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RESEARCH ARTICLE

Molecular Features and Targeted Therapy in KRAS wild-type Pancreatic Cancer

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Abstract

KRAS mutation is the major oncogenic event in approximately 90% of pancreatic ductal adenocarcinomas. The subset of patients with *KRAS* wild-type pancreatic ductal adenocarcinomas represent a distinct subgroup with a higher frequency of actionable genomic alterations. In this review article, we aim at exploring the more frequent molecular alterations found among *KRAS* wild-type pancreatic ductal adenocarcinomas, their prognostic implications, as well as the potential targetable therapeutic options beyond cytotoxic chemotherapy for this unique subset of patients.

Keywords: pancreatic cancer; *KRAS* wild-type; targeted therapy

Introduction

Pancreatic cancer, although deemed classically a somewhat rare tumor subtype in mist of other gastrointestinal primary cancers, has proven to have rapidly increasing incidence rates and is projected to become one of the leading causes of cancer mortality in the United States by 2030[1]. Currently, PDAC accounts for about 3% of all cancers in the United States (US) and about 7% of all cancer-related deaths[2]. It is the third leading cause of cancer-related deaths in the US, projected to become the second most common in the next decade worldwide[1]. Despite the 5-year relative survival rate for pancreatic cancer having increased from 3% in the mid-1970s to 12% in the last decade, it still carries the lowest cancer survival rates[2]. Among patients with advanced PDAC, the 5-year survival rate remains under 3%[3].

It is estimated that 90% of the cases of pancreatic cancer are histologically classified as pancreatic ductal adenocarcinoma (PDAC). Also, *KRAS* is the major oncogenic driver in PDAC and its mutations are present in 90% of cases, an early step in the carcinogenic process, as attested by its presence in common preneoplastic and precursor lesions, such as pancreatic intraepithelial neoplasias (PanINs) and intraductal papillary mucinous neoplasms (IPMNs)[4]. To date, the standard treatment strategies in PDAC therapy are based on chemotherapy combinations, specially regimens such as FOLFIRINOX[5] and gemcitabine associated with nab-paclitaxel[6], with modest increases in overall survival (OS)[5,6].

Interestingly, there is a subset of PDAC that lacks activating mutations in the *KRAS* gene and is considered *KRAS* wild-type (*KRAS* wt)[7]. This subgroup corresponds to approximately 10% of PDACs in general[5], but up to 16% to 18% among patients under age 50[8]. The absence of *KRAS* mutations in PDAC is more common in females, older (>50 years) patients, and tumors located in the body and tail of the pancreas[7,9]. *KRAS* wt PDAC may present other mutations, fusions and molecular alterations, brightening, therefore, the horizon on possible molecular targeted therapies considering the hypothesis that in the absence of *KRAS*-activating mutations, other molecular and genomic alterations drive carcinogenesis and may potentially be targetable[10]. On that note, retrospective data demonstrated that patients with PDAC derived survival benefit upon receiving therapies matched to alterations presented in their tumors[11].

In this review we aim to focus on the minority of cases of pancreatic cancer that are *KRAS* wt and may harbor a wide variety of genetic and molecular alterations. We aim to characterize such alterations while exploring their therapeutic implications as shown in Figure 1 and the main studies involving such molecular alterations as well as their outcomes are outlined in Table 1.

TABLE - 1

Drug	First author	Type of study	Number of PDAC patients	Best Response	Progression-free survival (months)	Overall survival (months)
Fusions						
NTRK						
Larotrectinib	REF 98 Drilon et al	Phase I/II prospective	1	PR (1)	Not reported	Not reported
Larotrectinib	Ref 99 Hong et al	Pooled analysis	2	PR (1)	Not reported	Not reported
Entrectinnib	REF 100 Demetri et al	Pooled analysis	4	ORR: 75%	12.8 months	22 months
ALK						
Crizotinib	REF 104 Singhi et al	Case series	4	SD (3)	Not reported	Not reported
Alectinib	REF 105 Ou et al	Case report	1	PR	Not reported	Not reported
RET						
Selpercatinib	REF 107 Subbiah et al	Phase I/II prospective	11	ORR 54.5%	Not reported	Not reported
Pralsetinib	REF 108 Subbiah et al	Phase I/II prospective	4	CR (1)	Not reported	Not reported
FGFR						
Pemigatinib	REF 112 Subbiah et al	Phase I/II prospective	4	ORR: 25%	Not reported	Not reported
NRG1						
Zenocutuzumab	REF 116 Schram et al	Phase I/II prospective	4	SD	Not reported	Not reported
MSI-H						
Pembrolizumab	Ref 84 Marabelle et al	Phase II prospective	22	CR (1)	2.1 months	4.0 months
Pembrolizumab	REF 85 Le et al	Phase II prospective	8	CR (2)	Not reported	Not reported
Dostarlimab	REF 86 Andre et al	Phase I prospective	11	ORR: 0%	Not reported	Not reported

Drug	First author	Type of study	Number of PDAC patients	Best Response	Progression-free survival (months)	Overall survival (months)
MAPK						
EGFR						
Nimotuzumab	REF 52 Schultheis et al	Phase IIb prospective	13	SD	Not reported	53.8% (12 months)
Nimotuzumab	REF 53 Qin et al	Phase III prospective	92	SD	4.2 months	10.9 months
BRAF						
Dabrafenib + trametinib	REF 80 Salama et al	Prospective	2	SD (1)	Not reported	Not reported
HRD						
Olaparib	REF 130 Golan et al	Phase III prospective	92	CR (50)	7.4 months	No difference
Rucaparib	REF 131 Reiss et al	Phase II prospective	42	CR (3)	13.2 months	23.5 months

Table 1: Main studies involving targeted molecular alterations in Pancreatic adenocarcinoma (mainly with *KRAS* wild-type) as well as their outcomes.

Abbreviations: PDAC: pancreatic adenocarcinoma; PR: partial response; CR: complete response; SD: stable disease; ORR: overall response rate

Methods

For this literature review, we conducted a broad search on Pubmed and abstracts published in the American Society of Clinical Oncology Annual Meeting, American Society of Clinical Oncology Gastrointestinal Cancers Symposium, European Society of Medical Oncology Annual Meeting, and European Society of Medical Oncology World Congress on Gastrointestinal Cancer, spanning from 2013 to 2022. For our search on English language articles and abstracts, we used the terms "wild-type KRAS pancreatic cancer"; "wild-type KRAS AND pancreatic cancer"; "wild-type KRAS AND pancreas cancer"; and "Molecular profile AND pancreatic cancer".

