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## A small molecule with big impact: MRTX1133 targets the KRAS<sup>G12D</sup> mutation in pancreatic cancer

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### Abstract

KRAS mutations drive oncogenic alterations in numerous cancers, particularly in human pancreatic ductal adenocarcinoma (PDAC). About 93% of PDAC have KRAS mutations, with G12D (~42% of cases) and G12V (~32% of cases) being the most common. The recent approval of sotorasib (AMG510), a small-molecule, covalent, and selective KRAS<sup>G12C</sup> inhibitor, for treating patients with non-small cell lung cancer represents a breakthrough in KRAS targeted therapy. However, there is a need to develop other much-needed KRAS mutant inhibitors for PDAC therapy. Notably, Mirati Therapeutics recently developed MRTX1133, a small-molecule, non-covalent, and selective KRAS<sup>G12D</sup> inhibitor through extensive structure-based drug design. MRTX1133 has demonstrated potent *in vitro* and *in vivo* antitumor efficacy against KRAS<sup>G12D</sup>-mutant cancer cells, especially in PDAC, leading to its recent initiation of a phase I/II clinical trial. Here, we provide a summary of the recent advancements related to the use of MRTX1133 for treating KRAS<sup>G12D</sup>-mutant PDAC, focusing on its efficacy and underlying mechanistic actions. Additionally, we discuss potential challenges and future directions for MRTX1133 therapy for PDAC, including overcoming intrinsic and acquired drug resistance, developing effective combination therapies, and improving MRTX1133's oral bioavailability and target spectrum. The promising results obtained from preclinical studies suggest that MRTX1133 could revolutionize the treatment of PDAC, bring about a paradigm shift in its management.

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Potential Conflict of interest

AM is listed as an inventor on a patent that has been licensed by Johns Hopkins University to Thrive Earlier Detection and serves as a consultant for Freenome and Tezcat Biosciences. The other authors declare no conflicts of interest.

## Keywords

Pancreatic Cancer; KRAS Targeted Therapy; MRTX1133

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## 1. Introduction

Pancreatic cancer, mainly pancreatic ductal adenocarcinoma (PDAC), currently ranks as the third-leading cause of cancer-related death in the United States, and it is projected to be the second -leading cause of such death by 2030 (1). PDAC carries a bleak prognosis, with a median survival duration of 8 months following diagnosis and a 5-year survival rate of less than 12% across all stages (2). These discouraging statistics primarily stem from the late detection of the disease, its resistance to conventional chemoradiotherapy, and the lack of effective targeted therapy. Despite the success of immune therapy for numerous other malignancies, immune therapy for PDAC has yielded disappointing results. This outcome can be largely attributed to PDAC's desmoplastic and immunosuppressive tumor microenvironment (TME), which consists of a dense extracellular matrix, cancer-associated fibroblasts (CAFs), and various immune cells, particularly cancer-associated macrophages (CAMs) and myeloid-derived suppressor cells (MDSCs). These components hinder the infiltration, activation, and elimination of tumor cells by effector T cells (3). Hence, there is an urgent need for novel therapeutic strategies that can significantly improve outcomes for patients with PDAC.

## 2. Oncogenic KRAS mutations drive PDAC development and progression

PDAC harbors a multitude of genetic alterations, including oncogenic KRAS mutations and inactivation of the CDKN2A, TP53, and SMAD4 tumor suppressors. These alterations pose significant challenges for molecular comprehension of PDAC and the development of targeted therapy for PDAC(4), as there are currently no effective inhibitors available for direct targeting of these driver alterations in clinical applications. Alternatively, researchers have been identifying and targeting downstream effectors of these driving oncogenic signaling pathways. For example, they have explored the use of MEK, EGFR, and PI3K inhibitors, but these approaches have shown limited clinical benefit in patients with PDAC (4–6). One promising PDAC targeted therapy utilizing small molecular inhibitors has been exemplified by olaparib. Olaparib, a small molecular inhibitor of poly (ADP-ribose) polymerase (PARP), has been approved as the first non-chemotherapy, targeted maintenance treatment for adult patients with BRCA-mutated metastatic pancreatic cancer based on the favorable outcomes in a phase III clinical trial ([NCT02184195](#)) (7).

KRAS mutations are considered a hallmark genetic alteration and represent one of the earliest events in pancreatic tumorigenesis (Fig. 1A). KRAS mutations are detected in 74% of human pancreatic intraepithelial neoplasia (PanIN) lesions and in 63% foci of acinar-ductal metaplasia associated with these PanINs (8), and approximately 93% of cases of human PDAC exhibit KRAS mutations (9). The mutations that promote cancer often occur at specific hotspots within the KRAS protein, such as codons 12, 13, and 61. The most common mutations are G12D (41.8%),G12V (31.6%), and G12R (16.1%), and G12C

mutation is less prevalent, accounting for less than 2% of the mutations in PDAC (10,11) (Fig. 1B), which is in contrast with the much higher frequency of G12C observed in non-small cell lung cancer (NSCLC) (~13%) (12,13). Studies using genetically engineered mouse models revealed that activation of Kras<sup>G12D</sup> in pancreatic epithelial lineage of cells leads to the development of a spectrum of PanIN lesions, which progress spontaneously, albeit at a low frequency, to invasive and metastatic PDAC (14); and that Kras<sup>G12D</sup> is required for PDAC progression and maintenance (15–17). In comparison to G12V, G12C, and other KRAS mutations, the G12D mutation displays the highest oncogenic potential in *in vitro* and *in vivo* models of pancreatic, lung, and colorectal cancers (18,19). This heightened potential likely stems from its favorable structure conformation that impacts KRAS downstream signaling (19,20). Patients with PDAC carrying the KRAS<sup>G12D</sup> mutation exhibit a shorter survival duration compared to those with KRAS wild-type, G12V, or G12R mutation (21).

