PROF. MARTIN OLOF BERGO (Orcid ID : 0000-0002-6915-7140)

TARGETING KRAS[G12C]-MUTANT CANCER WITH A MUTATION-SPECIFIC INHIBITOR

James G. Christensen,[1*] Peter Olson,[1] Tiffany Briere,[1] Clotilde Wiel,[2] and Martin O. Bergo.[2]

[1] Mirati Therapeutics, Inc., San Diego, CA, 92121, USA

[2] Department of Biosciences and Nutrition, Karolinska Institutet, SE-141 83 Huddinge, Sweden

* Corresponding author: Email: christensenj@mirati.com; Phone: +1-(858) 332-3410

ABSTRACT

The *RAS* genes, which include *H*, *N*, and *KRAS*, comprise the most frequently mutated family of oncogenes in cancer. Mutations in *KRAS*—such as the G12C mutation—are found in most pancreatic, half of colorectal, and a third of lung cancer cases and is thus responsible for a substantial proportion of cancer deaths. Consequently, KRAS has been the subject of exhaustive drug targeting efforts over the past 3–4 decades. These efforts have included targeting the KRAS protein itself but also its posttranslational modifications, membrane localization, protein-protein interactions, and downstream signaling pathways. Most of these strategies have failed and no KRAS-specific drugs have yet been approved. However, for one specific mutation, KRAS^{G12C}, there is light on the horizon. MRTX849 was recently identified as a potent, selective, and covalent KRAS^{G12C} inhibitor that possesses favorable drug-like properties. MRTX849 selectively modifies the mutant cysteine residue in GDP-bound KRAS^{G12C} and inhibits GTP-loading and downstream KRAS-dependent signaling. The drug inhibits the in vivo growth of multiple KRAS^{G12C}-mutant cell line xenografts, causes tumor regression in patient-derived xenograft models, and shows striking responses in combination with other agents. It has also produced objective responses in patients with mutant-specific lung and colorectal cancer. In this review, we discuss the history of RAS drug targeting efforts, the discovery of MRTX849, and how this drug provides an exciting and long-awaited opportunity to selectively target mutant KRAS in patients.

INTRODUCTION

KRAS is the oncogene most frequently mutated in cancer and it is the first of over 700 genes to be causally linked to cancer in humans (COSMIC) [1]. *KRAS* mutations are particularly common among the three deadliest types of cancer in the United States: pancreatic (95%), colorectal (45%), and lung (35%) [2]. The fact that these common mutations occur in a broad spectrum of aggressive cancers has stimulated intensive drug discovery efforts to develop drugs that block the function of the KRAS oncoprotein for cancer treatment. However, four decades of research have failed to produce a clinically viable KRAS cancer therapy. Recent developments, however, have stimulated a new wave of efforts to develop KRAS targeted therapies.

THE KRAS REVIVAL

The last decades have been plagued by drug development failures and a waning interest in and presumed intractability of targeting KRAS directly. But new technologies and strategies have stimulated a comeback for KRAS as a therapeutic target [3], [4]. Key to this revival is the KRAS^{G12C} mutation which is a well-validated driver mutation and the most common individual KRAS mutation in lung cancer [5]. The KRAS^{G12C} mutation results in a switch from glycine to cysteine at position 12 in the protein and is associated with poor prognosis and therapy resistance. Thus, this mutation represents an unmet clinical need and exciting opportunity. The KRAS^{G12C} mutation is causally linked to 14% of lung adenocarcinomas (~14,000 new US cases/year), 5% of colorectal adenocarcinomas (~5,000 new US cases/year) and smaller fractions of other cancer forms. All-in-all, KRAS^{G12C} mutations comprise a patient population with a worldwide yearly incidence of over 100,000 individuals. The rationale for targeting the KRAS^{G12C} oncoprotein was first described by Shokat and colleagues; they identified small molecules that bound irreversibly to the mutant reactive cysteine at codon 12 in a previously unappreciated binding pocket near the KRAS effector region. Small molecules that bind this pocket can inhibit KRAS by locking the protein in its GDP-bound inactive form [6], [7]. Irreversible targeting of this site was obviously an advancement, but it was also clear that identified molecules required substantial optimization in order to achieve drug-like potency and pharmaceutical properties. Therefore, Mirati Therapeutics Inc. (San Diego, CA) began a drug-discovery partnership with Array Biopharma (Boulder, CO; recently acquired by Pfizer Inc.). An intensive structure-based drug design effort lead to the identification of MRTX849 which became an Investigational New Drug (IND) track clinical candidate in 2017. MRTX849 possesses a drug-like cellular potency of ~5–10 nM; selectivity of > 1000× for KRAS^{G12C} compared to the wild-type form;

