Targeting KRAS for the potential treatment of pancreatic ductal adenocarcinoma: Recent advancements provide hope (Review)

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Abstract. Kirsten rat sarcoma viral oncogene homolog (*KRAS*) is one of the most frequently mutated oncogenes in solid tumors. More than 90% of pancreatic ductal adenocarcinoma (PDAC) are driven by mutations in the *KRAS* gene, suggesting the importance of targeting this oncogene in PDAC. Initial efforts to target KRAS have been unsuccessful due to its small size, high affinity for guanosine triphosphate/guanosine diphosphate, and lack of distinct drug-binding pockets. Therefore, much of the focus has been directed at inhibiting the activation of major signaling pathways downstream of KRAS, most notably the PI3K/AKT and RAF/MAPK pathways, using tyrosine kinase inhibitors and monoclonal antibodies. While preclinical studies showed promising results, clinical data using the inhibitors alone and in combination with other standard therapies have shown limited practicality, largely due to the lack of efficacy and dose-limiting toxicities. Recent therapeutic approaches for KRAS-driven tumors focus on mutation-specific drugs such as selective $KRAS^{G12C}$ inhibitors and son of sevenless 1 pan-KRAS inhibitors. While $KRAS^{G12C}$ inhibitors showed great promise against patients with non-small cell lung cancer (NSCLC) harboring $KRAS^{G12C}$ mutations, they were not efficacious in PDAC largely because the major *KRAS* mutant isoforms in PDAC are G12D, G12V, and G12R. As a result, $KRAS^{G12D}$ and pan-KRAS inhibitors are currently under investigation as potential therapeutic options for PDAC. The present review summarized the importance of KRAS oncogenic signaling, challenges in its targeting, and preclinical and clinical targeted agents including recent direct KRAS inhibitors for blocking KRAS signaling in PDAC.

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Abbreviations: PDAC, pancreatic ductal adenocarcinoma; KRAS, Kirsten rat sarcoma viral oncogene homolog; NSCLC, non-small cell lung cancer; GTP, guanosine triphosphate; GDP, guanosine diphosphate; RTK, receptor tyrosine kinases; PanIN, pancreatic intraepithelial neoplasia; mTOR, mammalian target of rapamycin; PDX, patient-derived xenograft; FTI, farnesyltransferase inhibitor

Key words: pancreatic cancer, PDAC, KRAS, MAPK, PI3K

Contents

1. Introduction

RAS oncogenes [*HRAS, NRAS,* Kirsten rat sarcoma viral oncogene homolog (*KRAS*)] comprise the most frequently mutated gene family in cancer, identified in ~30% of cancers. The RAS family plays a critical role in major cellular processes such as growth and proliferation; thus, an activating mutation in these genes can lead to tumor formation. Within this group, *KRAS* is the most frequently mutated isoform in cancer, identified in ~84% of all *RAS*-mutant cancers. *NRAS* mutation (11-17%) is also relatively prevalent, while *HRAS* is quite rare among *RAS*-mutated cancers (1).

KRAS is a small GTPase signal transduction protein that cycles between active guanosine triphosphate (GTP)-bound and inactive guanosine diphosphate (GDP)-bound states. In normal quiescent cells, RAS is largely GDP-bound and inactive, but the GTP-bound state is formed through extracellular stimuli activation of receptor tyrosine kinases (RTKs) as well as other cell-surface receptors. Activated KRAS maintains the engagement of effector proteins that then regulate several intracellular signaling networks that control mitogenic

processes. KRAS has been shown to play a central role in controlling tumor metabolism (2). Oncogenic *KRAS* genes are characterized by missense mutations that encode single amino acid substitutions at three primary locations: Glycine-12 (G12), glycine-13 (G13), or glutamine-61 (Q61). Among these, G12 mutations comprise 83% of all *KRAS* mutations, followed by G13 (14%) and Q61 (2%) mutations (3). The mutation subtypes at *KRAS* are mainly classified as *KRAS* G12D, G12V, G12C, G13D, G12R and G12A. *KRAS* mutations are most common in pancreatic cancer, NSCLC and colorectal cancer, and the profiles of *KRAS* mutation subtypes differ in different types of cancer. For example, G12C mutation is the most common subtype in NSCLC (41%), whereas G12D and G12V are the major subtypes in pancreatic cancer and colorectal cancer (4). All *KRAS* mutations render KRAS constitutively bound to GTP and active, overstimulating effector signaling pathways to drive uncontrolled growth of cells leading to cancer formation. This suggests that blocking KRAS has high therapeutic potential for several cancers. In the present review, the critical role of oncogenic KRAS signaling, challenges in its targeting, and preclinical and clinical studies targeting KRAS signaling, including its downstream signaling effectors or direct inhibitors, in pancreatic ductal adenocarcinoma (PDAC) were investigated. In the present review, a comprehensive literature search was performed using three databases, PubMed, Medline and Web of Science.

2. KRAS in pancreatic cancer

Comprising >90% of cases, the most common form of pancreatic cancer is PDAC, which has a 5-year overall survival (OS) rate of 11% (5). PDAC is the 3rd leading cause of cancer-related deaths in the US and has the highest frequency of *KRAS* mutations (>90%). PDAC displays two major *KRAS* mutations at G12D (41%) and G12V (34%), while other less frequent mutations are G12R (16%), Q61H (4%) and G12C (~1%) (6).

PDAC has four types of pre-neoplastic precursors: Intraductal papillary mucinous neoplasm, pancreatic mucinous cystic neoplasm, intraductal tubular papillary neoplasm, and pancreatic intraepithelial neoplasia (PanIN). PanIN is the most common precursor lesion and is classified as low-grade (PanIN-1A and PanIN-1B), intermediate-grade (PanIN-2), or high-grade (PanIN-3) before progressing to PDAC. *KRAS* mutation is an early event in the genetic onset of PDAC. The progression from normal pancreatic tissue to PDAC is typically initiated by advancing stages on noninvasive microscopic ductal lesions, PanINs. *KRAS* mutations are considered to be the driving force in the development of human PanINs and >90% frequency of *KRAS* mutations are identified in PanIN-1 lesions. Furthermore, Aguirre *et al* reported that the $KRAS^{G12D}$ mutation alone formed PanIN and the protracted onset of PDAC (7).

PanIN-to-PDAC progression is initiated by subsequent inactivation of tumor suppressor gene cyclin-dependent kinase inhibitor 2A, identified in PanIN-2, and then followed by inactivation of two other tumor suppressor genes, *TP53* and *SMAD4*, proteins unique to PanIN-3. KRAS-mediated signaling then further leads to the development of PDAC through several metabolic processes, providing the energy and biosynthetic building blocks necessary to drive uncontrolled tumor growth. Furthermore, previous studies revealed that *KRAS*-mutant PDAC tumor cells are capable of regulating autophagy to meet metabolic demand (8,9). Although *KRAS* mutations are the initiating genetic step, mutant *KRAS* is still involved in every step to maintain the growth of metastatic PDAC (2). The gradual progression of KRAS-mediated PDAC progression ultimately results in rapid growth and metastasis. Thus, there is high therapeutic potential in targeting the KRAS pathway for improving clinical PDAC therapy.

