04524702
	Evolocumab Atorvastatin Ezetimibe FOLFIRINOX	3 Inhibitors of cholesterol metabolism	No	M, L1	I	Recruiting NCT04862260
	GP-2250 GEM	GAPDH inhibitor	No	M or LA, L2	I	Recruiting NCT03854110
	SBP-101 GEM ABX	Polyamine metabolism inhibitor	GEM ABX	M, L1	II/III	Recruiting NCT05254171
	SLC-0111 GEM	CAIX inhibitor	No	M, CAIX-positive	I	Recruiting NCT03450018
Tumor micro-environment	(pamrevlumab + GEM ABX) OR Racemetyrosine	CTGF Tyrosine metabolism	GEM ABX or mFOLFIRINOX	M, L1-2	III	Recruiting NCT04229004
	GEM ABX + Ramucirumab	VEGFR2	No	M or LA, L1	I/II	Active, not recruiting NCT03745430
	NIS793 Spartalizumab GEM ABX	TGF-β signaling Anti-PD1	GEM ABX	M, L1	II	Recruiting NCT04390763
	NIS793 GEM ABX	TGF-β signaling	GEM ABX	M, L1	III	Recruiting NCT04935359
	NLM-001 Zalifrelimab GEM ABX	Hedgehog signaling Anti-CTLA4	No	M, L1	I/II	Recruiting NCT04827953
	CEND1 GEM ABX	Drug transport	GEM ABX	M, L1	II	Recruiting NCT05042128
	Vactosertib Nal-IRI + 5-FU	TGF-β signaling	No	M, L2	I	Recruiting NCT04258072
	XB2001 Nal-IRI + 5-FU	IL-1α inhibitor	Nal-IRI + 5-FU	M or LA, L2	I/II	Recruiting NCT04825288
Epigenetics	GEM ABX +/− Romidepsin +/− Azacytidine	HDAC DNA-methyltransferase	GEM ABX	M, L1	I/II	Recruiting NCT04257448

5-FU, 5-fluorouracil; ABX, nab-paclitaxel; CAIX, carbonic anhydrase IX; CTGF, Connective tissue growth factor; CDK, cyclin-dependent kinase; FGFR, fibroblast growth factor receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GEM, gemcitabine; HDAC, histone deacetylase; IL-1α, interleukin 1α; KRAS, Kirsten rat sarcoma viral oncogene homolog; L ≥2, second line or more; L1, first line; L2, second line; LA, locally advanced; M, metastatic; Nal-IRI, liposomal irinotecan; PD-1, programmed cell death protein 1; PDAC, pancreatic ductal adenocarcinoma; TGF-ß, transforming growth factor-ß; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor

Finally, angiogenic factors such as VEGF could contribute to the pathogenesis of PDAC. Combining chemotherapy with anti-angiogenic agents such as bevacizumab or ramucirumab, however, was not superior to chemotherapy alone in patients with metastatic PDAC.[54,55]

Altogether, combinations of targeted therapies with chemotherapy have not yet demonstrated clinical benefit in metastatic PDAC.

Immunotherapy

Cancer immunotherapy has attracted considerable attention due to the rise of checkpoint inhibitors. Other immunotherapies are emerging, however, such as therapeutic vaccines or chimeric antigen receptor (CAR) T cells. Ongoing trials evaluating immunotherapy in metastatic PDAC are reported in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2022.100638.

Checkpoint inhibitors. Checkpoint inhibitors are antibodies targeting cofactors of lymphocyte activation, such as PD-1, programmed death-ligand 1 (PD-L1) or CTLA4. These drugs demonstrated clinical benefit in several tumors, including colon and esophageal cancer. Check-point inhibitors in monotherapy, however, show no sign of efficacy in metastatic PDAC.[56,57]