Normally, the KRAS protein functions as a molecular switch, transitioning between binding with guanosine-5'-triphosphate (GTP) in an active state and with guanosine-5'-diphosphate (GDP) in an inactive state. Mutations in KRAS disrupt the intrinsic GTPase activity of RAS, resulting in elevated levels of GTP-bound KRAS. This sustained RAS signaling activates pro-tumorigenic signaling through downstream effector pathways, including the Ras/Raf/MAPK (MEK)/ERK pathway and the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway (22) (Fig. 2). These pathways play critical roles in cellular processes, including promoting cell proliferation and survival, enhancing angiogenesis, facilitating metastasis, reprogramming cellular metabolism to support the energy demands of rapidly dividing cancer cells (17,23–25), and inducing resistance to therapy (26). Furthermore, the activation of KRAS signaling in cancer cells can extend to and reprogram the surrounding microenvironment, thereby influencing cancer initiation, progression, and therapeutic response (27–30). These findings strongly indicate that directly targeting mutant KRAS could serve as an effective therapeutic strategy.

### 3. Identification of MRTX1133, a first-in-class small-molecule, non-covalent, and selective KRAS<sup>G12D</sup> inhibitor.

Given the high prevalence and functional significance of mutant KRAS in cancer, there has been considerable interest in developing therapeutic strategies to directly target mutant KRAS. However, three decades of effort have failed to identify clinically useful inhibitors of the KRAS proteins (31), because initial analyses of the KRAS proteins indicated that they lack a clear pocket for high-affinity binding of small molecules (32).

In 2013, Ostrem et al. reported a novel strategy to develop covalent inhibitors to target the reactive cysteins-12 (Cys-12) in the common KRAS<sup>G12C</sup> mutant (33). Through structure-based design, they identified ARS-1620, a highly potent small molecular inhibitor that covalently binds to Cys-12 in the switch-II pocket of KRAS<sup>G12C</sup>. This binding does not affect wild-type KRAS but subverts the native nucleotide preference to favor GDP over GTP and impairs binding to RAF, thus disrupting KRAS signaling (Fig. 2) (33). ARS-1620 demonstrated significant *in vitro* and *in vivo* activity against KRAS<sup>G12C</sup> tumors (34). This

discovery marked a milestone in targeting mutant KRAS, which was previously considered *undruggable*. Furthermore, using a structure-based design, Amgen developed a highly potent and selective KRAS<sup>G12C</sup> inhibitor called AMG510, also known as sotorasib (Lumakras<sup>™</sup>), for a phase I/II clinical trial (NCT03600883) initiated in August 2018 (35). Based on the significant anti-tumor activity and manageable side effects in heavily pretreated patients with non-small cell lung cancer (NSCLC) with KRAS<sup>G12C</sup> mutations in the clinical trial, the U.S. Food and Drug Administration (FDA) granted accelerated approval to sotorasib in May 2021 for the treatment of adult patients with locally advanced or metastatic NSCLC harboring the KRAS<sup>G12C</sup> mutations, who have received at least one prior systemic therapy. This approval represents a breakthrough in KRAS targeted therapy, which is also demonstrated by recently updated clinical report showing that sotorasib achieved an overall response rate of 41%, a progression-free survival of 6.3 months, an overall survival (OS) of 12.5 months, and a 2-year OS rate of 33% (36). In parallel, Mirati developed Adagrasib (MRTX849) (37), an oral, small-molecule, covalent, and GDP-bound KRAS<sup>G12C</sup> potent inhibitor with favorable pharmacological properties (38), for a phase I/II clinical trial in patients with advanced solid tumors with a KRAS<sup>G12C</sup> mutation (NCT03785249). The trial was initiated in January 2019, and a recently updated report showed that among 57 patients with measurable solid tumors (excluding NSCLC and CRC patients), the objective response rate was 35.1% (20/57). This includes 7 out of 21 (33.3%) responses in pancreatic cancer and 5 out of 12 (41.7%) responses in biliary tract cancers (39). Notably, these developments have shown that the switch-II binding pocket, present in all KRAS proteins, is a feasible binding surface for the development of such selective KRAS<sup>G12D</sup> inhibitors. The successful development of a KRAS<sup>G12C</sup>-specific inhibitors provides valuable insights into and inspiration for the development of small molecular inhibitors targeting KRAS<sup>G12D</sup>, which are desperately needed to treat PDAC.