3. KRAS and metabolic reprogramming in pancreatic cancer

The presence of *KRAS* mutations creates an ideal environment for the thriving of cancer cells, as it elevates the levels of glucose and glycolytic intermediates, cellular redox potential, and uptake of fatty acids and glutamate. Furthermore, mutant *KRAS* enhances micropinocytosis and promotes amino acid turnover (2,10-12). Autophagy becomes a vital mechanism for *KRAS* mutant PDAC cells, supporting tumor growth and survival during starvation (2,10-11). Additionally, mutant *KRAS* plays a role in reprogramming the tumor microenvironment (TME) by influencing the expression of enzymes that regulate glucose metabolism including HK1/2, phosphofructokinase 1, and LDHA (2,11,13,14). The KRAS/MAPK signaling pathway further amplifies the expression of MYC, leading to increased nonoxidative pentose phosphate pathway gene RPIA expression and sustained nucleotide biosynthesis (15). Furthermore, mutant *KRAS* orchestrates the reprogramming of noncanonical glutamine metabolism, resulting in a cellular redox state that strongly favors tumor growth (16,17). Collectively, these studies establish the crucial role of mutant KRAS in maintaining PDAC growth through the selective activation and adaptation of biosynthetic metabolic pathways. Consequently, exploring the metabolic reprogramming occurring in *KRAS* mutant PDAC presents an opportunity to gain insights into the underlying mechanisms of tumor progression and therapeutic resistance. Such knowledge can be harnessed to develop innovative therapeutic strategies.

4. Challenges in KRAS direct targeting

In theory, targeting KRAS signaling could potentially provide an effective approach to block the growth of KRAS-dependent tumors, but this was deemed unrealistic due to several barriers. First, KRAS is a member of a large family of related proteins sharing similar GTP/GDP binding domains, causing the development of specific targeted drugs very difficult. Second, KRAS has a very high picomolar affinity for GTP and GDP, both of which have very high intracellular concentrations. Third, the KRAS protein lacks accessible binding sites for the high-affinity binding of small-molecule inhibitors (18). Thus, not only do KRAS protein inhibitors have limited effectiveness due to chemical affinity, but they are also hindered by the structure of the target proteins. Due to these challenges, KRAS is widely considered undruggable, shifting focus to blocking downstream effector signaling.

Targeting downstream effector signaling has emerged as a promising alternative approach to block oncogenic KRAS signaling pathways. Oncogenic-activating *KRAS*

mutations regulate an intricate complex of cytoplasmic signaling networks. There are at least 11 different effector families, and tumorigenesis is likely the result of multiple integrated effector-signaling pathways (19). Thus, while it shows great promise, targeting downstream KRAS effector signaling is more complex than it seemed at first glance. Therapeutic approaches must decide which effector pathways are the best to target and if co-inhibition of multiple effectors is required. Based on the prominent role of KRAS in PDAC progression and difficulties in the direct targeting of KRAS, much of the efforts have centered on indirect strategies in hopes of finding a more effective treatment for PDAC.

5. Preclinical studies targeting KRAS signaling in pancreatic cancer

KRAS-GTP is known to activate several downstream oncogenic signaling pathways including PI3K/AKT/mTOR, MAPK, RAL-PLD1 and T1AM1-Rac, and crosstalk exists among these pathways (Fig. 1). Thus, the concept of targeting KRAS effector signaling is not without complications.

PI3K/AKT/mTOR pathway inhibition. Previous studies have shown that PI3K subunits play a critical role as effectors of mutant KRAS-driven oncogenesis (20,21). PI3K phosphorylates PIP2 and stimulates the formation of PIP3, which then phosphorylates and activates a multitude of proteins, including AKT 1/2/3, localizing it in the plasma membrane. AKT phosphorylates numerous other proteins that promote cell growth, in particular the mammalian target of rapamycin (mTOR) (22,23). The PI3K/AKT/mTOR pathway plays a pivotal role in a number of cellular processes such as proliferation, survival, and growth. Abnormalities in this pathway such as PI3K mutation/amplification, loss of PTEN, *AKT* mutation or RTK activation, have been implicated in cancer cell proliferation, invasion, survival, metastasis, epithelial-mesenchymal transition (EMT) and drug resistance (24,25). There have been conflicting studies regarding PI3K activation in driving PDAC development. A previous study revealed that 93% of rare *PIK3CA* mutations co-occur with a *KRAS* mutation, suggesting that activated *KRAS* is not sufficient to effectively activate PI3K (26). However, another study revealed that KRAS suppression did not alter AKT activation levels in a majority of *KRAS*-mutated PDAC cell lines (27).

Several drugs have been developed to target this pathway, including PI3K, PI3K/mTOR, mTOR, and AKT inhibitors (Table I). Wortmannin was the first PI3K inhibitor developed which was identified to be extremely effective in inhibiting PI3K, but it was revealed to be nonspecific, unstable and toxic in animals (28). In orthotopic PDAC xenografts, wortmannin promoted the antitumor activity of gemcitabine (tumor weight reduction relative to control was 1.4-fold by gemcitabine and 5-fold by gemcitabine plus wortmannin; P<0.001) supporting its potential as an adjuvant to conventional chemotherapy treatments of PDAC (29). LY294002, however, is a very specific synthetic inhibitor of PI3K and is chemically more stable but has been identified to be less potent than wortmannin in preclinical studies. In PDAC models, LY294002 inhibited *in vitro* cell proliferation, induced apoptosis and reduced *in vivo* tumor growth. Furthermore, LY294002 enhanced the effects of cisplatin both *in vitro* and *in vivo* (tumor volume decreased to 77, 70 or 44% of the volume in the controls by cisplatin, LY294002 or combination; P<0.05) (30). Subsequently, Wang *et al* reported the opposite effect of LY294002 by demonstrating that LY294002 (but not wortmannin) enhanced AKT phosphorylation in the gemcitabine-resistant PDAC cell lines (31). This study suggested that the PI3K inhibitors can be counterproductive with gemcitabine-resistant PDAC cells.

MK-2206, a novel AKT inhibitor, inhibited *in vitro* proliferation and induced apoptosis of PDAC cells. When combined with gemcitabine, it both enhanced the cytotoxic efficacy of gemcitabine and inhibited AKT phosphorylation (32). In *KRAS*-mutant patient-derived xenograft (PDX) models, a combination of MK-2206 with dinaciclib, an inhibitor of cyclin-dependent kinases, demonstrated a reduction in PDAC growth (by >90%; P<0.001) and the number of metastatic lesions (by >88%; P<0.001) (33). Another AKT inhibitor perifosine (KRX-0401) was revealed to inhibit *in vitro* cell growth and interacted synergistically with gemcitabine in PDAC cells including primary cultures (34).

There are three major mTOR inhibitors currently approved by the FDA: Sirolimus, everolimus and temsirolimus. Sirolimus (rapamycin) induced autophagy and apoptosis in PDAC cell lines (35) but it also led to resistance mediated by AKT phosphorylation (36). The second-generation mTOR inhibitors, such as KU63794 and PP242, also led to treatment resistance due to an increase in ERK activation (36). Rapamycin exhibited a dose-dependent radiosensitization effect (37) and synergistic antiproliferative and antiangiogenic effects in combination with EGFR inhibitor gefitinib on PDAC cells (38). *In vivo* studies in mice demonstrated that rapamycin was specifically effective on *KRAS*-mutant PDAC tumors that have loss of *PTEN* (median survival in controls, gemcitabine, rapamycin and gemcitabine plus rapamycin was 10, 14, 56 and 32 days, respectively), while *KRAS*-mutant tumors with mutant *p53* (KPC) did not respond (39). Metformin causes diverse effects on PDAC tumorigenesis in both mTOR-dependent and -independent manner. In the syngeneic mouse model using C57BL/6 mice and Pan02 cells, metformin and rapamycin both exhibited significant tumor growth reduction (tumor burden compared with control 0.90 g was 0.62 g with metformin and 0.25 g with rapamycin) (40). Everolimus, an analog of rapamycin, sensitized PDAC cells to the effects of gemcitabine in an *in vitro* study (41). *In vitro* studies demonstrated that gemcitabine-resistant PDAC cells were more sensitive to everolimus, in contrast to various EGFR, AKT and PI3K inhibitors (42). In another *in vitro* study in PDAC cell lines, everolimus had an antiproliferative effect, while its combination with sorafenib exhibited an antagonistic effect (43). Wei *et al* demonstrated that neither everolimus nor AZD8055, a 2nd-generation mTOR inhibitor, exerted any cell viability inhibitory effect on PDAC cell lines (44). In PDAC PDX models, everolimus displayed higher antitumor efficacy when combined with either sorafenib (the tumor/control ratio was 0.6, 0.5 and 0.2 for treatment with everolimus, sorafenib and the combination) (45) or trametinib (46). Temsirolimus (CCI-779), a water-soluble, more stable and specific mTOR inhibitor, showed a significant antiproliferative effect on PDAC cells (47) and exhibited synergistic antitumor response in combination with gemcitabine in PDAC xenograft models