broad-spectrum antitumor activity in nonclinical models; predicted oral bioavailability in humans of ~50%; and ~20 hours half-life and a 10-fold therapeutic index in nonclinical studies. On October 29, 2018, the IND application proposing clinical development of MRTX849 was submitted to the U.S. Food and Drug Administration (FDA) with first patient dosing soon after FDA approval of the IND application. The early non-clinical and clinical proof-of-concept data was published recently and will be discussed further below [8].

RAS IS A SMALL GTPASE THAT CYCLES BETWEEN INACTIVE AND ACTIVE FORMS

The RAS proteins belong to a family of small GTPases which can be activated by growth factors and other extracellular stimuli [9]. The RAS proteins control signaling through multiple intercellular pathways responsible for cell survival, growth, proliferation, migration, and differentiation. Activation of the RAS proteins occur at the inner surface of the cell membrane and results in the binding of key effector molecules, generation of signaling complexes, and initiation and propagation of intracellular signaling cascades including the RAF and PI3 kinase pathways. Because aberrant RAS function may result in enhanced or sustained signaling through these pathways and lead to malignant transformation, RAS protein activation and inactivation is tightly regulated in normal cells [9].

RAS proteins typically cycle between GTP-bound and GDP-bound states, where the GTP-bound conformation represents the "on" state and the GDP-bound the "off" state [9]. RAS proteins in the GTP-bound "on" state undergo marked structural changes in two regions called Switch I and Switch II, which then coalesce to form an interaction surface. The interaction surface is required for generating the signaling complexes with the RAF family and PI3 kinase family proteins which in turn trigger the downstream signaling cascades. The binding of GDP and GTP with RAS is very strong; the dissociation constants are in the picomolar range; thus, RAS is essentially always bound to either GTP or GDP. Moreover, the RAS proteins have low dissociation rates for GDP and low intrinsic enzymatic GTPase activity. Consequently, the conversion of GDP-bound inactive RAS to the GTP-bound the active form requires very high rates of nucleotide exchange which is facilitated by guanine nucleotide exchange-factors (GEFs); whereas conversion back to the GDP-bound inactive form requires GTPase-activating proteins (GAPs) which stimulates the intrinsic hydrolytic activity of the RAS proteins. The fact that RAS and other GTPases depend on GEFs and GAPs for switching them on and off allows both of these processes to be tightly controlled and to be rapidly responsive to diverse signals. By contrast, mutant forms of RAS which are constitutively active generally function by preventing GTP hydrolysis, thereby generating active GTP-bound RAS molecules that can't be

turned off. This sustained RAS signaling can pose severe consequences for the cell, including deregulated growth and malignant transformation. The picomolar affinity of RAS for GTP, the high GTP levels in cells (> 500 μM), and the inability of oncogenic RAS to hydrolyze GTP create significant challenges for traditional drug discovery approaches, as discussed below [4], [7], [10].

KRAS MUTATIONS IN CANCER

The RAS protein family has three founding members; KRAS, NRAS, and HRAS. Mutations in *RAS* genes are found in approximately one-fourth of all human cancers and account for up to one million deaths per year worldwide. Most of these missense mutations occur in KRAS (85%), and less frequently in NRAS (12%) and HRAS (3%) [9]. Most RAS mutations occur at amino acid residues/codons 12, 13, and 61. The frequency of mutation at these three residues, and the RAS isoform mutated, varies among different cancer types [9]. Most KRAS mutations occur at residue 12 which is normally occupied by a glycine residue. The mutation of this glycine at residue 12 to anything other than proline results in steric hindrance which prevents binding of GAP proteins to KRAS, reduces GTP hydrolysis, and thereby increases levels of the GTP-bound active form [9].