Diagnosis of KRAS mutation in pancreatic cancer

The oncogenic *KRAS* mutation is the major event in pancreatic cancer; it confers permanent activation of the KRAS protein, which results in the molecular switch for the GTP bound active state with a failure to convert GTP to GDP (inactive state). As a consequence, it constitutively activates a cascade of intracellular signaling pathways and transcription factors inducing cell differentiation, proliferation, migration, transformation, adhesion, and survival[12]. In clinical practice, *KRAS* mutation testing is currently applied in some epithelial cancers, such as colorectal cancers (CRC), for

therapeutic purposes, since monoclonal antibodies targeting epidermal growth factor receptor (EGFR) can only be administered in metastatic *KRAS* wt CRC[13]. The single-nucleotide variant (SNV) at codon 12 (exon 2) represents more than 80% of *KRAS* mutations in PDAC, with G12D, G12V and G12R being the most common ones[14]. SNVs can also occur less frequently at codons 11, 13, 61 or 146.

Several laboratory methods have been developed to detect *KRAS* mutations in biological samples (fresh tumor tissues, formalin- fixed paraffin- embedded tissue, fine needle aspiration [FNA] materials and cytology, pancreatic juice, total blood, plasma and urine), most of them with the use of PCR to amplify the appropriate region of the gene, including exons 2 and 3, and then employing different *KRAS* mutation detection techniques in key codons, such as codons 12 and 13[15]. There are some challenges for the 'first generation' of *KRAS* mutation assays (e.g.: restriction fragment length polymorphism plus sequencing or sequencing alone) to detect a mutant allele in specimens with poor cellularity or with a high desmoplastic environment. Various effective methods have been developed to address some of these challenges and to increase analytical sensitivity, which include quantitative PCR methods, allele-specific PCR, next-generation sequencing (NGS), real- time PCR methods (with specific probe technologies, such as peptide nucleic acids), and droplet digital PCR (ddPCR)[15]. In fact, the techniques available for that have different levels of limit of detection (LOD) of mutant alleles and sensitivity such as Sanger sequencing (LOD of 20%)[16], NGS (LOD of 1-6%)[17], amplification-refractory mutation system PCR (ARMS-PCR – LOD of 1%)[18], Enhanced-ice-COLD PCR (Co-amplification at a lower denaturation temperature)/mutant-enriched PCR (LOD of 0.1%) [19], and ddPCR (LOD of 0.1%)[20].

At this moment, the main data source for identification of *KRAS* mutations is based on CRC. In clinical practice, direct sequencing (PCR followed by sequencing) is still an important method for detecting mutations. Although direct sequencing is able to detect all mutations of interest, it requires a high allele frequency of mutation (LOD of 10–30%). The sensitivity of this assay may not be appropriate for clinical application[18,21].

TheraScreen *KRAS* kit (Qiagen), a test based on ARMS technology, was the first FDA-approved assay used to evaluate tumor-specific mutations in patients with CRC, which is able to detect seven mutations in codons 12 and 13 with higher sensitivity and specificity when compared to direct sequencing[22]. StripAssay (Vienna Labs), a mutant-enriched PCR followed by reverse hybridization, can detect 10 of the most common mutations with lower LOD and higher cost than direct sequencing[23]. There is another technique, known as SNaPshot, that can detect 12 mutations in codons 12 and 13 with lower sensitivity and cost than StripAssay[23]. The TaqMelt PCR assay Cobas (Roche) is able to detect 19 mutations in codons 12, 13 and 61, which is more sensitive and reproducible than the TheraScreen assay. Moreover, this assay has a rapid turnaround time[24]. NGS methodology has some advantages in this scenario, such as detecting uncommon

mutations by entire exon sequencing, which may be clinically relevant for prognostic and predictive information and harboring a greater sensitivity. Due to its high cost per sample, NGS panels usually analyze mutational hotspots in various oncogenes, being more likely to find actionable targets than only testing *KRAS*[25,26].

Furthermore, the search of *KRAS* mutation combined with cytopathology analysis in EUS-FNA materials has the potential to increase the sensitivity, the negative predictive value and the accuracy of cytopathology alone for the differential diagnosis of pancreatic cancer and benign conditions like autoimmune and chronic pancreatitis[27,28]. Also, a large number of studies have been conducted to assess the role of *KRAS* mutation assay in liquid biopsy samples for diagnosis, minimal residual disease, prognosis and monitoring during PDAC treatment. In the scenario of liquid biopsy, the development of new technologies with greater sensitivity is needed. A potential future approach will be the combination of several methods for detecting circulating tumors elements (e.g.: circulating tumor cells, circulating tumor DNA, circulating cell-free RNA, extracellular vesicles, and tumor-educated platelets), multi-omics analyses (i.e.: genomics, transcriptomics, proteomics), and machine learning methods[29–31].

Prognostic implications of *KRAS* wild-type pancreatic cancer

Kim and colleagues demonstrated that patients with *KRAS* wt advanced PDAC treated with gemcitabine-based chemotherapy showed a better objective response rate (ORR) and longer OS compared to *KRAS* mutant patients[32]. Another small study involving patients with loco-regional and metastatic PDAC demonstrated a longer OS for the *KRAS* wt subgroup independent of the age at diagnosis, gender, stage of disease, MMR status, and chemotherapeutic regimen[33]. Similar to previously reported studies, a large real-world data showed that the overall cohort of *KRAS* wt PDAC had a statistically significant prolongation of OS compared to the *KRAS* mutated counterpart, especially for the subgroup with metastatic disease. This survival advantage was observed for the subgroup treated with gemcitabine/nab-paclitaxel or fluorouracil/oxaliplatin[10]. Within the *KRAS* wt cohort, *TP53* wt status was the molecular alteration enriched in patients with longer OS[10]. Dai and colleagues evaluated the prognostic value of *KRAS* status in patients with early resectable PDAC and demonstrated that *KRAS* wt, which was more prevalent in the Chinese population (18.9%), had longer disease-free survival (DFS) and OS[34]. Patients with *KRAS*-G12D mutation exhibited shorter OS and DFS than patients in the other KRAS mutant subgroups[34].

In summary, *KRAS* mutations are identified in nine out of ten patients with PDAC and tend to be associated with reduced DFS and OS, regardless of the stage of PDAC or type of treatment[35]. Also, some data suggest that the *KRAS* mutation subtype such as G12D might negatively influence prognosis, regardless of systemic therapy[36].