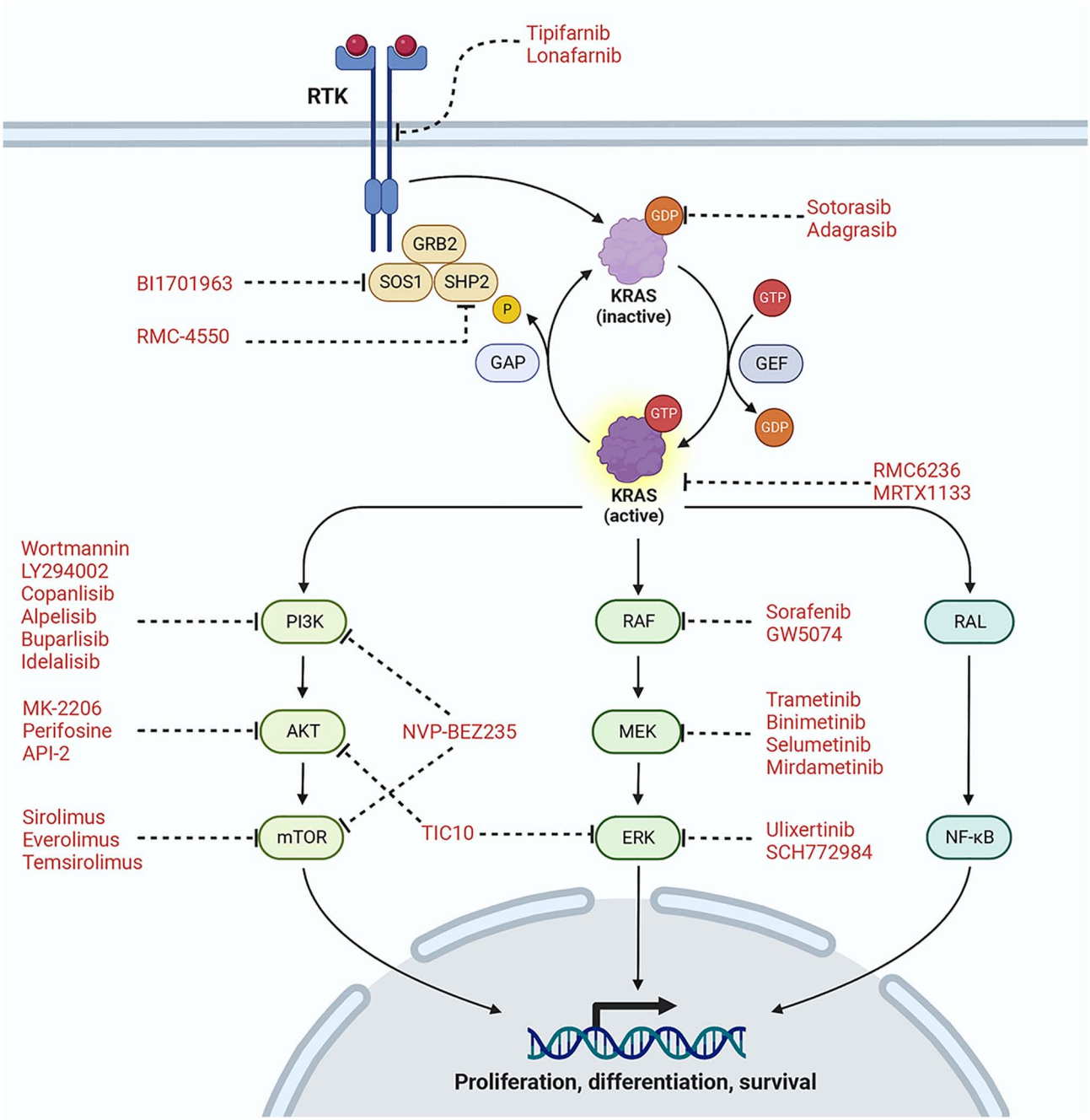

Figure 1. Preclinical and clinical targeted agents for blocking KRAS signaling in PDAC. When RTK is activated, the GRB2-SOS complex interacts with KRAS protein through SOS facilitating its activation by catalyzing the exchange of GDP for GTP. Once KRAS is mutated, it remains in GTD-bound active state causing persistent activation of the downstream PI3K/AKT/mTOR and RAS/RAF/MEK/ERK (MAPK) signaling cascade, resulting in increased cell proliferation, differentiation, and survival. To target each node of KRAS signaling pathways, various inhibitors of KRAS signaling have been developed as shown in red. Due to the challenges in direct KRAS targeting, the initial focus was blocking different components of PI3K/AKT/mTOR and MAPK pathways. Recent advancements have led to the development of direct KRAS inhibitors, including isoform-specific and pan-KRAS inhibitors.

(compared with control, decrease in tumor volume was 68%, P=0.0009 with a high dose of CCI-779; and 41%, P=0.0002 with CCI-779 plus gemcitabine (48).

Dual inhibition of PI3K-mTOR holds arguably the most potential. NVP-BEZ235, the primary proponent of this class of drugs, demonstrated significant delay in tumor growth (56, 36 and 46%) in three different orthotopic PDX models (P<0.05) (49). In the peritoneal dissemination animal survival model, NVP-BEZ235 enhanced gemcitabine response (median survival in days was 16, 21, 28 and 30 in control, NVP-BEZ235, gemcitabine, and combination; P<0.05) (50). In PDAC subcutaneous xenografts, NVP-BEZ235 exhibited synergistic tumor growth inhibition in combination with pan-histone deacetylase inhibitor panobinostat (51) or inRas37 antibody (52). *In vitro* study using PANC-1 and MIA PaCa-2 PDAC cells revealed that NVP-BEZ235 markedly induced the ERK/MEK pathway. The MEK inhibitors U126 and PD0325901 prevented ERK overactivation induced by NVP-BEZ235 and the combination of MEK inhibitors with NVP-BEZ235 produced a further inhibition of PDAC cell proliferation (53).

Table I. Preclinical results with KRAS downstream effector inhibitors in PDAC.