The most common mutation in non-small cell lung cancer (NSCLC) is KRAS^{G12C} (glycine to cysteine). This mutation comprises almost half of all KRAS mutations, followed by G12V (glycine to valine) and G12D (glycine to aspartic acid) [5]. As outlined earlier, the reactive cysteine in the KRAS^{G12C} mutant provides an opportunity for irreversibly targeting this variant with small molecule drugs. Genomic studies of lung cancer have indicated that KRAS mutations, including G12C, are mutually exclusive with other NSCLC oncogenic driver mutations, including EGFR, ALK, ROS1, RET, and BRAF which suggests that KRAS mutations make up a unique set of lung cancers that lack targeted therapy options [11], [12]. Studies in endogenous mouse models of lung cancer reveal that co-expression of oncogenic KRAS with other oncogenic driver mutations leads to oncogene-induced senescence, which likely explains why they are rarely detected in the same tumor [13], [14]. However, KRAS mutations in NSCLC frequently co-occur with mutations in STK11, KEAP1, and TP53 which cooperate with mutant RAS to transform cells into highly malignant and aggressive tumor cells [15], [16]. Functional genomic studies with shRNA technology to suppress the expression of thousands of gene products in hundreds of cancer cell lines have revealed that KRAS-mutant cancer cells depend on KRAS function for growth and survival [15], [16]. The results illustrate a central role for mutant KRAS as a causative agent in a large number of cancers and present an exciting opportunity for therapeutic agents that target KRAS to make a profound impact in treating these cancers.

A HISTORICAL PERSPECTIVE ON RAS TARGETING EFFORTS

Therapeutic strategies that directly target oncogene products have produced substantial benefits for a variety of cancer patients, including patients with NSCLC, breast cancer, melanoma, and some types of leukemia. Therapies that are currently under development include selective small molecule drugs that target oncogene products including mutant EGFR and BRAF; gene-amplified HER2; and ALK and ROS1 that have undergone gene rearrangements. However, one of the most attractive drug targets in cancer, mutant KRAS, is also the one that poses the most challenges. Historically, targeting mutant KRAS has proven to be extremely difficult. The challenges have included 1) lack of pockets large enough for small molecules to bind; 2) high affinity for GTP which prevents direct targeting of the nucleotide-binding pocket; 3) high intracellular GTP concentrations and fast nucleotide exchange cycles that hinders competition; and 4) potential toxicity arising from the indiscriminate inhibition of wild-type KRAS proteins [4], [7], [10]. Strategies that indirectly target KRAS have also been evaluated and include inhibiting the targeting of KRAS to the cell membrane and targeting downstream signaling molecules including RAF, MEK, ERK, and PI3K family proteins.

These indirect targeting strategies have also faced severe challenges including 1) low therapeutic index which arises from the targeting of essential cell growth and survival pathways; 2) compensatory escape mechanisms; and 3) feedback regulation of signaling pathways and redundancy due to tight regulation of these essential pathways [4]. Here, we will discuss the following primary categories of challenges for KRAS targeting strategies: *1) Reducing the proportion of active RAS-GTP (direct); 2) Disrupting protein–protein interactions (direct); 3) Reducing RAS plasma membrane targeting (indirect); 4) Targeting downstream effector signaling; and 5) Synthetic lethality.*

DIRECT TARGETING STRATEGIES

Reducing the proportion of active RAS-GTP

Early RAS-drugging efforts were based on misconceptions; the most devastating was the hypothesis that the function of all RAS proteins is identical. The three canonical *RAS* oncogenes (*HRAS*, *NRAS*, and *KRAS*) encode four main RAS proteins (HRAS, NRAS, KRAS4A, and KRAS4B). The oncogenic potential of all four proteins was well established but scientists paid a disproportionate attention to HRAS, an isoform for which many reagents were already readily available; these reagents included antibodies, vectors, cell lines, and transgenic mouse models. Thus, HRAS was an ostensible representative of the RAS protein family but later turned out to be the least clinically relevant; the most clinically relevant isoform is KRAS.

As outlined earlier, RAS GTPases exist in a GDP-bound "off" state and a GTP-bound "on" state. Due to its GTP-dependent activation, the earliest ideas on how to target mutant RAS focused on reducing the levels of active RAS-GTP. It was reasoned that if a small-molecule inhibitor could preferentially bind to the RAS-GTP pocket, it would likely be an effective inhibitor of mutant RAS. But this strategy was unsuccessful and further biochemical studies revealed that the high picomolar affinity of RAS for GTP along with the high intracellular GTP concentrations (~500 μM), made it unlikely that the drug would successfully compete for binding in the nucleotide-binding pocket [4], [10]. By contrast, the affinity of the "druggable" protein kinase families with their natural co-substrate, ATP, is in the micromolar range (i.e., a million-fold difference compared with RAS and GTP); the ATP concentration in cells range between 1 and 10 mM [17]. Moreover, the featureless surface of RAS, which was recently visualized through crystallography, did not provide new ideas for how to target the protein. The relatively smooth surface lacks pronounced hydrophobic pockets into which a drug

can bind securely; this realization supported notions of the intractability of oncogenic RAS.

