EDITORIAL

Glimmers of hope for targeting oncogenic KRAS-G12D

KRAS mutations are one of the most common genetic abnormalities in cancer, especially lung, colon, and pancreatic cancers. Strategies targeting the oncogenic KRAS pathway include direct and indirect approaches. KRAS-G12C inhibitors developed based on binding to the switch II pocket structure of KRAS mutant protein represent a breakthrough in the development of targeted therapeutic strategies against oncogenic proteins previously considered undruggable. The covalent KRAS-G12C inhibitors sotorasib (AMG510) and adagrasib (MRTX849) are used to treat patients with *KRAS-G12C*-mutated non-small cell lung cancer. Emerging research shows that other host point mutations in KRAS can also be directly targeted by small-molecule compounds. Recently, through extensive structure-based drug design from Mirati Therapeutics, a novel non-covalent KRAS-G12D inhibitor, MRTX1133, showed significant preclinical antitumor activity in *KRAS-G12D*-bearing tumor cells, especially pancreatic ductal adenocarcinoma. Here, we discuss the selectivity, efficacy, toxicity, and potential application challenges of this novel targeted protein inhibitor.

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Cancer is a very ancient disease, the oldest written description of this disease dates back to ancient Egypt around 3000 BC [1]. However, due to the discovery of the first human oncogene cellular Src kinase (*c-Src*) by the American microbiologists John Michael Bishop and Harold Varmus, the genetic paradigm of cancer was not proposed until the 1970s [2]. To date, scientists have identified more than 70 human oncogenes in different cancers [3]. Among them, the Kirsten rat sarcoma virus gene (*KRAS*) is the most frequently mutated oncogene, present in approximately 30% of human tumors [4]. The most hotspot oncogenic *KRAS* mutations are at position 12 (especially *G12D*, *G12V*, and *G12C*), which are constitutively activated due to their defective ability to hydrolyze guanosine triphosphate (GTP), leading to activation of the downstream RAF-MEK-ERK and PI3K-AKT-MTOR pathways [4]. However, KRAS has long been considered a challenging therapeutic target, or even "undruggable", because its protein surface does not have deeper clefts [4]. This dilemma was broken in 2013, when the American chemical biologist Kevan Shokat discovered that the KRAS-G12C protein displays a pocket structure in the switch II domain that can be covalently bound by small-molecule drugs [5]. This class of targeted drugs, now collectively known as KRAS-G12C inhibitors, includes Amgen's sotorasib (AMG510) and Mirati's adagrasib (MRTX849) for the treatment of patients with *KRAS-G12C*-mutated non-small cell lung cancer [6–8]. These groundbreaking studies on KRAS-G12C inhibitors represent a breakthrough in oncoprotein-targeted therapy [9], although drug resistance remains a challenge [10].

Compared to *G12C*, *G12D* is most commonly seen in pancreatic ductal adenocarcinoma (PDAC), a dismal disease with an average 5-year survival rate of less than 10% due to difficult early diagnosis and lack of effective treatment [11]. KRAS-G12C inhibitors rely on a reactive warhead to form a stable covalent bond with mutant Cys12 [12, 13]. In contrast, the KRAS-G12D protein lacks reactive residues adjacent to the switch II pocket, as demonstrated by recent attempts, thus requiring a new approach to identify selective inhibitors [14]. Recently, *Nature Medicine* published a study led by Mirati's James G. Christensen showing encouraging preclinical data for the small molecule compound MRTX1133 as a KRAS-G12D inhibitor in treating *KRAS-G12D* mutant cancers, particularly PDAC (Fig. 1) [15].