Prognosis of *KRAS* mutation has also been evaluated by detection in liquid biopsies. In most studies, the detection of mutation in

plasma is significantly associated with a poor prognosis, especially for OS[29]. In the case of early stage PDAC, a significant association with disease recurrence has been noted[37]. Although promising, these data require further validation in order to reach clinical practice.

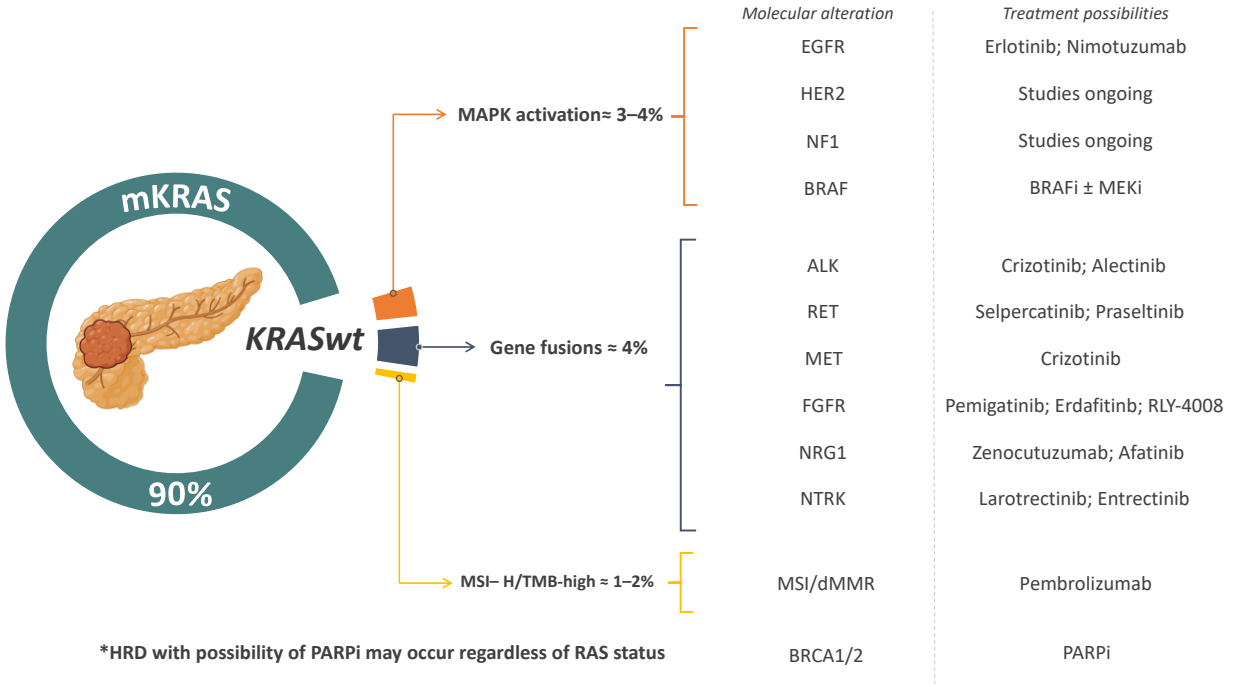

Figure 1: *KRAS* wt PDAC represents approximately 10% of all PDAC. The main molecular alterations of *KRAS* wt PDAC are represented in the figure along with possible targeted therapeutic strategies.

Abbreviations: EGFR: epidermal growth factor receptor; HER2: Human Epidermal Growth Factor Receptor 2; NF1: neurofibromatosis 1; *BRAF* inhibitor; MEKi: MEK inhibitor; HRD: Homologous Recombination Repair Deficiency; MSI-H microsatellite instability high; TMB-high: tumor mutational burden high; dMMR: Microsatellite instability; MSI: mismatch repair deficiency PARPi: PARP inhibitor.

Subgroups of *KRAS* wt Pancreatic Cancer

The subset of PDAC considered *KRAS* wt represents a distinct molecular subtype of PDAC. In an analysis of 2,483 unselected PDAC patients, amongst the KRAS wt population (233 patients), the most frequent mutated gene was *TP53* (44.5%), followed by *BRAF* (13.0%) as well as DNA-damage repair pathway genes, and genes involved in cell-cycle regulation, chromatin remodeling and amplifications[10].

KRAS wt PDAC can be mainly divided into three different groups based on the genetic alterations encountered: approximately 4% being PDAC with altered Mitogen-Activated Protein Kinase (MAPK) pathway other than *KRAS* mutation; 1-2% of PDAC with microsatellite instability or DNA mismatch repair defects, accompanied by a high mutational tumor burden; and another 4% presenting with tumors with kinase gene fusions or rearrangements, totalizing 10% of

all wt *KRAS* PDAC[38]. These data highlight the importance of comprehensive molecular profiling including RNA-based assays for identification of gene fusions in this highly actionable subgroup of PDAC.

Considering that *KRAS* and *BRAF* mutations are virtually mutually exclusive in PDAC, the presence of *BRAF* mutations in such tumors suggest that the MAPK pathway can be alternatively activated, leading to oncogenesis regardless of the presence of *KRAS* mutations[39]. Therein, it is reasonable to consider molecular targeted therapy for this subset of patients.

Microsatellite instability (MSI)/mismatch repair deficiency (dMMR) is another genetic alteration that is enriched in the *KRAS* wt PDAC population. Indeed, MSI/dMMR PDAC harbor less frequent *KRAS* mutations[40] and present with higher tumor mutational burden[10]. The prevalence of MSI/dMMR PDAC can be higher in some subsets, such as IPMN-derived carcinomas and medullary and mucinous/colloid variants[41,42]. Nonetheless, it is important to note that approximately 30% of MSI/dMMR PDAC can also present with *KRAS* mutation[41].

Lastly, yet not less important, is the third group of *KRAS* wt PDAC presenting with kinase gene fusions. Although it is possible to encounter *KRAS* mutant PDAC with a gene fusion, most of them will be found among *KRAS* wt tumors[9].

However, in addition to the aforementioned subgroups, PDAC patients may also present with yet another molecular alteration in the DNA repair pathways such as Homologous Recombination Repair Deficiency (HRD) and the genetic alterations herein involved. In this context, it appears that among PDAC patients, HRD may have a slightly more frequent presentation in *KRAS* wt PDAC when compared to *KRAS* mutated PDAC.