Disrupting protein–protein interactions

Following the increased understanding of how extraordinary the RAS-GTP binding kinetics are, research efforts switched focus away from the RAS nucleotide-binding pocket to other surface regions most notably the sites of effector protein-protein interaction. In its oncogenic conformation, disrupting the interactions between mutant RAS-GTP and effector proteins could potentially prevent the hyperactivation of downstream signaling pathways. However, the development of clinically effective inhibitors that disrupt such interactions has been challenging, primarily because small molecules can't cover a large enough portion of the protein–protein interaction, and they would be too chemically weak to effectively mask the interaction sites. Two studies have reported the discovery of small molecules that bind RAS and disrupt its interaction with the GEF SOS [18], [19]. Compounds that block GEF binding to mutant RAS, inhibit its hyperactivation by altering the nucleotide cycling equilibrium in favor of the inactive GDP-bound state. However, the activity of this class of molecules has not proven efficient enough to advance to clinical development.

A second approach to inhibit KRAS signaling by disrupting protein–protein interactions is to block the dimerization of RAS and RAF. This strategy was predicted to be effective because the primary effectors of RAS signaling is the RAF family of serine-threonine kinases (ARAF, BRAF, and CRAF). Following activation of RAS, RAS-GTP recruits RAF to the plasma membrane and thereby promotes RAF dimerization and activation of downstream signaling pathways [9]. If RAS-GTP dimerization or multimerization is required for binding to downstream effectors, disrupting this complex could be an effective strategy to inhibit mutant RAS hyperactivation [4], [9]. This concept has however not advanced past academic proof of principle [20].

INDIRECT TARGETING STRATEGIES

Reducing RAS plasma membrane targeting – prenylation inhibitors

In light of the results that indicated that RAS could not be inhibited directly, efforts shifted toward inhibiting RAS indirectly. The most promising of these focused on earlier findings that RAS has to localize to the plasma membrane to function properly. The carboxyl terminus of the four main RAS isoforms contains a so-called *CAAX* motif where *C* is a cysteine residue; A are aliphatic amino acids; and X can vary. The *CAAX* motif triggers three posttranslational modification steps: First, a 15-carbon farnesyl lipid is covalently attached to the cysteine residue (i.e., the *C* in *CAAX*) by

farnesyltransferase (FTase). Second, the last three amino acids (i.e., the –*AAX*) are cleaved off by RAS converting enzyme 1 (RCE1). Third, the newly-exposed farnesylcysteine residue is methylated by isoprenylcysteine carboxyl methyltransferase (ICMT). These posttranslational processing steps render the RAS carboxyl terminus more hydrophobic which increases membrane interactions. NRAS and HRAS subsequently acquire a second membrane-targeting signal in the form of palmitoylation of upstream cysteine residues; KRAS relies on a polybasic stretch of lysines to stimulate electrostatic interactions with the negatively charged phospholipid heads on the inner leaflet of the membrane.

All *CAAX* processing enzymes have been evaluated for therapeutic purposes, but most efforts focused on FTase. The rationale was simple: if membrane localization is a requirement for RAS function, an FTase inhibitor (FTI) should attenuate oncogenic activity as a consequence of RAS displacement [3], [4]. Indeed, studies had shown that mutating the cysteine of the *CAAX* to serine to create a so-called "*SAAX* mutant", prevented farnesylation and membrane targeting, and eliminated RAS-induced oncogenic transformation in cell culture experiments [21], [22].

Intense efforts from multiple companies and academic institutions yielded many FTIs that demonstrated potent inhibition of FTase activity and HRAS-driven cancer cell growth in both *in vitro* and *in vivo* models (and in recent clinical trials selectively enrolling HRAS mutated tumors). FTIs even caused regression of established tumors in MMTV-HRAS transgenic mice [23]. Two inhibitors advanced as far as phase III clinical trials where, unfortunately, they failed to demonstrate efficacy in KRAS-driven pancreatic, colorectal, and lung cancers. Unlike HRAS, the prenylation of KRAS and NRAS can be catalyzed by another enzyme—geranylgeranyltransferase type I (GGTase-I)—when FTase is inhibited, resulting in intrinsic resistance of KRAS-mutant cancers to FTIs. This realization prompted efforts to develop GGTase-I inhibitors (GGTIs) and dual-prenylation inhibitors (DPIs) [24], [25], which didn't advance very far due to problems with toxicity (GGTase-I processes more substrates than FTase, including RHO family proteins RHOA, RAC1, and CDC42).