Mirati's research team discovered MRTX1133 through a structure-based drug design strategy, which is a non-covalent inhibitor that can bind to the inactive and activated states of KRAS-G12D protein, resulting in KRAS pathway inhibition [16]. MRTX1133 is highly selective for KRAS-G12D protein, more than 1000-fold higher than wild-type KRAS protein, resulting in an estimated K_D of 0.2 pM for KRAS-G12D (Fig. 1). Using in-cell western assay of downstream phosphorylated extracellular signal-related kinase (ERK), the authors found that MRTX1133 inhibited 24 of 25 *KRAS-G12D* mutant cancer cell lines (median IC_{50}: 6.1 nM). In contrast, in 11 non-*KRAS-G12D* mutant cell lines, the median IC_{50} of MRTX1133 was >3000 nM. The combination of western blot and CellTiter-Glo assay also confirmed that MRTX1133 selectively blocks KRAS activation and suppresses cell proliferation in HPAC cells (human *KRAS-G12D*-mutated PDAC cell line). These cell-free assays and cellular experiments strongly demonstrate that MRTX1133 is a highly selective KRAS-G12D inhibitor.

Does MRTX1133 block KRAS-G12D protein with similar selectivity in vivo? To answer this question, the authors first performed a dose-response experiment (3, 10, 30 mg/kg) and then examined the effects of a single dose of 30 mg/kg (intraperitoneally [IP], twice daily [BID]) MRTX1133 in a series of mouse xenograft tumor models with *KRAS-G12D* mutations in human cancer cell lines or patient-driven tumor tissues. This large-scale animal screening study showed that in 25 *KRAS-G12D*-mutated tumor models, MRTX1133 resulted in more than 30% tumor shrinkage in 11 models. The anticancer activity of MRTX1133 was particularly evident in the PDAC model, with 8 out of 11 models (73%) showing tumor shrinkage greater than 30%. In contrast, MRTX1133 did not show significant antitumor efficacy in 4 non-*KRAS-G12D*-mutated models in vivo. Subsequent orthotopic PDAC (using AsPC-1 cells) mouse model further confirmed the anticancer activity of MRTX1133 at 30 mg/kg (IP, BID). Notably, MRTX1133 alone at 30 mg/kg (IP, BID) was well tolerated in repeated-dose studies up to 28 days, with no evidence of weight loss or overt symptoms of toxicity. Therefore, the antitumor activity of MRTX1133 in vivo also largely depends on the *KRAS-G12D* mutation, and no significant side effects were observed during testing.

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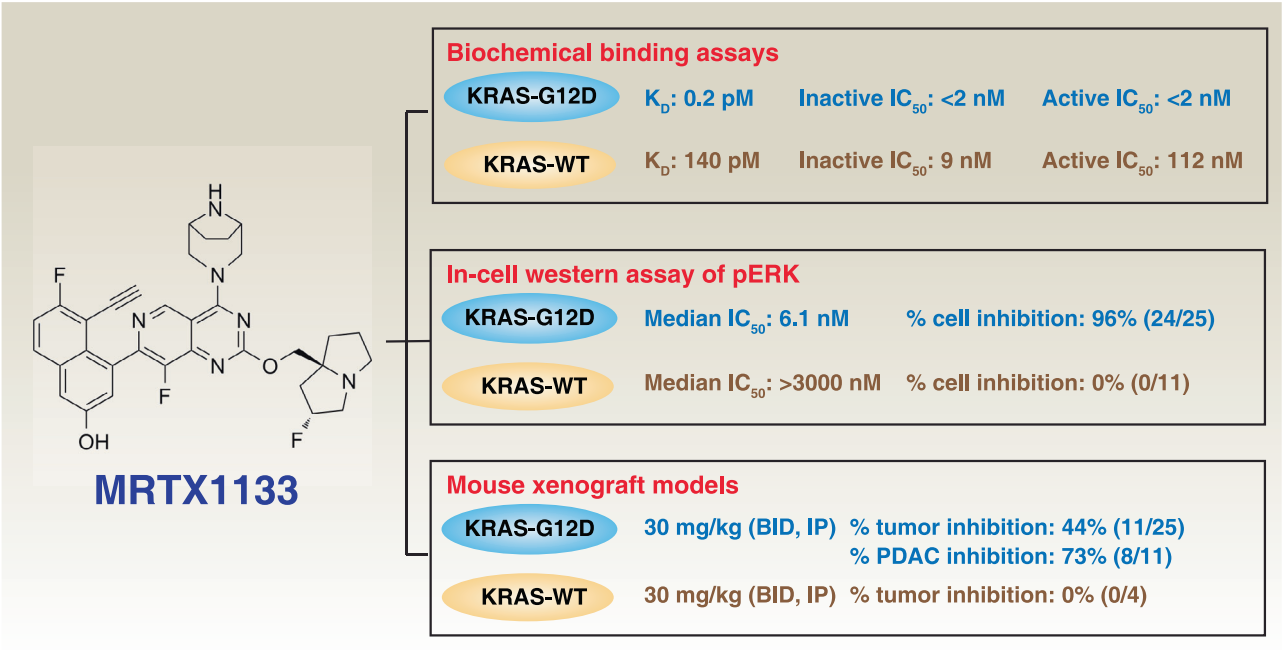