Using a structure-based design and optimization, Mirati successfully developed a potent and selective non-covalent KRAS<sup>G12D</sup> inhibitor called MRTX1133 (Fig. 3) in 2021 (40,41). Recent exciting results from preclinical studies indicate that the application of MRTX1133 has the potential to bring about a paradigm shift in the treatment of PDAC.

#### 4. Recent advances using MRTX1133 to target mutant KRAS<sup>G12D</sup> in pancreatic cancer

##### MRTX1133 is a highly potent and selective non-covalent inhibitor of KRAS<sup>G12D</sup> in vitro.

In a biochemical homogeneous time-resolved fluorescence assay, MRTX1133 exhibited binding to the GDP-bound, inactive form of KRAS<sup>G12D</sup> with a half maximal inhibitory concentration (IC<sub>50</sub>) below 2nM, which was the lower limit of the detection of the assay (40). The selectivity of MRTX1133 for binding to the GDP-bound inactive KRAS<sup>G12D</sup> was approximately 700-fold higher compared to that of MRTX1133 to the GDP-bound wild type KRAS (KRAS<sup>WT</sup>). Additionally, MRTX1133 also inhibited the binding of a RAF-RAS binding domain peptide to the active form of KRAS<sup>G12D</sup>.

Functionally, MRTX1133 effectively reduced cell viability and inhibited key signaling molecules in a dose-dependent manner in the KRAS pathway, such as phosphorylated

extracellular signal-regulated kinase 1 and 2 (pERK1/2) and phosphorylated S6 (pS6), in KRAS<sup>G12D</sup>-mutant pancreatic and other cancer cell lines, with a median IC<sub>50</sub> value of approximately 5 nM (bioRxiv 2023.03.18.533261). But MRTX1133 treatment was found to upregulate the expression and phosphorylation of EGFR and HER2 in KRAS<sup>G12D</sup>-mutant mouse and human pancreatic cancer cell lines (42). MRTX1133 exhibited selectivity over 1,000-fold larger for KRAS<sup>G12D</sup> compared to that for KRAS<sup>WT</sup> cell lines. However, certain established or patient-derived PDAC cell lines show intrinsic or acquired resistance to MRTX1133 treatment (42) (bioRxiv 2023.03.18.533261).

Notably, MRTX1133 demonstrated significantly higher efficacy in inhibiting cell proliferation in the three-dimensional (3D) organoid cell cultures compared to such two-dimensional (2D) cultures, even at concentrations that had no effect in the 2D setting (40) (bioRxiv 2023.03.18.533261). This finding suggests that anchorage-dependent cell growth may overcome KRAS dependency, which is consistent with the observed potent anti-tumor efficacy of MRTX1133 shown in *in vivo* studies (40)(bioRxiv 2023.03.18.533261).

### **MRTX1133 demonstrates KRAS<sup>G12D</sup>-mutant tumor regression in xenograft mouse models of pancreatic cancer.**

To evaluate the efficacy of MRTX1133 treatment on tumorigenesis, various preclinical models of pancreatic cancer have been used. In a xenograft model derived from the KRAS<sup>G12D</sup>-mutant human HPAC cell line, MRTX1133 treatment via intraperitoneal injection demonstrated dose-dependently anti-tumor efficacy. The higher dose (30 mg/kg administered twice daily) resulted in a near-complete response, with an 85% regression rate without weight loss or overt signs of toxicity observed for up to 28 days. The antitumor effect was consistent with a dose-dependent reduction in a fraction of pERK- and pS6-positive cells, as well as a decrease in KRAS activity and increase in apoptosis in the tumor tissues (40).

In a panel of xenograft models derived from human KRAS<sup>G12D</sup>-mutant cell-lines and patient-derived cells, MRTX1133 had excellent anti-tumor activity, especially in models of pancreatic cancer. Eight out of 11 (73%) models tested had at least showed 30% tumor regression, whereas only 2 out of 8 (25%) colorectal cancer models exhibited a similar response (40).

Interestingly, MRTX1133 displayed potent antitumor efficacy in xenograft tumors derived from patient-derived MRTX1133-resistant cells in 2D culture (EC<sub>50</sub>>1 μM) (bioRxiv 2023.03.18.533261). This finding highlights the sustained effectiveness of MRTX1133 in a 3D context and suggests that anchorage-dependent cell growth may contribute to the resistance observed in 2D cultures. Furthermore, MRTX1133 showed no significant anti-tumor efficacy in all four non-KRAS<sup>G12D</sup>-mutant tumor models, further confirming the high-targeting specificity of MRTX1133 (40).

### **MRTX1133 is effective in immunocompetent models of pancreatic cancer.**

Immune surveillance plays a crucial role in the development and progression of pancreatic cancer, and the presence of an immunosuppressive TME contributes to the limited efficacy of immune therapies in treating PDAC. To assess the efficacy of MRTX1133

in immunocompetent models of KRAS<sup>G12D</sup>-mutated pancreatic cancer, both implantable and autochthonous PDAC models with an intact immune system were used. MRTX1133 demonstrated significant tumor regression in all tested models, leading to complete or near-complete remissions within 14 days. Evidence of tumor regression was observed as early as 2 days after MRTX1133 treatment initiation (43).