Thus, such molecular findings prompt a complementation in the diagnosis of PDAC demonstrating the need to go beyond histological diagnosis and routinely determine the *KRAS* status of PDAC. Whole genome sequencing (WGS) with RNA-sequencing (RNA seq) for all metastatic PDACs, therefore, a broadened molecular profile would be warranted and such PDAC subtypes could be considered to guide therapeutic strategies.

KRAS wt with altered MAPK pathway other than *KRAS* mutation

EGFR mutated *KRAS* wt Pancreatic Cancer

Events involving the epidermal growth factor receptor (EGFR; also known as HER1 – Human Epidermal Growth Factor Receptor 1) are common in PDAC. Silent mutations have been described in up to 81% of PDAC [44]. *EGFR* overexpression and activating *EGFR* mutations occur in 30-95% and 0.5-4% of PDAC, respectively[43–46]. In the largest analysis so far, *EGFR* activating mutations were detected in 0.5% (n=16) of patients with PDAC using circulating tumor DNA (ctDNA)[46]. Among these patients, 73% had *KRAS* wt tumors, demonstrating that *EGFR* mutations act as an alternative mechanism to activate the MAPK pathway. Mutations were distributed along exons 18 (n=3), 19 (n= 3), 20 (n= 6), and 21 (n=4). Importantly, 75% of these

mutations were known or predicted to be sensitizing to EGFR small tyrosine kinase inhibitors (EGFR TKIs). Additionally, rare fusions involving the *EGFR* have also been reported [10].

The use of anti-EGFR therapy in non-selected patients with PDAC did not translate into clinically meaningful results[49,50]. Even for tumors with high levels of EGFR expression, anti-EGFR therapy was not associated with improved outcomes[47,48]. However, the use of anti-EGFR therapy for selected patients with tumors harboring activating *EGFR* mutations seems justified. Multiple case reports describe responses to EGFRs TKIs in patients with PDAC with activating *EGFR* mutations[49,50]. Additionally, in one small randomized trial from Taiwan, 88 patients were randomized to treatment with gemcitabine with or without erlotinib[51]. In the subgroup of patients with tumors harboring activating *EGFR* mutations (N = 49), the addition of erlotinib was associated with improved median progression-free survival (PFS; 5.9 *vs* 2.4 months; p = 0.004) and overall survival (OS; 8.7 *vs* 6.0 months; p = 0.044). However, the results of this trial have been seen with caution due to the very high rate of activating *EGFR* mutations in this population. It is not known whether these results are due to differences in tumor biology between ethnic groups or differences in the *EGFR* mutation detection techniques.

In 2017, Schultheis and colleagues conducted a multi-institutional, placebo-controlled, randomized phase IIb trial that demonstrated the effect of the association of gemcitabine with nimotuzumab, an anti-EGFR humanized IgG1 monoclonal antibody, compared to gemcitabine plus placebo in first-line treatment setting in locally advanced or metastatic PDAC patients[52]. This study randomized 192 unselected PDAC patients and presented a median OS of 8.6 months vs. 6.0 months (HR: 0.69, P = 0.03) in favor of the nimotuzumab plus gemcitabine arm. The 12-month OS survival rate in the general population also favored the nimotuzumab arm (34% *vs* 19%, P = 0,0341). Also, there was an increase in PFS with a median PFS of 5.1 vs. 3.4 months (HR: 0.68, P = 0.02) but no difference in objective response rates.

When analyzing the subpopulation of *KRAS* wt PDAC (26.5%), the 12-month OS rate was significantly improved with the addition of nimotuzumab to gemcitabine (53.8% *vs* 15.8%, P = 0,026). This OS result was not demonstrated in the *KRAS* mutated population (27.8% *vs* 17.9%, P non-significant). Also, the OS gain was demonstrated in the subpopulation of patients presenting with *EGFR* overexpression in the treatment arm when compared to placebo (36.4% vs. 8.3%, P = 0,045), improvement not shown in the subgroup of normal *EGFR* expression PDAC. Therefore, showing that activating *EGFR* mutations may be targeted in PDAC *KRAS* wt patients with interesting perspectives.

More recently, Qin and colleagues[53] published data of a phase III randomized trial that tested the combination of nimotuzumab and gemcitabine (versus gemcitabine combined with placebo) in 92 Chinese patients diagnosed with locally advanced or metastatic *KRAS* wt PDAC. The authors

presented that OS was significantly improved in the nimotuzumab-gemcitabine group (10.9 *vs* 8.5 months, p = 0.025) with a 1-year survival rate of 43.6% in the treatment group compared to 26,8% in de control group and 13.9% vs. 2.7% at three years. Regarding disease progression, the median PFS was significantly improved in the nimotuzumab-gemcitabine group (4.2 *vs* 3.6 months, p = 0.013). However, the authors did not find significant differences in ORR between the two study arms.

ERBB2/HER2 Alterations

Aberrations in other members of the Human Epidermal Growth Factor Receptor family (HER family) have been described in PDAC, especially in HER2. Studies report a wide range of HER2 expression rates in PDAC (0-82%)[54], probably due to tumor heterogeneity and differences in the methodology used to assess HER2 expression. Recent studies using the criteria used for gastroesophageal cancers have revealed that 5-11% of PDAC have strong (3+) membrane HER2 staining on immunohistochemistry[54–56]. It is important to note that staining is frequently heterogeneous, especially for HER2 negative or HER2 low (1+ or 2+) tumors[54]. *In situ* hybridization (ISH) studies have demonstrated ERBB2 (the gene codifying HER2) amplification in 2.1-23.8% of the tumors (pooled = 7.7%)[57]. However, in genomic studies, ERBB2 amplification has been described in lower frequency both in *KRAS* wt (3.8%) and mutant (2.4%) PDAC[9]. Interestingly, patients with ERBB2-amplified PDAC are characterized by the lack of liver metastasis and preponderance of lung and brain metastasis[58]. Finally, rare cases of *ERBB2*, *ERBB3*, and *ERBB4* mutations have been described in *KRAS* wt PDAC [10].