Drug	Target	Effect as a single agent	Effect in combination therapy	(Refs.)
Wortmannin	PI3K	Effective in inhibiting PI3K, but relatively non-specific, unstable, and toxic	Promoted antitumor activity of Gem in orthotopic xenografts	(28,29)
LY294002	PI3K	Specific, stable but less potent, antiproliferative, opposite effect in Gem-resistant PDAC cell lines	Synergistic antitumor effects with cisplatin (CDX)	(30,31)
MK-2206	AKT	Antiproliferative and pro-apoptotic effects in PDAC cell lines	Synergistic antitumor response with CDK inhibitor dinaciclib (PDX); MEK inhibitor trametinib and chemotherapy (*in vitro*, CDX)	(32,33,73)
Perifosine (KRX-0401)	AKT	Inhibited growth of PDAC cells including primary cultures	Enhanced activity in combination with Gem (*in vitro)*	(34)
API-2	AKT	Radiosensitization of PDAC cells	Synergistic antitumor effects with MEK inhibitor mirdametinib (CDX)	(70)
Sirolimus (Rapamycin)	mTOR	Antiproliferative and radiosensitization effects (*in vitro*); efficacy in KC PTEN mice but not in KPC mice (GEMM)	Synergistic tumor growth inhibition with metformin (CDX); and EGFR inhibitor gefitinib (*in vitro*)	(35-40)
Everolimus	mTOR	Inhibited growth of Gem-resistant PDAC cell lines	Higher efficacy in combination with Gem (*in vitro*); trametinib (PDX); sorafenib (PDX)	(41-46)
Temsirolimus (CCI-779)	mTOR	Antiproliferative effects on PDAC cell lines	Synergistic antitumor efficacy in combination with Gem (CDX)	(47,48)
NVP-BEZ235	PI3K-mTOR	Antiproliferative activity (*in vitro*); tumor growth inhibition (orthotopic PDX)	Synergistic antitumor activity with Gem (*in vitro*, CDX); panobinostat (CDX); inRas37 antibody (CDX); PD0325901 (*in vitro*)	(49-53)
Tipifarnib (R115777)	Ras	Antiproliferative and proapoptotic activity (*in vitro*)	N/A	(54)
Lonafarnib (SCH66336)	Ras	Antiproliferative activity (*in vitro*)	Synergistic antitumor activity in combination with taxanes (*in vitro*)	(55)
Sorafenib (BAY43-9006)	Raf	Antiproliferative and proapoptotic activity (*in vitro*)	Synergistic antitumor activity with vitamin K (*in vitro*); melatonin (*in vitro*, CDX); Gem (*in vitro*, CDX)	(56-59)
GW5074	c-Raf	Antiproliferative activity (*in vitro*); tumor growth inhibition (*in vivo*)	Synergistic antitumor activity with DFMO (*in vitro*, CDX)	(60)
Trametinib	MEK	Antiproliferative and pro-apoptotic effects (*in vitro*)	Increased the antitumor effects of lapatinib (orthotopic PDX); NPT plus Gem with or without MK-2206 (CDX); HDAC inhibitors MPT0E028 or SAHA (*in vitro*, CDX)	(61-63,73)
Mirdametinib (PD-0325901)	MEK	Tumor growth inhibition (CDX); radiosensitization of PDAC cells	Synergistic antitumor effects with clusterin inhibition (*in vitro*); AKT inhibitor API-2 (CDX)	(64)
SCH772984	ERK	Antiproliferative effect on PDAC cells	Synergistic antitumor effects with cucurbitacin (*in vitro*, CDX); autophagy-inhibiting agents	(27,65-68)

Table I. Continued.

Drug	Target	Effect as a single agent	Effect in combination therapy	(Refs.)
			(*in vitro*, PDX, organoid); CDK4/6 inhibitor palbociclib (*in vitro*, organoid)	
Ulixertinib (BVD-523)	ERK	Inhibition of PDAC cell viability (*in vitro*)	Synergistic effect with Gem, pan-HER1/2/3 inhibitor afatinib or pan-PI3K inhibitor GDC-0941 (CDX)	(69)
TIC10 (ONC201)	AKT/ERK	Antiproliferative, proapoptotic activity (*in vitro*); tumor growth inhibition (*in vivo*)	Synergistic antitumor effects with Gem (CDX)	(72)

KRAS, Kirsten rat sarcoma viral oncogene homolog; PDAC, pancreatic ductal adenocarcinoma; Gem, gemcitabine; CDX, cell-derived xenografts; PDX, patient-derived xenografts; GEMM, genetically engineered mouse model; DFMO, difluoromethylornithine; NPT, nab-paclitaxel.

RAS/RAF/MEK/ERK pathway inhibition. Another hallmark downstream pathway through which KRAS signaling occurs is the Ras-Raf-MEK-ERK (MAPK) pathway which is the key effector in the initiation, progression, and maintenance of KRAS-dependent tumors. Thus, several drugs targeting different components of this pathway have been explored extensively for therapeutic intervention in *KRAS*-mutant PDAC (Table I).

Ras proteins undergo farnesylation by the enzyme farnesyl-protein transferase for their biological or transforming functions. Therefore, farnesyltransferase inhibitors (FTIs), tipifarnib (R115777) and lonafarnib (SCH66336) have been assessed in PDAC preclinical models. Tipifarnib suppressed the growth of human PDAC cell lines through modulation of the STAT3 and ERK pathways (54). The FTI, lonafarnib, synergized with taxanes to inhibit cell proliferation in *KRAS*-mutant and *KRAS*-wild-type PDAC cells (55).

Raf kinases (ARaf, BRaf, and CRaf/Raf1) comprise the most significant effectors of KRAS-driven PDAC. In PDAC cell lines, Raf inhibitor sorafenib (BAY 43-9006) that also targets VEGFR2 and PDGFR-b, demonstrated strong antiproliferative and proapoptotic effects, either alone (56) or in combination with vitamin K (57). Sorafenib also synergized with melatonin to suppress the growth of PDAC both *in vitro* and *in vivo* (58). Sorafenib alone or in combination with gemcitabine and EMAP inhibited PDAC cell proliferation. This study also showed enhancement in animal survival by combination treatment of sorafenib, gemcitabine and EMAP (median survival in controls, gemcitabine, sorafenib, EMAP and gemcitabine + sorafenib + EMAP was 22, 29, 23, 25 and 36 days; P=0.004) (59). In PDAC orthotopic xenografts, a selective c-RAF inhibitor, GW5074, exhibited a significant decrease in tumor weight, either alone or in combination with polyamine biosynthesis inhibitor difluoromethylornithine, but it had no effect on improving animal survival (60).

Downstream to RAF, inhibitors for MEK and ERK have been assessed extensively. In *KRAS*-mutant and wild-type orthotopically implanted patient-derived tumors, the MEK-inhibitor, trametinib, showed a significant reduction in tumor growth and liver metastasis that was increased by the EGFR/HER-2 inhibitor lapatinib (61). In *KRAS*-mutant PDAC cell-derived xenograft (CDX) models, trametinib increased the effects of nab-paclitaxel-based chemotherapy by inhibiting tumor growth and enhancing animal survival (compared with controls, increase in animal survival was 95 and 145% by nab-paclitaxel + gemcitabine and nab-paclitaxel + gemcitabine + trametinib) (62). Chao *et al* observed that MEK inhibitors (PD98059 or trametinib) increased the antiproliferative and proapoptotic effects of HDAC inhibitors (MPT0E028 or SAHA) in *KRAS*-mutant and wild-type PDAC cell *in vitro* studies. Furthermore, in AsPC-1 subcutaneous xenografts, MPT0E028 and PD98059 combination revealed enhanced antitumor activity (63). Recently, MEK inhibitor mirdametinib (PD0325901) exerted antitumor efficacy in MIA PaCa-2 PDAC xenografts at day 4 and became refractory within a week after treatment due to the involvement of clusterin expression (64). SCH772984 was the first ERK inhibitor studied in PDAC, and it displayed the ability to suppress PDAC xenograft growth (27). SCH772984 in combination with cucurbitacin, a natural tetracyclic triterpene, synergized to induce growth inhibition and apoptosis of PDAC cells *in vitro* and reducing tumor growth in PDAC subcutaneous xenografts (percent inhibition in tumor volume by cucurbitacin, SCH772984 and combination treatment was 63.8, 54.7 and 85, respectively) (65). Furthermore, SCH772984, in combination with autophagy inhibition, synergistically reduced PDAC growth *in vitro* and *in vivo* models (66,67). Recently, SCH772984 has been demonstrated to combine synergistically with CDK4/6 inhibitor palbociclib in *KRAS*-mutant PDAC cell lines and organoid models (68). Another ERK inhibitor, ulixertinib, has been shown to effectively inhibit the growth of multiple PDAC cell lines and potentiate the cytotoxic effect of gemcitabine *in vitro* and *in vivo*. This study also showed that concurrent inhibition of HER (with afatinib) or PI3K proteins (with GDC-0941) synergizes with ulixertinib in suppressing PDAC cell growth in subcutaneous xenograft models (69). Thus,

MEK and ERK inhibitors showed promise in preclinical studies in mitigating the progression of PDAC.