In addition to inducing tumor cell apoptosis and halting proliferation, MRTX1133 treatment induced notable changes in the TME. These changes included reprogramming CAFs and the extracellular matrix, an increase in CD8-positive effector T cells, and a decrease in neutrophils or myeloid cells (43) (44) (bioRxiv 2023.03.18.533261). The contribution of the immune-mediated antitumor effect elicited by MRTX1133 treatment was supported by the finding that MRTX1133 treatment resulted in potent tumor regression in syngeneic mouse models but not in immune-deficient mouse models (bioRxiv 2023.03.18.533261). The presence of T cells was essential for MRTX1133's full antitumor effect, as T-cell depletion expedited tumor regrowth after therapy. Consistent with previous report (40), MRTX1133 treatment had no effect on tumor growth in immunocompetent mice carrying KRAS<sup>G12C</sup> mutant pancreatic or lung tumors, or KRAS<sup>WT</sup> colon tumors (43).

Furthermore, in autochthonous genetic mouse models with a KRAS<sup>G12D</sup> mutation, MRTX1133 treatment resulted in the regression of both established pancreatic intraepithelial neoplasia (PanIN) and advanced PDAC. The regression of advanced PDAC relied on the presence of CD8-positive T cells and combining MRTX1133 with immune checkpoint blockade therapy (iCBT) targeting CTLA-4 and PD-1 (i.e., anti-CTLA-4 and anti-PD-1 antibodies) synergistically eradicated PDAC and extended overall survival compared to treatment with MRTX1133 alone (44). The ability of MRTX1133 to regress PanIN suggests that such inhibitors might be considered for use in a high-risk interception setting.

Mechanistically, the inhibition of KRAS<sup>G12D</sup> by MRTX1133 in PDAC cell lines or tumor models resulted in the suppression of KRAS signaling and reduction of MAPK-dependent target gene expression, cell cycle regulator gene expression, and pro-survival gene expression, while pro-apoptotic and Fas genes were significantly upregulated in cancer cells (40) (44). MRTX1133 treatment also activated interferon- $\gamma$  signaling and enhanced major histocompatibility complex (MHC) antigen presentation in the tumors, thus facilitating CD8-positive T cell-mediated cell death (44)(bioRxiv 2023.03.18.533261).

The promising results of MRTX1133 in preclinical studies have prompted exploration of its translation to human disease. MRTX1133 obtained clearance as an investigational new drug by the FDA in October 2021 for a phase I/II clinical trial (NCT05737706) that was initiated on March 20, 2023, targeting patients with advanced solid tumors (PDAC, NSCLC, colorectal cancer, and others) with KRAS<sup>G12D</sup> mutations. This ongoing trial is expected to be completed on August 30, 2026.



## 5. Challenges and future directions

Despite the potent and selective antitumor effects observed *in vitro* and *in vivo* against KRAS<sup>G12D</sup>-mutated pancreatic cancer cells, targeted therapy using MRTX1133 poses several challenges and necessitates further studies.

**First**, one of the major challenges in MRTX1133 treatment lies in overcoming intrinsic and acquired resistance to MRTX1133. Studies have clearly shown that not all PDAC cells with KRAS<sup>G12D</sup> mutations are sensitive to MRTX1133 treatment (40) (bioRxiv 2023.03.18.533261), and cancer cells often develop resistance to the therapy over time (42,45–47). Understanding the underlying mechanisms of resistance and identifying strategies to overcome it will be crucial to achieving long-term success in MRTX1133 therapy.

In addition to a high frequency of oncogenic KRAS mutations, other alterations including inactivation of the CDKN2A, TP53, SMAD4, and PTEN tumor suppressors (4,48), activation of PIK3CA mutation (present in 3%–5% of PDAC) (49–52), contribute to the aggressiveness and therapeutic resistance of PDAC. In KRAS<sup>G12C</sup>-targeted therapy for cancer patients, several mechanisms of primary and acquired resistance have been identified, including co-mutations in KEAP1, SMARCA4, CDKN2A, and STK11 (36,53,54), activation of YAP1 signaling (39,41,47,48), reactivation or feedback activation of the RAS/MAPK/PI3K pathway, acquired additional genetic alterations, such as in KRAS G12D/R/V/W, G13D, Q61H, R68S, H95D/Q/R, and Y96C, a high level of amplification of the KRAS<sup>G12C</sup> allele; activation mutations in NRAS, BRAF, MAP2K1, EGFR, and RET, and loss-of-function mutations in NF1 and PTEN (45). These findings may shed new light on our understanding of the resistance to MRTX1133 targeted therapy in PDAC and designing novel strategies to overcome such resistance.

Bioinformatics analyses have shown that cancer cells with lower PTEN and CDKN2A RNA expression are relatively insensitive to MRTX1133 treatment (40). SgRNAs-CRISPR/Cas9 mediated screening indicated that loss of tumor suppressors, including *PTEN*, *KEAP1*, *NF1*, and *RBI*, may confer partial resistance to MRTX1133 treatment in PDAC cells (40). In preclinical models, inhibition of mutant KRAS leads to feedback compensation to promote the expression of ERBB receptors or activation of EGFR/RAS<sup>WT</sup> signaling was identified as a potential mechanism for acquired resistance of cancer cells to MRTX1133 treatment (46) (42). Therefore, elucidating the mechanisms for therapeutic resistance in MRTX1133 therapy would be facilitated by establishing MRTX1133-resistant cancer cell lines/clones or model systems, particularly the use of patient-derived samples, and exploring CRISPR/Cas9-based screening, single-cell RNA sequencing, and functional reverse-phase protein array (RPPA) approaches for comprehensive analysis, (40) (42).