Most of the previous efforts to evaluate anti-HER2 treatments in PDAC have focused on non-selected populations. The combinations of gemcitabine plus trastuzumab plus erlotinib and lapatinib plus chemotherapy (either capecitabine or gemcitabine) have yielded disappointing results in prospective single-arm studies[59–61]. However, while HER2 expression or ERBB2 amplification have not been definitely associated with prognosis in PDAC [54,57], HER2 expression might have direct therapeutic implications. To date, only one study has evaluated the activity of trastuzumab in a selected group of patients with PDAC expressing HER2 (3+ or 2+ and ISH+)[55]. However, results were frustrating, with a median PFS of only 65 days for the combination of capecitabine plus trastuzumab in the first line setting. To date, the combination of trastuzumab with other anti-HER drugs has not been formally tested in PDAC. One case report describes a patient with HER2-expressing PDAC who sustained stable disease for nine months with the combination of trastuzumab and pertuzumab[62]. Currently, trastuzumab plus pertuzumab is being tested in patients with PDAC harboring *HER2/HER3* amplification, mutation, or overexpression in an expanded cohort of the TAPUR (Targeted Agent and Profiling Utilization Registry) study (NCT02693535). However, it is important to highlight that since *KRAS* mutant pancreatic cancer constitutively activates MAPK and other pathways in the absence of HER2/3

stimulation, medications that simply inhibit these receptors might not be active in the in the setting of *KRAS* mutation, as recently seen in biliary tract cancer[63].

Recent breakthroughs in drug development have boosted the activity of anti-HER2 agents. Especially important in this scenario is the antibody-drug conjugate (ADC) trastuzumab deruxtecan, which has shown impressive activity against a broad range of tumors with HER2 overexpression. Limited evidence suggests promising activity of trastuzumab deruxtecan in HER2 positive PDAC. In a dose-expansion phase I study evaluating the activity of trastuzumab deruxtecan in multiple solid tumors, one patient with HER2+ (2+ and ISH+) PDAC achieved a partial response [64]. Another case report describes a deep response after treatment with trastuzumab deruxtecan plus nivolumab [62]. Given its unique mechanism of action and the ability to tackle tumors with heterogeneous HER2 expression (so called bystander effect)[65], trastuzumab deruxtecan is expected to become another option in the treatment of this group of patients. Currently, two studies are enrolling patients with advanced solid tumors, including pancreatic adenocarcinoma with HER2 overexpression (DESTINY-PanTumor02, NCT04482309) or *ERBB2* mutations (DESTINY-PanTumor01, NCT04639219) to receive trastuzumab deruxtecan.

Finally, chimeric antigen receptor modified T cells (CAR T cells) targeting HER2 have also been evaluated in two patients with HER2 positive PDAC enrolled into a small phase I Chinese study [66]. The best response for these patients was stable disease, with PFS times of 5.3 and 5.8 months. Despite the small sample size and the use of different criteria to assess HER2 positivity, this study demonstrates that, besides ADCs, CAR T cells might play a role in the treatment of this subgroup of patients in the near future.

NF1

The *Neurofibromatosis-1 (NF1)* gene codifies the NF1 protein, a GAP (GTPase-activating protein) responsible for boosting the weak intrinsic RAS GTPase activity [67]. Therefore, deficiencies in NF1 activity increase MAPK pathway signaling (so termed RASopathy). Germline mutations in *NF1* are the underlying genetic mechanism responsible for type I neurofibromatosis (von Recklinghausen's disease). However, the association between neurofibromatosis and an increased risk of gastrointestinal tumors, including PDAC, is uncertain [68]. *NF1* alterations occur in 3 to 25% of PDAC [67] and are related to significant levels of MAPK signaling in *KRAS* wt PDAC, and most missense and nearly half of the nonsense mutations in *NF1* are predicted to culminate in proteins with lower or absent RAS GTPase-activating domain. Importantly, almost all these nonsense mutations (7 out of 8) occur in patients with *KRAS* wt PDAC. Furthermore, confirming its role in PDAC pathogenesis, recent *in vitro* studies have shown that concomitant NF1 and TP53 inactivation are sufficient to trigger full-blown PDAC in the absence of *KRAS* mutations[67].

MEK inhibitors have been used with success in the treatment of *NF-1*-associated plexiform neurofibromas in the setting of type 1

neurofibromatosis[69]. Recently, activity against other tumors in the spectrum of the type 1 neurofibromatosis has also been described [70]. However, as of today, there is no clinical datum to support the use of MEK inhibitors for patients with *KRAS* wt PDAC harboring *NF1* alterations. The NCI-sponsored MATCH trial (subprotocol S1) is currently investigating the efficacy of trametinib in patients with hematological and solid tumors, including PDAC, with *NF1* genetic changes (NCT04439318). Additionally, another study is evaluating the combination of a SHP2 inhibitor (PF-07284892) with binimetinib in solid tumors with mutations in *RAS*, *NF-1,* or *BRAF* (NCT04800822).

BRAF

The Mitogen-Activated Protein Kinase (MAPK) pathway is activated in 38% of *KRAS* wt PDAC as a result of molecular events involving components other than *KRAS* in this signaling pathway [9]. As a group, alterations in *BRAF* comprise the most frequent molecular event affecting the MAPK pathway, being present in 11-13% of *KRAS* wt PDAC[9,10]. Indeed, *BRAF* alterations, especially *BRAF*/*RAF1* fusions, are particularly common in acinar pancreatic adenocarcinomas[71,72]. These alterations are considered to be virtually exclusive with *KRAS* mutations (occurring in only 0.4% of *KRAS* mutant PDAC).

BRAF V600E mutation, the most frequent *BRAF* mutation found in other tumor types such as melanoma and CRC, is responsible for a minority of *BRAF* alterations found in PDAC. Also, despite similar prognosis[72], the type of *BRAF* alteration seems to have direct therapeutic implications. *BRAF* mutations can be classified into three different classes according to the kinase activity, RAS-dependency, and dimerization status[73]. In addition, fusions and deletions involving *BRAF* can lead to analogous biological consequences. Class I mutations (such as the V600D/E/K/R mutations) result in strong kinase activity (~500-700-fold compared to wildtype *BRAF*), independent of RAS signaling and BRAF dimerization[73]. Short deletions near the αC helix of the kinase domain (such as N486_P490del) have similar biological consequences to class I mutations. Class II mutations have intermediate kinase activity leading to RAS-independent signaling through protein dimerization. Activating *BRAF* fusions lead to constitutively active BRAF dimers that signal similarly to class II mutant *BRAF*. Finally, class III mutations have low intrinsic kinase activity compared to *BRAF* wt and lead to *BRAF* mutant heterodimerization with CRAF or BRAF wt with signaling transduction dependent on RAS activation by upstream effectors in the MAPK pathway.