Due to the extensive crosstalk and compensatory mechanism between PI3K/AKT and MAPK pathways, simultaneous targeting of these two pathways has been explored in PDAC. In PDAC subcutaneous xenografts, a combination treatment with MEK inhibitor mirdametinib and AKT inhibitor (API-2) induced activation of apoptotic pathways, radiosensitized pancreatic cancer cells and maximized tumor growth inhibition (70). Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has been shown to induce apoptosis of cancer cells by engaging its cell surface death receptors (71). A TRAIL-inducing compound TIC10 (ONC201) that causes dual inhibition of AKT and ERK-inducing TRAIL pathway-mediated cell death, inhibited PDAC cell survival and proliferation *in vitro*, and demonstrated potent antitumor activity in PDAC xenografts as well as synergized gemcitabine activity (72). Subsequently, Awasthi *et al* demonstrated that the combined inhibition of PI3K and MAPK signaling with MK-2206 and trametinib, respectively, displayed an enhanced nab-paclitaxel plus gemcitabine (NPT+Gem) chemotherapy antitumor response in PDAC *in vitro* and *in vivo* models. In this study, compared with controls, enhancement in animal survival by NPT + Gem and NPT + Gem + MK-2206 + trametinib treatment was 67 and 129%, respectively (73). These findings indicate the potential of dual inhibition of downstream targets of *KRAS*-mutation-driven signaling in combination with current treatments for clinical PDAC therapy.

6. Clinical studies targeting KRAS signaling in pancreatic cancer

Dense desmoplastic stroma which comprises >90% of the tumor mass is one of the hallmarks of PDAC. This stroma plays a major role in PDAC progression, metastasis, and therapy resistance (74). The TME in PDAC preclinical models and patients with PDAC have several differences including composition, oncogenic mutations and expression of oncogenic growth factors and cytokines (75,76). Therefore, inhibiting the major signaling pathways downstream of KRAS sometimes has different effects on PDAC tumors in preclinical settings compared with their effect on the progression and survival of patients with PDAC (76).

PI3K/AKT/mTOR pathway inhibition. Considering the activation of the PI3K/AKT/mTOR signaling pathway in *KRAS*-mutant PDAC progression and survival, several clinical trials evaluated the antitumor efficacy of inhibitors of this pathway in clinical studies (Table II).

Copanlisib (BAY 80-6946), a pan-class I PI3K inhibitor, in combination with gemcitabine or cisplatin plus gemcitabine in solid tumors including PDAC demonstrated favorable clinical efficacy (out of 4 evaluated patients with PDAC, 1 partial response, 2 stable disease) with an acceptable toxicity profile in a phase I study (77). In a phase I trial of advanced patients with PDAC who have not received any cytotoxic chemotherapy except as adjuvant therapy, alpelisib (BYL719), an α-specific PI3K inhibitor, in combination with nab-paclitaxel plus gemcitabine, was safely administered and the median progression-free survival (PFS) and OS were 5.36 and 8.74 months (78). Another pan-class I PI3K inhibitor buparlisib (BKM120), in a phase I study which included patients with metastatic PDAC with *RAS*- or *BRAF*-mutation, in combination with trametinib showed minimum clinical activity (best overall response was stable disease) (79). In a phase I study of patients with refractory solid tumors including PDAC, buparlisib combination with mFOLFOX6 caused an increase in toxicity and one patient with stage IV PDAC exhibited a 47% decrease in measurable disease from baseline (80). A phase Ib study of PI3K inhibitor idelalisib alone or in combination with nab-paclitaxel or mFOLFOX6 in patients with PDAC was terminated prematurely due to severe toxicity issues observed in a phase III clinical study of idelalisib for hematological malignancies (81).

Perifosine (KRX-0401) and MK-2206 are the most studied AKT inhibitors for PDAC clinical therapy. Although perifosine exhibited significant activity in PDAC preclinical studies, two phase II clinical trials using this drug in patients with advanced PDAC who were previously untreated (82) or had one prior systemic therapy (83) were halted prematurely due to a lack of efficacy and high toxicity. A phase II study compared MK-2206 plus MEK inhibitor selumetinib with mFOLFOX in gemcitabine-refractory patients with metastatic PDAC (84). The median OS was shorter in the MK-2206 plus selumetinib versus mFOLFOX (3.9 vs. 6.7 months; P=0.15) and the median PFS was also inferior (1.9 vs. 2.0 months; P=0.02) (84). Subsequently, a phase I study of MK-2206 plus CDK inhibitor dinaciclib in patients with previously treated metastatic PDAC demonstrated disappointing results (median survival 2.2 months; survival rates at 6 and 12 months 11 and 5%, respectively) (85).

The mTOR inhibitor everolimus in a phase II study in gemcitabine refractory metastatic PDAC patients demonstrated minimal clinical activity and median OS and PFS were 4.5 and 1.8 months, respectively (86). A phase II study of temsirolimus in gemcitabine refractory PDAC patients was closed to accrual due to significant adverse effects and median OS and PFS were 44 and 19 days, respectively (87). Considering the disappointing results of mTOR inhibitors as monotherapy, several clinical studies evaluated mTOR inhibitors in combination with other cytotoxic or targeted agents.

A phase II study of everolimus plus erlotinib in patients with advanced PDAC who received at least one prior gemcitabine-based regimen showed minimal clinical activity with an OS of 87 days and a PFS of 49 days (87). The oral regimen with the combination of everolimus and capecitabine in a phase II trial of patients with advanced PDAC who were untreated (first-line) or had prior chemotherapy (second-line) demonstrated a moderate activity (median OS of 8.9 months and a PFS of 3.6 months) with an acceptable toxicity profile (88). Temsirolimus plus gemcitabine in previously untreated patients with advanced PDAC failed to show any meaningful clinical response in a phase II study (OS, 4.95 months; PFS, 2.69 months) (89). Furthermore, the combination of temsirolimus and docetaxel in patients with refractory solid tumors including PDAC did not meet its primary objective due to dose-limiting toxicities in a phase I study (90).

The combination of PI3K/AKT/mTOR inhibitors with other targeted therapies has also been evaluated in several clinical studies. A phase I study evaluated everolimus and the

Table II. Clinical results with KRAS downstream effector inhibitors.