**Second**, developing effective and mechanism-based combination therapy regimens, especially with immunotherapy, is essential to maximizing the antitumor efficacy of MRTX1133 and holds great promise for tumor elimination. For instance, MRTX1133 induces long-term regression of established tumors in immune-competent mice, which is not observed in immune-deficient mice. This indicates that, apart from tumor cell death induced

by KRAS<sup>G12D</sup> inhibition, an adaptive immune response is involved in the potent antitumor effect induced by MRTX1133 in immune-competent preclinical mouse model (43). These findings are supported by the observations that T-cell depletion accelerated tumor regrowth after monotherapy with MRTX1133 (43) and combination therapy with iCBT synergized with MRTX1133 to eradicate PDAC and prolong overall survival in a preclinical mouse model, whereas iCBT alone has been proven ineffective in treating PDAC (44). These results provide the rationale for future development of combination therapy of MRTX1133 with iCBT and other immunotherapies in clinical trials.

Since MRTX1133 treatment has been found to induce KRAS-dependent signaling pathway reactivation and/or feedback compensation (40,46) (42), combining MRTX1133 with inhibitors that target key KRAS-signaling components, both upstream and downstream effectors, such as the pan-ERBB inhibitor afatinib or EGFR inhibitor cetuximab, the PI3K $\alpha$  inhibitor BYL719, CDK4/6 inhibitors, or YAP1 inhibitors, could be explored in future clinical trials (40). These combination therapies have the potential to enhance the efficacy of MRTX1133 treatment or overcome resistance based on the results of preclinical studies and the findings from KRAS<sup>G12C</sup> targeted therapy (40,46,55–57) (42). Given that KRAS<sup>G12D</sup> mutant PDAC cells can potentially develop the ability to bypass oncogenic KRAS dependency, utilizing mechanisms such as YAP activation, USP21 amplification, and HDAC5-mediated remodeling of TME (58–60), it is plausible to consider that a combination targeting of these altered KRAS bypass signaling pathways may enhance or sensitize the efficacy of MRTX1133 treatment in PDAC. Additionally, combining MRTX1133 with conventional chemotherapy and radiotherapy is also expected to enhance its therapeutic efficacy in treating patients with PDAC (61).

**Third**, there is additional need to develop novel, more potent, and broader KRAS mutant inhibitors for improved KRAS-targeted therapy. MRTX1133 has its limitations, including low oral bioavailability and high selectivity for G12D, as reported previously(40). To improve the low oral bioavailability of MRTX1133, researchers have designed and synthesized a series of prodrug compounds. These prodrugs aim to mask the amino group (refer to Fig. 3, highlighted in green) that is believed to be responsible for the molecule's poor absorption in the gastrointestinal tract. Among these prodrugs, prodrug 9 has been discovered to have significantly better pharmacokinetic properties, oral bioavailability, and effective antitumor activity in a xenograft mouse model of human pancreatic cancer with KRAS<sup>G12D</sup> mutation after oral administration(62). The high selectivity of MRTX1133 may restrict its broad use in treating patients with PDAC, such as for such patients whose tumors carry different variants of KRAS mutation due to heterogeneity or mutations acquired during treatment.

A recent study using isogenic “Ras-less” cell lines that express a single Ras allele demonstrated that, in addition to being highly sensitive to KRAS<sup>G12D</sup>, MRTX1133 also exhibited significant activity against several other KRAS mutations (G12C, G12V, G13D) and wild-type KRAS but had no effect on HRAS (WT and G12D) and NRAS (WT and G12D) in the cells. Further studies indicated that while the 12D mutant residue is important, it is not essential to MRTX1133 activity. It is also important to note that although MRTX1133 exhibited activity against other KRAS mutations and wild-type KRAS, the



concentrations of MRTX1133 needed to inhibit non-G12D variants were significantly higher and may not be pharmacologically achievable/relevant. The selectivity of MRTX1133 is associated with its binding to H95 on KRAS, a residue that is not conserved in HRAS and NRAS (63). This finding could be exploited for the development of the next generation of MRTX1133-like pan-KRAS inhibitors.

Moreover, the discovery of MRTX1133 has sparked a new wave of development of various KRAS<sup>G12D</sup> inhibitors with alternative binding models, different allele specificities, selectivity for KRAS mutant variants, and distinct mechanistic actions (Table 1). Continuously characterizing, optimizing, and examining these inhibitors in cellular and animal models and translating them into clinical applications may usher in a new era of improved KRAS-targeted therapy for PDAC. It is reasonable to speculate, for instance, that combining MRTX1133 with ASP3080, a degrader of the KRAS<sup>G12D</sup> protein, could lead to a synergistic or additive antitumor effect, helping overcome the therapeutic resistance caused by overactivation or feedback compensation of KRAS-dependent signaling.