Gene knockout studies in mice were used to understand the relative impact of blocking FTase and GGTase-I, and both enzymes, in wild-type and KRAS-mutant cells [26]–[29]. Whole-body knockout of FTase and GGTase-I is lethal in mice, but conditional knockout of either enzyme significantly reduces KRAS-driven lung cancer development, but only by ⊐25%. However, in cells lacking either FTase or GGTase-I, KRAS can reach the plasma membrane and function normally; thus the anti-tumor effect is mediated by inhibiting the prenylation of non-RAS substrates [27]; a result that fits well

with findings that some FTIs inhibit the growth of non-RAS-mutant cancer cells [30], [31]. Interestingly, conditional knockout of both FTase and GGTase-I abolishes KRAS prenylation and plasma membrane targeting, and essentially eliminates KRAS-driven lung tumor development [27]. Unfortunately, targeting both enzymes is associated with significant toxicity in most other tissues [27]. Thus, the general idea of targeting KRAS prenylation and decreasing membrane localization is valid and supported by in vitro and in vivo genetic studies, but the toxicity associated with the inhibition of both FTase and GGTase-I makes the strategy difficult. Nevertheless, the failed clinical trials with FTIs resulted in abandonment of drug discovery research directed at modifying KRAS function, and activities shifted toward targeting proteins that mediate signaling downstream of KRAS.

Targeting downstream effector signaling

RAS is a central hub for signaling to more than 11 downstream effector families. With regard to RAS-driven cancers, the most studied are the RAS-RAF-MEK-ERK pathway and the RAS-PI3K-AKT-mTOR pathway. In the majority of tumors, RAS mutations leads to hyperactivation of RAS-RAF-MEK-ERK signaling. The interest in targeting RAS-RAF-MEK-ERK signaling increased when BRAF mutations were shown to cause malignant melanoma and other cancer types which suggested that blocking this pathway could be effective also in cancers without RAS mutations [32]. Indeed, the subsequent development of BRAF inhibitors and BRAF-MEK inhibitor combinations led to the approval of several drugs and drug combination strategies useful for treating BRAF-mutant melanoma; some of those strategies have shown promise in other BRAF-mutant cancers including NSCLC [33]. In contrast, the efficacy of BRAF and MEK inhibitors in the treatment of KRAS-mutant cancer has been disappointing. Inhibition of MEK1 and -2 was previously considered a highly promising KRAS-targeting strategy, but it failed to deliver in clinical trials [34]. The clinical failure of MEK inhibitors is attributed to difficulty of achieving a therapeutic index that enables significant inhibition of the pathway (which stems from the important role of MEK in normal cells), but also to compensatory pathway activation which provides an escape for cancer cells exposed to the inhibitors. Blocking BRAF in KRAS mutated cells is actually contraindicated because of the paradoxical activation of ERK signaling which is triggered by compensatory feedback activation of RAF1 in the setting of activated KRAS [33]. Recently, a new generation of drugs that inhibit RAF-MEK-ERK pathway signaling have entered clinical trials and include dual BRAF/RAF1 inhibitors and ERK1/2 inhibitors. These drugs may be associated with lower levels of compensatory pathway activation and have shown some clinical activity; however, it is not yet known whether these agents

can produce optimal levels of target inhibition and single-agent antitumor activity simply because the target signaling proteins are important for the function of normal cells [33], [35]. Consequently, it is highly likely that these drugs will have to be combined to effectively inhibit KRAS-mutant cancer, but it is too early to speculate on whether any of those approaches will be effective.

Synthetic lethality

Synthetic lethal interaction partners of RAS are defined as proteins that are essential for the survival of RAS-mutant cells but dispensable in wild-type cells. A great deal of attention in the last decade has been given to large-scale screens designed to identify RAS synthetic lethal partners. The outcome of such screens has been a host of new potential targets for targeting RAS-mutant cancer. Unfortunately, the most promising hits could not be reproduced when conditions were altered, which was yet another blow to RAS drug discovery efforts [3], [4]. In the most recent efforts to identify synthetic lethal RAS targets, two comprehensive RNAi screens, Project DRIVE and Project Achilles, used improved strategies, reagents, and computational approaches of past screens to enhance the ability to map cancer gene dependencies. Project DRIVE used a high-density shRNA library to knock down 7,837 genes in 398 cell lines and classify genetic, expression, metabolic, and synthetic lethal relationships among cancer genes [15], [16]. Aside from mutant NRAS and BRAF, Project DRIVE confirmed that KRAS-mutant cells depend on KRAS for growth and survival. However, the screen failed to identify new independent genes that are synthetic lethal with mutant KRAS and that could be used to target mutant KRAS function indirectly, which again illustrates the likely requirement for direct inhibition of KRAS to block its oncogenic properties.