Drug	Target	Effect as single agent	Effect in combination therapy	(Refs.)
Copanlisib (BAY 80-6946)	PI3K	N/A	Phase I, with Gem or Gem plus cisplatin: manageable safety profile, favorable PK and clinical efficacy	(77)
Alpelisib (BYL719)	PI3Ka	N/A	Phase I, with NPT plus Gem: manageable safety profile; PFS, 5.36 mo; OS, 8.74 mo	(78)
Buparlisib (BKM120)	PI3K	N/A	Phase I, minimum activity with trametinib or binimetinib, significant toxicity with mFOLFOX6	(79,80,117)
Idelalisib	PI3K	Phase I, no conclusion: study terminated early	Phase I, with NPT or mFOLFOX6: early termination of the study due to severe toxicity concerns	(81)
Perifosine (KRX-0401)	AKT	Phase II, halted due to high toxicity and low efficacy	N/A	(82,83)
MK-2206	AKT	N/A	Phase II, with selumetinib vs. mFOLFOX, OS 3.9 vs. 6.7 mo, PFS 1.9 vs. 2.0 mo; phase I, with dinaciclib, OS 2.2 mo	(84,85)
Sirolimus	mTOR	N/A	With vismodegib: minimum activity, only SD in a subgroup of patients	(92)
Everolimus	mTOR	Phase II, Gem refractory patients, OS 4.5 mo, PFS 1.8 mo	Phase II, with erlotinib (OS 87 days, PFS 49 days); phase II, with capecitabine (OS 8.9 mo, PFS 3.6 mo); phase I, with ribociclib (OS 3.7 mo, PFS 1.8 mo); cetuximab plus capecitabine (phase I/II, OS 5 mo)	(86-88,91,95)
Temsirolimus (CCI-779)	mTOR	Phase II, Gem refractory patients, OS 44 days, PFS 19 days	Ineffective with Gem (phase II, OS 4.95 mo, PFS 2.69 mo); docetaxel (phase I); erlotinib (phase I)	(87,89,90, 94)
Tipifarnib (R115777)	Ras	Phase II, untreated patients: OS 19.7 weeks, PFS 4.9 weeks; Phase II, OS 2.6 mo, PFS 1.4 mo	No benefit: Monotherapy vs. with Gem (phase III, OS 193 vs. 182 days, PFS 112 vs. 109 days); CXRT vs. CXRT + tipifarnib (phase II, OS 11.5 vs. 8.9 mo)	(96-99)
Sorafenib (BAY 43-9006)	c/b-Raf	Phase II, well tolerated but no clinical activity (OS 4.3 mo, PFS 2.3 mo)	Phase III, with or without Gem, OS 9.2 vs. 8 mo, PFS 5.7 vs. 3.8 mo; phase II, cisplatin plus Gem with and without sorafenib, OS 7.5 vs. 8.3 mo, PFS 4.3 vs. 4.5 mo; phase I, with concurrent RT and Gem, OS 12.6 mo, PFS 10.6 mo	(100-104)
Trametinib	MEK	N/A	Phase II, with Gem vs. Gem alone, OS 8.4 vs. 6.7 mo (p=0.453), PFS 16 vs. 15 weeks (p=0.349); phase I, with lapatinib, no activity; phase II, with erlotinib, OS 7.3 mo, PFS 1.9 mo; phase II, with RT plus pembrolizumab vs. RT plus Gem, OS 14.9 vs. 12.8 mo, PFS 8.2 vs. 5.4 mo; phase II, with GSK2256098, OS 3.6 mo, PFS 1.6 mo	(105-109,116)
Selumetinib	MEK	Phase II, Gem refractory patients, compared with capecitabine (OS 5.4 vs. 5.0 mo); phase II, $KRAS^{G12R}$-mutant patients with ≥2 chemotherapy, OS 9 mo, PFS 3 mo	Phase II, with MK-2206 vs. mFOLFOX, OS 3.9 vs. 6.7 mo, PFS 1.9 vs. 2.0 m	(84,110,111)

Table II. Continued.

Drug	Target	Effect as single agent	Effect in combination therapy	(Refs.)
Binimetinib	MEK	N/A	Phase I, terminated due to toxicity issues with avelumab; study ongoing with hydroxychloroquine	(112,113)
Ulixertinib (BVD-523)	ERK	N/A	Phase I, with NPT plus Gem, OS 12.23 mo, PFS 5.46 mo, study terminated due to toxicity; study ongoing with palbociclib	(114,115)

KRAS, Kirsten rat sarcoma viral oncogene homolog; N/A, not applicable; Gem, gemcitabine; NPT, nab-paclitaxel; RT, radiotherapy; PK, pharmacokinetics; PFS, progression-free survival; OS, overall survival; mo, months; CXRT, concurrent radiation with gemcitabine and paclitaxel; PR, partial response; SD, stable disease.

CDK4/6 inhibitor ribociclib as a third-line therapy in patients with PDAC after disease progression on both 5-fluorouracil and gemcitabine. This study demonstrated favorable tolerability with a decrease in CDK4/6-regulated genes but median OS and PFS were only 3.7 and 1.8 months, respectively (91). A phase I clinical study is currently ongoing to evaluate the combination of the PI3K/mTOR inhibitor, gedatolisib, and the CDK4/6 inhibitor, palbociclib, in patients with solid tumors (including PDAC) that is metastatic or unresectable and resistant to standard therapy (NCT03065062). Based on suggestive crosstalk between PI3K/AKT/mTOR and hedgehog (Hh) signaling, the mTOR inhibitor sirolimus was assessed in combination with the Hh inhibitor vismodegib in patients with advanced PDAC. However, in contrast with preclinical results, the combination of vismodegib and sirolimus only showed stable disease in a subgroup of patients (92). EGFR overexpression has been reported in up to 95% of patients with PDAC. The small molecule EGFR inhibitor erlotinib in combination with gemcitabine is an approved therapy for PDAC (93). Because PI3K/mTOR signaling is a well-established resistance mechanism to erlotinib, the combination of mTOR inhibitors and erlotinib was assessed in PDAC clinical trials. However, the combination of erlotinib with everolimus (87) or temsirolimus (94) did not demonstrate any meaningful clinical benefit. In addition, a phase I/II study of the combinations of temsirolimus with the EGFR monoclonal antibody cetuximab and capecitabine in patients with advanced PDAC demonstrated no clinical benefit with a median OS of 5 months (95).

RAS/RAF/MEK/ERK pathway inhibition. Mutations of *KRAS* or *BRAF* play a major role in the activation of the MAPK pathway. The role of inhibitors of the MAPK pathway has been investigated extensively in clinical studies for improving PDAC therapy (Table II).

Among FTIs, tipifarnib (R115777) has been assessed in PDAC clinical trials. In a phase II study, treatment with tipifarnib in previously untreated patients with metastatic PDAC resulted in partial inhibition of farnesyltransferase activity but it did not exhibit antitumor activity (median OS, 19.7 weeks; PFS, 4.9 weeks; and the estimated 6-month survival rate was 25%) (96). Another phase II study of tipifarnib in previously untreated patients with metastatic PDAC did not show any clinical efficacy and the median OS, PFS and 6-month survival rate were 2.6 months, 1.4 months and 19%, respectively (97). In a phase III trial, the tipifarnib combination with gemcitabine did not exhibit any clinical benefit compared with gemcitabine alone (median OS, 193 vs. 182 days; median PFS, 112 vs. 109 days; 6-month and 1-year survival rates were 53 and 27 vs. 49 and 24%, respectively) (98). In a phase II trial, weekly paclitaxel, gemcitabine and radiation (CXRT) was compared with CXRT plus maintenance tipifarnib. This study demonstrated that the addition of tipifarnib was associated with a broad range of toxicities and there was no clinical benefit (median OS, 11.5 vs. 8.9 months for the CXRT and CXRT + tipifarnib, respectively) (99).