Theoretically, class I mutations and short deletions near the αC helix of the kinase domain render the tumor sensitive to BRAF inhibitors (with or without MEK inhibitors). Indeed, multiple case reports describe response to dabrafenib (BRAF inhibitor) with or without trametinib (MEK inhibitor), vemurafenib (BRAF inhibitor) plus trametinib, and encorafenib (BRAF inhibitor) plus binimetinib (MEK inhibitor) in patients with PDAC[74–78]. Another MEK inhibitor, cobimetinib, has also been combined with

chemotherapy (gemcitabine plus nab-paclitaxel) with a complete radiological response in one patient[79]. In the largest study so far, clinical responses have been described in all 3 patients with exon 15 (class I) mutations[72]. On June 23rd, 2022, based on the results of the ROAR (Rare Oncology Agnostic Research) cohorts and the NCI-MATCH trial subprotocol H, the FDA granted accelerated approval for the combination of dabrafenib plus trametinib as an agnostic treatment for patients with unresectable or metastatic solid tumors with *BRAF* V600E mutations. In the latter study, two patients with PDAC were treated with this combination, and one patient experienced progressive disease as the best response and the other had an ongoing stable disease for approximately 2.5 months[80].

Also, it has been shown that patients with the short deletion N486_490Pdel can benefit from BRAF inhibitors[81]. In the previously mentioned analysis, 40% (2/5) of the patients with tumors with BRAF N486_490Pdel had clinical response after single-agent MEK inhibitors (one partial radiological response). Among patients with *BRAF/RAF1* fusion, 80% (4/5) of the patients experienced clinical response after single-agent MEK inhibitor (two partial radiological responses). Currently there is no data on the activity of BRAF or MEK inhibitors in PDAC with class II or III mutations. However, given that class III mutations frequently co-occur with aberrations that lead to activation of RAS, the combination of BRAF (and MEK inhibitors) with other drugs targeting upstream components of the MAPK pathway seems necessary [82]. Finally, it is important to highlight that the presence of other confounding tumor drivers likely abolishes the benefit provided by anti-BRAF treatments in these patients [72].

MSI/dMMR

Overall, microsatellite instability high (MSI-H)/ defective DNA mismatch repair (dMMR) are infrequently seen among PDAC with an estimated frequency of 1-2% with the majority of these cases due to Lynch Syndrome[41,83]. In a systematic review including 34 studies and 8323 PDAC patients, 2.61% of them had MSI-H/dMMR tumors. After eliminating studies focusing on PDAC subtypes apparently enriched by this molecular feature, the prevalence of MSI-H/dMMR tumors was 2.53%, yet higher than expected, which might be explained by the fact that only 6 of the 34 included studies used the suggested and standardized IHC antibodies and/or MSI PCR markers. When only studies based on NGS were considered, the MSI/dMMR prevalence varied from 0% to 1.6%. Nonetheless, the presence of MSI-H/dMMR tumors was strongly associated with medullary and mucinous/colloid histology, as well as with *K-RAS* wt and *P53* wt PDAC [5]. A very recent study reported on WGS results of 2483 PDAC samples, including not only 2297 PDAC, but also less common histologies, such as 120 mucinous, 45 squamous / adenosquamous, 11 acinar, 7 sarcomatoid, 2 pseudopapillary and 1 pleomorphic. Overall, 10.7% of the tumors were *KRAS* wt, especially in acinar (81.8%) and pseudopapillary (100%) histologies. Indeed, *KRAS* wt PDAC were more likely to be MSI-high/MMR-deficient (4.7% vs 0.7%; $p<0.05$) [10]. Taken together,

these data indicate that the group of patients with *KRAS* wt PDAC is enriched for the presence of MSI/dMMR.

Those MSI-H/dMMR tumors typically accumulate thousands of mutations, which encode potential neoantigens, featuring a hypermutated genome and high activity of immune checkpoint inhibitors (ICI) in an agnostic fashion[84]. In the study by Le et al., 86 patients with MSI-H/dMMR tumors that had progressed to at least one line of therapy, including 8 with PDAC, were treated with anti-PD-1 antibodies. Among PDAC, ORR was 62%, including two patients with complete response [85]. However, less robust benefit was reported for PDAC in the phase II Keynote-158 trial, where 233 patients with non-colorectal MSI-H/dMMR tumors who had progressed to at least one prior therapy were treated with pembrolizumab, including 22 individuals with PDAC. ORR was modest at 18.2%, while mOS was only 4 months for PDAC, meaning that PDAC achieved the worst outcomes among the different investigated cancers[84].

Dostarlimab, an anti-PD1 antibody, was also evaluated in refractory MSI-H/dMMR tumors, including 11 patients with PDAC who participated in the cohort F of the GARNET phase I trial. ORR was 45.5% for PDAC, consistent with the other solid tumors [86]. Additionally, a retrospective study evaluated the efficacy of ICI among 9 patients with MSI-H PDAC detected by a plasma-based circulating tumor DNA liquid biopsy. Indeed, 8 of them received pembrolizumab, while one was treated with nivolumab and ipilimumab. Interestingly, ORR was 77% [87].

Unfortunately, there is no further information on histologies or the status of *KRAS* for patients included in those immunotherapy studies. In accordance with other tumor types, the presence of MSI in PDAC might also confer resistance to chemotherapy, although further studies are needed [88]. So far, the US Food and Drug Administration (FDA) and other regulatory agencies have approved the PD-1 immune checkpoint inhibitor pembrolizumab for the 'site-agnostic' treatment of MSI/dMMR tumors. However, it remains unknown whether MSI-H PDAC should be treated with pembrolizumab alone or if they require a combination treatment with either chemotherapy or anti-CTLA4 monoclonal antibody.

Gene fusions and rearrangements

It has been well-known the group of PDAC lacking *KRAS* mutation is enriched in highly actionable alterations, which act as oncogenic drivers. In recent years, cases of exceptional responses to targeted therapy mainly in *KRAS* wt PDAC harboring a variety of oncogene fusions have been reported, suggesting that those genetic alterations are more likely to be encountered among *KRAS* wt PDAC[89–92].