Several mechanisms have been proposed to explain PDACs resistance to checkpoint inhibitors.[58] First, lymphocyte activation requires the presence of tumor neoantigens. Tumors with a low TMB, such as pancreatic cancer, are less immunogenic. Beyond the TMB, tumors exhibiting dMMR have demonstrated increased sensitivity to checkpoint inhibitors. Consequently, the PD-1 inhibitor pembrolizumab obtained a tumor-agnostic approval for the treatment of dMMR tumors. In PDAC, however, the prevalence of dMMR is $<1\%$.[15]

Alternatively, checkpoint inhibitors possibly fail in PDAC due to the immunosuppressive microenvironment. Therefore, several trials are investigating combinations of checkpoint inhibitors with other therapies, with the aim to render the tumor sensitive to checkpoint blockade.[59] A phase I/II trial evaluated the combination of ipilimumab, a CTLA4 inhibitor, with the PARP inhibitor niraparib as a maintenance therapy for metastatic patients with stable disease after 16 weeks of chemotherapy: 59.6% of patients were progression free after 6 months compared with a predefined endpoint of 44%. There was, however, no appropriate comparator arm in this study.[60]

Therapeutic vaccines. Therapeutic vaccines are designed to release a large amount of tumor antigens, in order to elicit tumor-specific immune responses. Existing vaccines are approved for the treatment of prostate cancer, bladder cancer, and melanoma. GV1001 is a peptide vaccine derived from hTERT, an enzyme overexpressed in PDAC. The phase III TELOVAC trial evaluated the addition of GV1001 to a chemotherapy regimen combining gemcitabine and capecitabine, versus gemcitabine and capecitabine alone. The trial, which included 1062 treatment-naive patients with locally advanced or metastatic PDAC, was negative.[61] Another phase III trial tested the combination of GV1001 and chemotherapy in patients with elevated eotaxin. Patients in the control arm received gemcitabine and capecitabine alone. The trial was positive, with a median OS of 11.3 and 7.5 months in the experimental and control arms, respectively ($P = 0.021$).[62]

Adoptive cell therapy. Adoptive cell therapy consists of the *in vitro* expansion and reinfusion of autologous immune cells. For example, CAR T cells are lymphocytes engineered to express a receptor directed against a tumor-specific antigen. Lymphocytes collected from the patient by leukapheresis are genetically modified to express CARs, and then reinfused to the patient. Attempts to generate CAR T cells against solid tumors, however, have faced several challenges. Contrary to lymphomas, solid tumors do not express highly specific antigens: certain 'tumor-associated antigens', are overexpressed in solid tumors, but they are also expressed at lower levels in normal tissues. This lack of specificity results in a reduced homing of CAR T cells to the tumor.[63] The immunosuppressive tumor microenvironment also contributes to the insufficient homing of infused cells to the tumor.

Claudin-18 is a protein involved in tight junction formation. The Claudin 18.2 isoform is overexpressed in 50%-70% of PDACs. An early phase I study evaluated Claudin 18.2-specific CAR T cells in advanced Claudin 18.2-positive pancreatic or gastric cancers. Patients received one to five cycles of a lymphodepletion pretreatment with or without nab-paclitaxel followed by CAR T cell infusion. A total of 12 patients were included (7 gastric cancers and 5 PDACs). Among them, one patient with gastric cancer had a complete response and three showed partial responses (two gastric adenocarcinomas and one PDAC).[64] The objective response rate was 33.3%.

CONCLUSION

To date, the largest gain in life expectancy for patients with metastatic PDAC came from chemotherapy with FOLFIRINOX or gemcitabine-nab-paclitaxel: these regimens expanded the median OS from 4 to nearly 12 months. Targeted therapies provide no benefit in unselected patients with metastatic PDAC. They are, however, efficient in PDACs carrying specific mutations, such as: mutations affecting DNA homologous repair, *KRAS* wild-type PDAC, or the *KRAS G12C* mutant. Combining chemotherapy with targeted therapies has not yet demonstrated a clinical benefit in metastatic PDAC. Available immunotherapies are not efficient in PDAC, but this paradigm could potentially change due to the development of alternative approaches such as therapeutic vaccines or CAR T cells.

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