RMC-6236 was identified as a first-in-class, orally bioavailable, selective KRAS (ON) inhibitor, exhibited high-antitumor efficacy in RAS-addictive cancer cells and RAS-driven cancer models, particularly KRASG12X (G12X includes G12A/C/D/R/S/V)(64). In pancreatic cancer, RMC-6236 treatment suppressed the phosphorylation of ERK kinases, leading to growth inhibition and apoptosis in several human cancer cell lines *in vitro*, and oral administration of RMC-6236 in cell line-derived and patient-derived xenograft models of KRAS-mutant PDAC resulted in profound and durable tumor regressions at doses that were well-tolerated (65). The promising preclinical data have led to a phase I clinical trial of RMC-6236, initiated on May 31, 2022, in adults with KRAS<sup>G12X</sup> (exclude G12C) mutant advanced solid tumors (NCT05379985). BI-2865 is another example of non-covalent, selective pan-KRAS inhibitor, which blocks nucleotide exchange to impair the activation of KRAS<sup>WT</sup> and a broad range of KRAS mutants, including G12A/C/D/F/V/S, G13C/D, V14I, L19F, Q22K, D33E, Q61H, K117N and A146V/T, while sparing NRAS and HRAS. BI-2865 treatment exhibited significant inhibition of KRAS signaling and cell proliferation that was restricted to KRAS-mutant cancer cells and led to KRAS-mutant tumor growth suppression in xenograft mouse models (66). If successfully translated into clinical applications, RMC-6236 and BI-2865 may be used to treat patients with PDAC carrying a variety of KRAS mutations with a lower frequency or delayed development of therapeutic resistance.

In summary, significant progress is being made rapidly in both preclinical and clinical studies regarding the use of MRTX1133, a non-covalent, selective, and potent KRAS<sup>G12D</sup> inhibitor, for treating PDAC. However, it is crucial to elucidate and overcome treatment resistance, develop novel and more potent KRAS inhibitors, and explore combination approaches to optimize the efficacy of KRAS-targeted therapy and improve patient outcomes in PDAC. Furthermore, ongoing research and clinical trials are likely to reveal additional challenges, necessitating tremendous effort in the future.