Raf, a signaling protein downstream of Ras, is an important target for cancer therapy. Sorafenib (BAY 43-9006) was extensively assessed in combination with chemotherapy in several clinical studies of metastatic PDAC. A phase I study of sorafenib plus gemcitabine in advanced unresectable or metastatic PDAC revealed favorable tolerability with 56.6% of patients (n=13) achieving disease stabilization (100). Subsequently, a phase II trial of sorafenib alone versus sorafenib plus gemcitabine in patients with metastatic PDAC who received no prior chemotherapy or completed prior adjuvant chemotherapy >6 months before study entry, demonstrated no clinical benefit (median OS, 4.3 vs. 6.5 months; median PFS, 2.3 vs. 2.9 months) (101). A phase III trial of gemcitabine plus sorafenib and gemcitabine plus placebo in previously untreated patients with advanced PDAC exhibited a median OS of 9.2 and 8 months, and a median PFS of 5.7 and 3.8 months (102). Another phase II study of cisplatin plus gemcitabine with and without sorafenib in patients with metastatic PDAC also showed no clinical activity of sorafenib addition (median OS, 7.5 vs. 8.3 months; median PFS, 4.3 vs. 4.5 months) (103). A phase I study demonstrated modest clinical activity of sorafenib with concurrent radiation therapy and gemcitabine in advanced PDAC patients. The median OS and PFS for 25 evaluable patients were 12.6 and 10.6 months, respectively (104).

A phase II trial of gemcitabine plus trametinib or placebo in previously untreated patients with PDAC revealed no significant clinical benefits with an observed median OS of 8.4 vs. 6.7 months (P=0.453), a median PFS of 16 vs. 15 weeks (P=0.349), and an overall response rate 22 vs. 18%. These outcomes were independent of *KRAS* mutations determined

by circulating free DNA (105). A phase I study of patients with *KRAS*-mutant solid tumors including PDAC showed manageable toxicity of trametinib plus lapatinib but there was no preliminary sign of antitumor activity in patients with PDAC (106). Based on preclinical evidence of synergistic antitumor response of MEK and EGFR inhibition in PDAC, a phase II study evaluated trametinib plus erlotinib in chemotherapy-refractory PDAC patients. This trial demonstrated a modest clinical efficacy of this combination in PDAC (median OS, 7.3 months; median PFS, 1.9 months) (107). Recently, a phase II study in locally recurrent, *KRAS*-mutant and PD-1 positive PDAC patients after surgery followed by chemotherapy (mFOLFIRINOX or 5-FU) demonstrated promising clinical efficacy after receiving the combination of radiotherapy plus pembrolizumab and trametinib (median OS and PFS of 14.9 and 8.2 months, respectively) compared with radiotherapy plus gemcitabine (median OS and PFS of 12.8 and 5.4 months, respectively) (108). Recently, a phase II trial of patients with advanced PDAC whose disease progressed after first-line chemotherapy with the combination treatment of trametinib and an oral FAK inhibitor, GSK2256098, demonstrated dismal clinical activity with a median PFS of 1.6 months and an OS of 3.6 months (109). A phase I trial is currently ongoing to evaluate trametinib and hydroxychloroquine in patients with PDAC (NCT03825289).

The MEK inhibitor, selumetinib, was compared with capecitabine in a phase II study in patients with PDAC who failed first-line gemcitabine therapy. In this trial, selumetinib was well tolerated with a manageable safety profile but showed no significant difference in OS compared with capecitabine (5.4 vs. 5.0 months) (110). A recent phase II study assessing selumetinib in $KRAS^{G12R}$-mutant PDAC patients who received two or more lines of systemic chemotherapy (~87.5% patients) demonstrated dismal clinical activity, revealing a 3-month median PFS and a 9-month median OS (111). A phase I study evaluating the MEK inhibitor, binimetinib plus the anti-PD-L1 antibody avelumab in patients with metastatic PDAC after 1-2 prior lines of therapy was terminated due to toxicity issues (112). A phase I study of binimetinib plus hydroxychloroquine in patients with metastatic PDAC who had at least one line of systemic therapy and harbor a *KRAS* mutation is currently ongoing (113).

The ERK inhibitor ulixertinib in combination with gemcitabine plus nab-paclitaxel in untreated patients with metastatic PDAC showed potentially similar efficacy (the median PFS and OS were 5.46 and 12.23 months, respectively) as chemotherapy and the study was terminated due to treatment-related adverse events (114). A phase I study of ulixertinib and palbociclib is currently ongoing in patients with advanced solid tumors including patients with PDAC who have received at least one line of therapy in the metastatic setting (115).

Attributed to the synergistic antitumor response in preclinical studies, simultaneous targeting of MAPK and PI3K pathways was assessed in several clinical studies in patients with *KRAS*-mutant PDAC. A phase I clinical trial of patients with solid tumors including PDAC with the PI3K/mTOR inhibitor GSK2126458 and trametinib demonstrated poor tolerability and limited antitumor efficacy (only 2 out of 7 patients with PDAC had stable disease) (116). As aforementioned in the previous section, a phase II study on gemcitabine-refractory PDAC patients demonstrated no clinical benefit of MK-2206 plus selumetinib therapy compared with mFOLFOX (84). Furthermore, a phase I study evaluating the combination of BKM120 plus binimetinib did not report any clinical efficacy in patients with advanced PDAC (117).

7. Direct KRAS targeting: New developments

Until recently, direct KRAS targeting by small molecule inhibitors was not possible and indirect therapeutic strategies including targeting RAS signaling and metabolic pathways demonstrated disappointing results in clinical trials in treating patients with *KRAS*-mutant cancers. Therefore, recently, numerous drugs with the potential of direct KRAS targeting have been evaluated in *KRAS*-mutant cancers including PDAC (Table III).

Advancements in drug discovery and significant efforts by several groups led to a breakthrough by successfully developing compounds to target the $KRAS^{G12C}$-mutant allele (118), paving the way for the development of two inhibitors of this class, sotorasib (AMG510) (119) and adagrasib (MRTX849) (120). These two inhibitors are more relevant in NSCLC where the mutation frequency of $KRAS^{G12C}$ is 13.8% but less relevant in colorectal and pancreatic cancer where $KRAS^{G12C}$ mutation is 3.2% and <1%, respectively.

Sotorasib demonstrated significant clinical activity in a phase II trial in $KRAS^{G12C}$-mutated lung cancer patients and received FDA approval for this indication (121). In preclinical models (*in vitro* tumor cell lines and *in vivo* tumor cell-derived xenografts) of $KRAS^{G12C}$-mutant cancers including PDAC, sotorasib exhibited antitumor activity and significantly synergized with the proapoptotic agent DT2216 (122). In a phase I/II trial in patients with $KRAS^{G12C}$-mutant PDAC who had received at least one previous systemic therapy, sotorasib demonstrated an acceptable safety profile and promising antitumor activity (median PFS, 4 months; median OS, 6.9 months) (123). Adagrasib also exhibited clinical efficacy in a proof-of-concept study in $KRAS^{G12C}$-mutated lung and colon adenocarcinoma patients (120). In a phase I/II (KRYSTAL-1) trial in previously treated patients with metastatic PDAC harboring $KRAS^{G12C}$ mutation, adagrasib demonstrated clinical efficacy in all 10 evaluable patients. Median PFS was 6.6 months, and treatment was ongoing in 50% of patients with PDAC at the time of data reporting (124). These findings are highly encouraging given that the median PFS was <3 months and a response rate of 10% with approved second-line therapy for advanced PDAC (125).

Given that the $KRAS^{G12D}$ mutation is the most predominant variant (~41%) in PDAC, $KRAS^{G12D}$ inhibitors have a high therapeutic potential for PDAC clinical therapy. MRTX1133 (126), a small-molecule inhibitor for $KRAS^{G12D}$, demonstrated marked tumor regression (≥30%) in 8 out of 11 $KRAS^{G12D}$-mutated PDAC CDX and PDX (127). Recently, Kemp *et al* demonstrated the specificity, potency and efficacy of MRTX1133 in immunocompetent $KRAS^{G12D}$ mutant PDAC models. In this study, MRTX1133 exhibited marked tumor regression in all *in vivo* models tested (128). Several other groups are also developing $KRAS^{G12D}$ mutant-specific inhibitors (129).