In one of the largest molecular analyses of PDAC, 1164 patients had their tumors sequenced and 144 (12.4%) were *KRAS* wt. Targetable fusions were encountered in 22%, while 52% harbored pathogenic mutations. Additionally, 5 patients had potentially targetable amplifications. The following fusions were described: *BRAF* (n=10), *RAF1* (n=2), *MET* (n=1), *FGFR2* (n=6), *FGFR3* (n=1), *ERBB2* (n=1), *EGFR* (n=1), *NRG1* (n=2),

RSPO3 (n=1), *ALK* (n=3), *ROS1* (n=1), *RET* (n=3) and *NOTCH* (n=1) [93]. Interestingly, fusions were largely exclusive of other drivers.

In another retrospective analysis of 100 patients with PDAC sequenced at Moffitt Cancer Center, 13% had *KRAS* wt tumors. Among those, 31% were identified with targetable gene fusions, including one patient with a *MET* fusion who achieved an ongoing complete response with crizotinib. Meanwhile, none of the *KRAS* mutant tumors harbored gene fusions[94]. This was further validated in two different cohorts of patients, where 19 and 20% of individuals with *KRAS* wt tumors had gene fusions identified (AACR Genie and TCGA cohorts, respectively)[94].

Another study evaluated the role of a novel fusion detection algorithm with high sensitivity and short runtime collecting (RNA seq samples from a total of 803 individuals across 18 studies on PDAC[95]. Matched whole genome sequencing (WGS) was available for 327 samples. The authors detected 30 potential driver fusions in the RNA-seq data, which were confirmed in WGS data. Fusions involving the following oncogenes were encountered: *BRAF* (n=4), *NRG1* (n=4), *NTRK3* (n=4), *PRKACA* (n=4), *RAF1* (n=4), *FGFR2* (n=3), *ALK* (n=2), *RET* (n=2), *NTRK1* (n=1), *RASGRP1* (n=1), and *ROS1* (n=1). Only 4 fusions were detected among *KRAS* mutant PDAC. Fusions were significantly associated with *KRAS* wt PDAC and, interestingly, some of the involved proteins were direct interaction partners of *KRAS*, such as *BRAF*, *RAF1*, and *RASGRP1*. Those findings indicate that the fusion proteins phenocopy the effect of *KRAS* activating mutations [95].

Testing for gene fusions makes sense since most of them are amenable to targeted therapy, as already shown in several tumor types. As the most well-known example, tropomyosin receptor kinase (TRK) inhibitors, such as larotrectinib and entrectinib, are indicated agnostically and approved by several regulatory agencies for metastatic patients harboring *NTRK* rearrangements. Indeed, patients with PDAC had been included in studies with TRK inhibitors, with reported benefit[96–100]. In a series of 400 PDAC which underwent WGS and RNAseq, 3 patients had an *NTRK* fusion (2 EML4-NTRK3 in a *KRAS* wt tumor and a single novel KANK1-NTRK3 fusion in the setting of a subclonal *KRAS* mutation). In this study, the overall prevalence of *NTRK* fusions in PDAC was 0.8%, while in *KRAS* wt tumors, it was 6.25% (2/32)[101]. Entrectinib is also a ROS inhibitor and has shown activity in a patient with PDAC harboring a *ROS-1* fusion[92].

ALK inhibitors, such as crizotinib and alectinib, are already successfully implemented in clinical practice for treating non-small cell lung cancer (NSCLC) harboring either *ALK* or *ROS* fusions [102,103]. In a study including 3,170 patients with PDACs who underwent comprehensive genomic profiling, 5 cases harboring an ALK translocation were identified, all of them in young patients (<50 years-old) with *KRAS* wt tumors[104]. Among those 5 patients with *ALK* fusions, 4 were treated with an ALK inhibitor and 3 of them achieved clinical benefit. In addition, a case report described a 34-year-old male with *ALK* rearrangement-positive and *KRAS* wt PDAC had a remarkable response to crizotinib after

resistance to prior chemotherapy and achieved a further response to alectinib after developing brain metastases[105]. Crizotinib is also a MET inhibitor and a complete response to this drug has also been reported in PDAC harboring a novel RDX-MET fusion[94].

RET gene rearrangements are more commonly found in pancreatic acinar cell carcinoma (ACC) and exclusive of *KRAS* mutation. In a study with 40 acinar cell spectrum tumors (36 pure ACC), 7.5% harbored a *RET* fusion[106]. Meanwhile, the recently published phase I/II LIBRETTO-001 trial showed that selpercatinib, a RET inhibitor, has interesting tumor-agnostic efficacy with an overall response rate of 43.9% [107]. In this phase I study, 12 (27%) of patients had PDAC. In a similar fashion, the phase I/II ARROW trial evaluated pralsetinib, also a RET inhibitor, in 29 patients with 12 different RET-fusion positive solid tumors, including 4 individuals with PDAC who achieved objective response including 1 complete response[108].

In biliary tract cancer, especially intrahepatic cholangiocarcinoma, *FGFR2* fusion has been reported in up to 15% of cases [109] and many respond to FGFR inhibitors. In a phase II trial, pemigatinib demonstrated a 35% overall response rate (ORR) in previously treated intrahepatic cholangiocarcinoma harboring *FGFR2* fusions or rearrangements, leading to its approval by some regulatory agencies[110]. In a similar population of patients, RLY-4008, a first highly selective, potent FGFR2 inhibitor designed to target both driver alterations and *FGFR* resistance mutations led to an extraordinary ORR of 88%[[111]. In advanced PDAC harboring FGFR alterations, a phase I/II trial with 4 PDAC patients demonstrated an ORR of 25%[112]. Also, in trials including PDAC patients with *FGFR2* fusions, remarkable responses with erdafitinib have been reported as well[94,113,114].

As for neuregulin-1 gene (*NRG1*), their fusion proteins are known oncogenic drivers in PDAC. They bind to HER3, leading to HER2/HER3 heterodimerization and consequent ErbB-mediated pathway activation[115]. There are many promising targeted therapies for *NRG1* fusion-positive tumors under investigation, such as EGFR-tyrosine kinase inhibitor, HER3, and HER2 antibodies[116]. In a multicenter phase II study and early access program investigating the efficacy of zenocutuzumab, a bispecific antibody targeting *NRG1* fusion signaling in *NRG1* fusion positive tumors, 4 out of 10 patients with *KRAS* wt PDAC responded and 90% achieved disease control [116]. In another study, two previously treated and refractory patients with *NRG1* rearranged PDAC achieved clinical response with ERBB inhibition, one with afatinib and the other with the combination of erlotinib and pertuzumab[89]. Similarly, 2 other patients with NRG1 fusion-positive PDAC responded to afatinib[90].