Fig. 1 MRTX113 is a selective inhibitor of KRAS-G12D protein. The activity and function of MRTX113 in targeting KRAS-G12D and KRAS-WT proteins were compared in cell-free systems, cellular, and xenograft models.

To better understand the mechanism of action of MRTX1133 in vivo, the authors used several high-throughput techniques to search for potential molecular biomarkers or modulators associated with antitumor activity. Unfortunately, although RNA-sequencing found that lower RNA expression of phosphatase and tensin homolog (*PTEN*, a tumor suppressor) and cyclin-dependent kinase inhibitor 2 A (*CDKN2A*, the gene encoding the cell-cycle inhibitor protein p16) was associated with reduced antitumor activity of MRTX113, neither trend reached statistical significance. Remarkably, the authors also used CRISPR–Cas9 screening to analyze the feedback signaling pathway and bypass pathway stimulated by MRTX1133, and revealed that the activation of epidermal growth factor receptor (EGFR), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA, also known as PI3Kα), and protein tyrosine phosphatase non-receptor type 11 (PTPN11, also known as SHP2) signaling pathways may facilitate resistance to MRTX1133. Combining MRTX1133 with the EGFR inhibitor cetuximab or the PIK3CA inhibitor alpelisib (also known as BYL-719) showed enhanced anticancer activity in various xenograft models, including the AsPC-1 PDAC model. These findings establish additional combinatorial strategies to overcome resistance to MRTX1133, although the underlying signaling details are still not fully understood.

In conclusion, this study may provide preclinical evidence of efficacy, selectivity, tolerability, and safety for the design of future clinical trials using MRTX1133 to target KRAS-G12D protein in PDAC patients. New lead discovery of therapeutic targets, especially those that have been undruggable in the past, is indeed a daunting task that requires multidisciplinary and team best practices to be successful. Additional efforts are needed in the future to address basic and clinical questions to better optimize the activity of MRTX1133 and overcome its resistance. For example, how can this information from MRTX1133 be used to design KRAS-G12D covalent inhibitors [17]? Given that the animal experiments used in this study were immunodeficient mice, what are the effects of MRTX1133 treatment on the tumor immune microenvironment [18]? Why MRTX1133 is more sensitive to *KRAS-G12D*-mutated PDAC cells than *KRAS-G12D*-mutated colorectal cancer cells in vivo? Does MRTX1133 synergize with current PDAC first-line treatments (such as FOLFIRINOX) or cytotoxic drugs to induce cell death or inhibit autophagy [19–24]? Does blocking other components of the KRAS pathway, such as RAF, MEK, ERK and advanced glycosylation end-product specific receptor (AGER/RAGE), enhance the anticancer activity of MRTX1133 [25]?

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AUTHOR CONTRIBUTIONS

D.T. and R.K. designed the concept, wrote the manuscript, and approved the final manuscript.

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COMPETING INTERESTS

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ADDITIONAL INFORMATION

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