KRAS missense mutation variants in human malignancies are in the following order, G12D (35%), G12V (29%), G12C

Table III. Efficacy of direct KRAS inhibitors in PDAC.

Drug	Target	Effects in preclinical studies	Effects in clinical studies	(Refs.)
Sotorasib (AMG510)	$KRAS^{G12C}$	Antitumor efficacy, synergistic effects with a proapoptotic agent DT2216 (*in vitro*, CDX)	Phase I/II, $KRAS^{G12C}$-mutant PDAC patients who had at least 1 systemic therapy: OS 6.9 mo, PFS 4 mo	(122,123)
Adagrasib (MRTX849)	$KRAS^{G12C}$	N/A	Phase I/II, $KRAS^{G12C}$-mutant previously treated PDAC patients: PFS 6.6 mo, treatment ongoing in 50% of patients	(124)
MRTX1133	$KRAS^{G12D}$	$KRAS^{G12D}$-mutant PDAC: tumor regression (≥30%) in CDX and PDX models; antitumor activity in immunocompetent models	N/A	(127,128)
RMC-6236	Cyclophilin A	Significant antitumor efficacy in multiple CDX and PDX models	Ongoing phase I study in solid tumor patients with specific *KRAS* mutations	(131, NCT05379985)
RMC-4550	SHP-2	Antitumor efficacy in combination with ERK inhibitor LY3214996 *in vitro* and multiple CDX and PDX models	Ongoing phase I study in solid tumor patients with *KRAS* mutations	(132, NCT04916236)
BI 1701963	SOS1	N/A	Phase I, *KRAS*-mutant patients: with trametinib, study ongoing; with adagrasib, study completed, results awaited	(133, NCT04975256)
mRNA-5671/ V941	$KRAS^{G12D}$ $KRAS^{G12V}$ $KRAS^{G13D}$ $KRAS^{G12C}$	N/A	Phase I, *KRAS*-mutant solid tumor patients including PDAC: monotherapy and in combination with pembrolizumab, study completed, results awaited	(NCT03948763)

KRAS, Kirsten rat sarcoma viral oncogene homolog; PDAC, pancreatic ductal adenocarcinoma; CDX, cell-derived xenografts; PDX, patient-derived xenografts; N/A, not applicable; PFS, progression-free survival; OS, overall survival; mo, months.

(21%), G12A (7%) and G12S (3%), indicating that recent advancements in targeting $KRAS^{G12C}$ mutated tumors represent a relatively small portion of *KRAS*-mutant tumors. Therefore, targeting all *KRAS* mutation alleles with a single drug may be an ideal therapeutic strategy for most solid tumors where relative frequencies of different *KRAS* mutation subtypes are very diverse (130). A small molecule pan-RAS inhibitor RMC-6236, has been reported, that binds to an intracellular chaperone protein, cyclophilin A, resulting in an inhibitory binary complex that binds active GTP-bound RAS to form an inactive tricomplex, suppressing RAS signaling by disrupting the interaction with signaling partners such as RAF kinases. In a preclinical study, RMC-6236, an inhibitor of multiple *RAS* mutations and wild-type RAS, demonstrated profound and durable tumor regression in multiple CDX and PDX models of *KRAS*-mutant PDAC (131). A phase I study of RMC-6236 in patients with solid tumors harboring specific *KRAS* mutations (including *KRAS G12A, G12D, G12V, G12R, G12S; excluding G12C)* is currently ongoing (NCT05379985). Recently, the combined inhibition of SHP2, upstream of KRAS, using the inhibitor RMC-4550 and of ERK, downstream of KRAS, using LY3214996, demonstrated significant tumor growth inhibition in multiple PDAC mouse models including CDX, PDX and orthotopic mouse models (132). Based on these results, a phase I trial is currently enrolling patients with *KRAS*-mutant solid tumors including PDAC to evaluate this drug combination (NCT04916236). In addition, a novel pan-KRAS inhibitor, BI 1701963, an inhibitor of the son of sevenless 1 protein that is a guanine nucleotide exchange factor involved in the activation of KRAS, is currently being investigated alone and in combination with trametinib in a phase I trial of patients with advanced solid tumors including PDAC harboring all major *KRAS* mutation subtypes (133). Recently, a phase I trial of BI 1701963 in combination with adagrasib has been completed in patients with $KRAS^{G12}$-mutant advanced solid tumors and results are awaited (NCT04975256). Furthermore, results are

also awaited for a recently completed phase I study evaluating an mRNA-based vaccine (mRNA-5671/V941), targeting G12D, G12V, G13D or G12C *KRAS* mutation subtypes, as monotherapy and in combination with pembrolizumab in patients with *KRAS*-mutant advanced or metastatic NSCLC, colorectal cancer or PDAC (NCT03948763).

8. Conclusions and future perspectives

Targeting KRAS signaling has great potential for therapeutic intervention in several solid tumors including PDAC. While downstream effector signal inhibitors have shown promise in preclinical studies, these drugs displayed limited clinical benefits, largely due to differences in the TME in these settings leading to enhanced toxicity and induction of adaptive resistance mechanisms in patients. Recent advances in preclinical models such as PDX and tumor organoids which better recapitulate patient tumor biology have helped design promising combination therapies targeting downstream KRAS effectors to overcome toxicity and resistance issues and improve PDAC clinical therapy. A combination of BRAF inhibitor vemurafenib plus sorafenib (phase II), binimetinib plus hydroxychloroquine, and binimetinib plus palbociclib are currently under clinical investigation (NCT05068752, NCT04132505, NCT04870034) in *KRAS*-mutant PDAC patients. Due to limited success with downstream KRAS inhibition strategy, attention has recently shifted to direct mutant-specific KRAS inhibitors and pan-KRAS inhibitors. Novel drugs such as sotorasib and adagrasib have been effective at inhibiting *KRAS* at G12C, but these are largely ineffective in PDAC as the major mutant isoforms are found at other locations. Recent advancements in $KRAS^{G12D}$-mutant-specific inhibitors (such as MRTX1133) are very encouraging and their success could be a game changer in PDAC clinical therapy. A phase I study is evaluating mesenchymal stromal cells-derived exosomes with $Kras^{G12D}$siRNA (iExosomes) in metastatic PDAC patients with $KRAS^{G12D}$ mutation (NCT03608631). In addition, the upstream pan-KRAS inhibitors RMC-6236, RMC-4550 and BI 1701963 are currently under clinical investigation in *KRAS*-mutant PDAC (NCT05379985, NCT04916236, NCT04975256). These pan-KRAS inhibitors that have the ability to target a broad range of oncogenic *KRAS* variants, including all major G12 and G13 subtypes, could make a marked clinical impact in the majority of patients with PDAC irrespective of their *KRAS* mutation subtypes. Additionally, KRAS targeting therapeutic vaccines including peptides, dendritic cells and mRNA vaccines have recently emerged as a promising therapy and these are currently under clinical investigation (NCT05013216, NCT03592888, NCT03948763) for potential improvement of PDAC clinical therapy.

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Authors' contributions

JZ and NA contributed to the conception and design of the article and interpretation of the relevant literature. JZ and NA prepared the original draft of the manuscript. JZ, LD, MSH and NA performed formal analysis. JZ, UvH and NA wrote, reviewed, and edited the manuscript. NA acquired funding. Data authentication is not applicable. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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