Meanwhile, gene fusions affecting *BRAF* or *RAF1* have been increasingly reported as potential therapeutic targets, especially in ACC, as previously described[117]. In a study involving comprehensive genomic profiling of 44 ACCs, recurrent rearrangements involving BRAF and RAF1 were identified in approximately 23% of tumors[118]. Another study evaluated 1,062 PCs and 3 of them featured *BRAF* fusions (2 ACC and 1 PDAC)[71].

Those *BRAF* and *RAF1* fusions lead to RAS/MAPK pathway activation and may be amenable to BRAF and/or MEK inhibition.

Since most gene fusions have been reported in patients with *KRAS* wt PDAC, systematic testing of the *KRAS* mutation and further screening for fusions only if *KRAS* wt has been recommended. However, a very small proportion of fusions may be lost with this approach. Therefore, we recommend, whenever available, WGS with RNAseq for all metastatic PDACs, not only to detect potential fusions, but also other targetable genetic alterations. Fusion detection is still challenging, and an RNA sequencing method with a good prediction accuracy is important to prevent false negative results[119].

HRD

HRD is characterized by a defect in the homologous recombination repair (HR) pathway is one of the mechanisms of DNA repair and is responsible for the correction of double-strand DNA breaks[120].

Classically, the core HR pathway genes are *BRCA1/2* and *PALB2*. Germline mutations of these genes (gBRCA1/2 and gPALB2) have been identified in approximately 5% of unselected cases of PDAC[121]. Classically, germline genetic alterations are more frequently identified than somatic mutations in PDAC (15% vs 5%)[122] and PDAC is the third most common cancer type amongst the cancer types related to the breast-cancer related (BRCA) gene mutations[123]. Within the genetic familial syndromes with high risk for pancreatic cancer, BRCA2 mutations have been seen in up to 17%[124].

However, there are other genetic alterations that are involved in DNA repair pathways that may also contribute to the HRD phenotype in PDAC, such as *RAD51*, *ATM*, *BARD1*, *BRP1*, *CHECK2* and *FANC* genes[121] A systematic review and prevalence meta-analysis of HRD in unselected PDAC patients involving 60 studies with 21,842 participants demonstrated the following germline and somatic mutations: *BRCA1* (0.9%), *BRCA2* (3.5%), *PALB2* (0.2%), *ATM* (2.2%), *CHEK2* (0.3%), *FANC* (0.5%), *RAD51* (0.0%), and *ATR* (0.1%)[125]. The aforementioned study also demonstrated that the prevalence of HRD alterations ranged between 14.5%-16.5% through targeted NGS and 24%-44% through WGS or whole-exome sequencing (WES)[125], suggesting that HRD is likely to go beyond point mutations in the core genes and that other molecular mechanisms are still to be uncovered.

Interestingly, in *KRAS* wt patients, the prevalence of some HRD genes seems to be slightly more common in comparison with *KRAS* mutated patients. A study analyzing 2,426 PDAC tumors (15% *KRAS* wt) demonstrated a significant higher frequency of *BRCA1* mutations in *KRAS* wt patients when compared *KRAS* mutated PDAC (9% vs. 3%, p = 0.05) [126]. Also, a more recent study analyzing ctDNA of 2,000 PDAC patients (1,000 *KRAS* wt and 1,000 *KRAS* mutated) demonstrated that HRD related mutated genes, such as *ATM,* appear to be more frequent in *KRAS* wt. patients (26% vs. 15%, $P < 0.05$). Additionally, albeit not statistically significant, *BRCA1/2* (12% vs. 11%) and *CHEK2* (6% vs. 5%) appeared to be numerically more common in *KRAS* wt PDAC. When regarding germline

mutations, *ATM* pathogenic alterations were significantly more frequent in *KRAS* wt compared to *KRAS* mutated tumors (3.8% vs. 2.1%, p = 0.04)[94]. However, other HRD pathogenic germline alterations, although more frequent in some cases, were not significant[94].

Studies have demonstrated the benefit of platinum-based therapeutic strategies in gBRCA1/2 mutated PDAC treatment in both neoadjuvant and first-line metastatic settings[127–129]. Associated to platinum-based chemotherapy strategies, the use of the PARP inhibitor (PARPi) olaparib is approved as maintenance therapy for patients with *gBRCA1/2* advanced or metastatic PDAC whom did not present disease progression (sustaining disease control) on a previous platinum-based treatment regimen. This approval was based on the results of the phase III POLO trial conducted with 154 PDAC patients with *gBRCA1/2* mutations and which demonstrated an improvement in median PFS of olaparib maintenance therapy when compared to placebo (7.4 vs 3.8 months; hazard ratio for disease progression or death: 0.53; P = .004). However, albeit positive in its primary endpoint, the POLO trial failed to demonstrate a significant improvement in OS and also in quality of life[130]. More recently, a Reiss and colleagues[131] published a phase II trial evaluating maintenance therapy with rucaparib, another PARPi, in 42 patients with advanced PDAC harboring both somatic or germline mutations of *BRCA1/2* and *PALB2*. Patients were included in this trial if they had not demonstrated disease progression (no evidence of tumor growth or elevation of tumor marker) within a minimum of 8 weeks of platinum-based chemotherapy and demonstrated promising median PFS and OS of 13.2 and 23.5 months, respectively[131].

It is important to note that only the core HR genes (*BRCA1/2*, *PALB2, RAD51C, and RAD51D*) are clinically validated biomarkers in PDAC. Besides, it has been shown that PDAC with *BRCA* mutations that present platinum-based chemotherapy resistance may also present PARPi resistance[132,133]. As such, identifying primary and secondary therapeutic resistance mechanisms in PDAC with HRD is of utmost importance, such as seeking additional molecular alterations of the HRD phenotype with NGS techniques. Combination strategies with immunotherapy may be a strategy in overcoming such resistance mechanisms to both platinum chemotherapy and PARPi and are in development.

CONCLUSIONS

Pancreatic cancer is classically associated with one of the worst prognoses among all solid tumors and the mainstay of treatment for advanced disease is cytotoxic chemotherapy. The subset of patients with *KRAS* wild-type tumors comprises 10% of PDAC cases and is characterized by a higher frequency of actionable genomic alterations when compared to KRAS mutated PDAC. An increasing body of data has described the efficacy of targeted therapeutic strategies in this unique subset of patients, highlighting the importance of genetic testing in this group of tumors.

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