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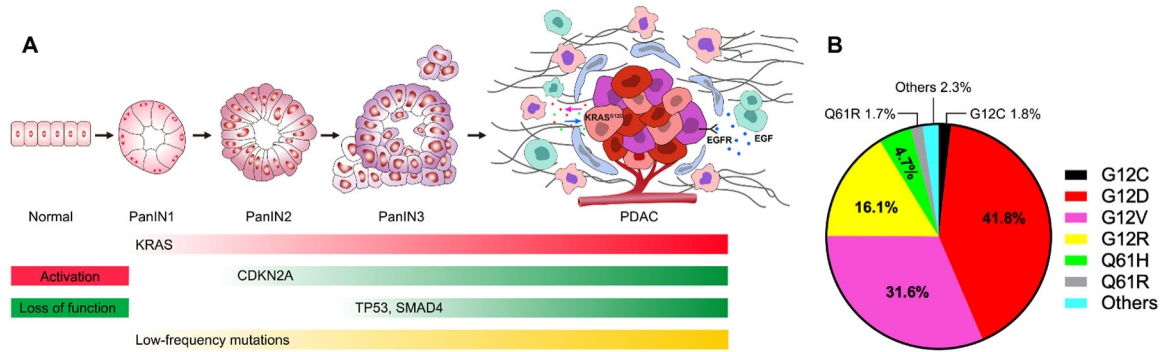
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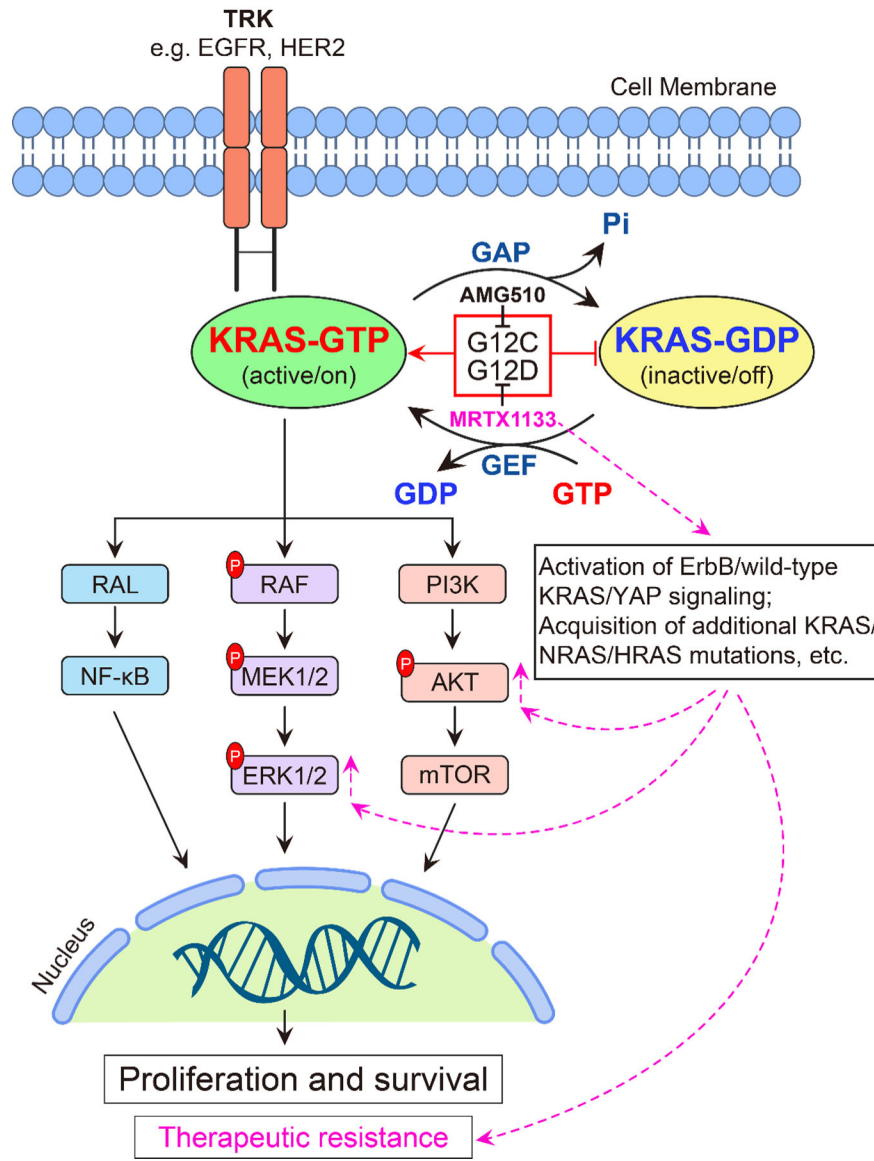
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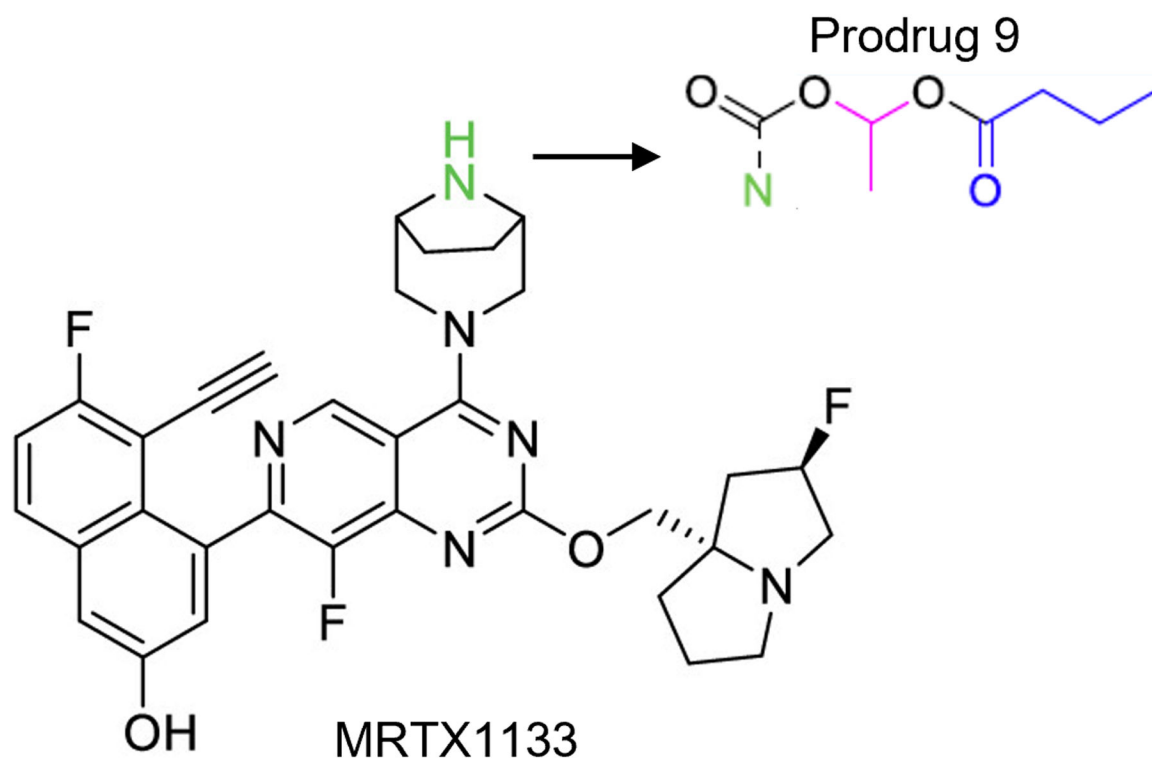


**Figure 1. Genetic mutations in PDAC initiation and development.**

**(A)** Multi-stage initiation and progression model of PDAC. As cells acquire mutations in genes like KRAS, CDKN2A, TP53 and SMAD4, along with other less commonly mutated genes, the lesion progresses from low grade pancreatic intraepithelial neoplasia (PanIN1) through PanIN2 and high grade PanIN3, and ultimately to become invasive adenocarcinoma. In PDAC, intrinsic signals such as the KRAS<sup>G12D</sup> mutation and extrinsic signals like EGFR activation contribute to an immune-suppressive and desmoplastic microenvironment through reciprocal interactions between tumor cells and diverse stroma cells. Examples of these stroma cells include cancer-associated fibroblasts (blue), cancer-associated macrophages (pink), myeloid-derived suppressor cells (green), among others. **(B)** Prevalence of KRAS mutant variants in human pancreatic cancer.



**Figure 2. KRAS protein functioning switch and key downstream signaling pathways.** In normal conditions, when cells receive extracellular signals through transmembrane receptors, RAS-selective guanine nucleotide exchange factors (GEFs) and GTP-activating proteins (GAPs) regulate GDP- and GTP-bound KRAS cycling. In cancer cells, KRAS mutations disrupt its intrinsic GTPase activity, causing it to be locked in the GTP-bound and active form. This leads to the constitutive activation of downstream signaling pathways, such as the RAF/MEK/ERK pathway and the PI3K/AKT/mTOR pathway, promoting cancer cell proliferation and survival. The small molecular inhibitor AMG 510 specifically targets the KRAS<sup>G12C</sup> mutation, while MRTX1133 specifically targets the KRAS<sup>G12D</sup> mutation. However, cancer cells can potentially develop resistance to KRAS inhibitors through multiple mechanisms that may include compensation or feedback activation of the ErbB/wild-type KRAS and YAP signaling, acquisition of additional KRAS/NRAS/HRAS mutations, and etc.



**Figure 3. Chemical structure of MRTX1133 and the orally effective prodrug 9.**

The amine moiety highlighted in green is believed to be a major contributor to its poor absorption in the gastrointestinal tract. Prodrug 9, which incorporates specific modifications to the amino group, demonstrated enhanced oral bioavailability compared to MRTX1133. Refer to ref. 62 for details.

**Table 1.**New KRAS<sup>G12D</sup> inhibitors under development and investigation

Drug	Sponsor	Properties	Status	Reference
ASP3082	Astellas	Binds to and targets KRAS <sup>G12D</sup> for proteasome-mediated degradation	Phase I (NCT05382559)	Nagashima et al. (67)
HRS-4642	Hengrui Medicine	KRAS <sup>G12D</sup> inhibitor	Phase I (NCT05533463)	
RMC-9805 (RM-036)	Revolution Medicines	Covalent, GTP-bound and orally bioavailable KRAS <sup>G12D</sup> (ON) inhibitor	IND-enabling	Jang et al. (68)
BI-2582	Boehringer Ingelheim	Non-covalent KRAS <sup>G12D</sup> inhibitor, with nanomolar binding affinity to GTP-bound KRAS <sup>G12D</sup> .	Preclinical	Tran et al. (69)
ERAS-4	Erasca	KRAS <sup>G12D</sup> inhibitor	Preclinical	GlobeNewswire - 23 Aug 2022
THZ835	Tsinghua University	Inhibitor forms a salt bridge with Asp 12 of KRAS <sup>G12D</sup> , induces switch-II fit pocket in KRAS <sup>G12D</sup> and has off-target effect because inhibition is not fully dependent on KRAS mutation status.	Preclinical	Mao et al. (70)
QTX3046	Quanta Therapeutics	Non-covalent and orally bioavailable KRAS <sup>G12D</sup> inhibitor, picomolar-level binding affinity (0.01nM) to inactive KRAS <sup>G12D</sup>	Preclinical	Vo et al. (71)
QTX3034	Quanta Therapeutics	Selective, orally bioavailable, and picomolar-level binding affinity (0.6 nM) to GDP-bound KRAS <sup>G12D</sup>	Preclinical	Zhang et al. (72)
VRTX153		Selective KRAS <sup>G12D</sup> inhibitor that binds to GDP-bound state, with a nanomolar-level IC <sub>50</sub> .	Preclinical	Bhavar et al. (73)
Compound 3		KRAS <sup>G12D</sup> inhibitor with IC <sub>50</sub> at nanomolar-level.	preclinical	Wang et al. (74)
MRC-6236	Revolution Medicines	Orally bioavailable, selective inhibitor for active RAS (ON) form of both wild and mutant variants of the canonical RAS isoforms (HRAS, NRAS, and KRAS) (pan-RAS/ RAS <sup>MULTI</sup> inhibitor).	Phase I (NCT05379985)	Gustafson et al. (65)
JAB-23425	Jacobio	Orally bioavailable, selective multiple KRAS mutant (G12A/C/D/R/V and Q61H) inhibitor, with sub-nanomolar-level binding affinity to GDP- and GTP-bound KRAS states. No inhibition of HRAS and NRAS (pan-KRAS/ RAS <sup>MULTI</sup> inhibitor).	Preclinical	Wang et al. (75)
BI-2865	Boehringer Ingelheim	Non-covalent, selective inactive form of KRAS (both wild and mutant) inhibitor, no effect on HRAS and NRAS (pan-KRAS or RAS <sup>MULTI</sup> inhibitor)	Preclinical	Kim et al. (66)

GTP, guanosine-5'-triphosphate; GDP, guanosine-5'-diphosphate; IND, investigative new drug.