DIRECT TARGETING: IN ACTION

Covalent irreversible KRAS^{G12C} inhibition

For decades the absence of defined pockets in the KRAS oncoprotein prevented the identification of effective inhibitors. Recently, however, software and computer modeling strategies have improved the prediction of affinities of small molecules for proteins harboring a smooth surface topology without a pocket that facilitates drug binding. Virtual computer modeling allowed for the design of fragment-based library screens which resulted in the discovery of the allosteric Switch II Pocket (S-IIP) region [6]. Interestingly, the S-IIP is proximal to the often mutated glycine residue at position 12. With these observations in mind, Shokat and coworkers designed a screening strategy for inactive GDP-bound KRAS^{G12C}. To accomplish this, they used a library of electrophilic fragments to identify

small molecules that would react with the cysteine residue specific to the G12C mutation. They identified small molecules which induced substantial structural modifications. Crystallography of KRAS[G12C] bound to one of these fragment molecules revealed a previously undetected S-IIP binding pocket next to the reactive cysteine residue and also revealed that the compound formed hydrophobic contacts and hydrogen bonds which facilitated its orientation in the S-II pocket [6]. The hit compounds were optimized and were confirmed to form covalent bonds with the reactive cysteine of KRAS[G12C] and to lock KRAS in its GDP-bound inactive form [6], [36]–[38]. However, there is still no information on whether these molecules have advanced to IND-track development.

IND-track of the mutant KRAS-selective Inhibitor MRTX849

Intensive structure-based drug design efforts which included more than 150 unique co-crystal structures, along with synthesis and evaluation of >2000 discrete small molecules, led to the identification of MRTX849. This clinical candidate is a potent and orally available small molecule KRAS[G12C] inhibitor which advanced to an IND application in Q4, 2018. MRTX849 has drug-like potency and blocks KRAS-dependent signal transduction and cancer cell viability with EC_{50} values of ~5-10 nM, and selectivity of >1000× for KRAS[G12C] compared with wild-type KRAS as judged by cellular and whole-cell proteomic screening approaches. Moreover, MRTX849 has broad-spectrum antitumor activity (it showed >30% tumor reduction in 65% of 26 models tested) across a panel of KRAS[G12C]-positive patient- and cell-derived tumor models implanted in mice at well-tolerated dose levels, including complete tumor responses in some models. The drug showed deep responses in KRAS-mutant tumor models that exhibit co-occurring mutations including STK11, KEAP1, and TP53. Pharmacodynamic and pharmacogenomic analyses in sensitive and partially resistant non-clinical models led to the identification of mechanistic factors that underlie the drug's anti-tumor activity including KRAS nucleotide cycling and pathways that trigger feedback reactivation and/or bypass KRAS dependence. These factors included RTK activation, dysregulation of the cell cycle, and bypass of KRAS dependence. Combining MRTX849 with inhibitors of RTKs, mTOR, or cell cycle revealed enhanced responses and led to substantial tumor regression in multiple tumor models, including models that were refractory to MRTX849 single therapy [8]. Collectively, these results support the notion that KRAS-mutant tumors depend on KRAS for growth and survival and suggest that simultaneously targeting KRAS and factors that cooperate with KRAS in cell transformation can markedly increase antitumor activity. MRTX849 has a predicted oral bioavailability in humans of ~50%, a ~20-hour half-life, and a therapeutic index of up to 10-fold in rodent and non-rodent repeat-

administration toxicology studies. Consequently, MRTX849 exhibit significantly improved potency and antitumor activity compared with previously reported KRAS-mutant-selective inhibitors. MRTX849 entered the clinic in January 2019 and thereby provided a long-awaited option for targeted therapy in patients whose tumors are driven by the KRAS[G12C] mutation.

PROOF OF PRINCIPLE IN PATIENTS AND CONCLUSIONS

Recently, MRTX849 was demonstrated to produce objective responses in patients with KRAS[G12C]-positive lung and colon adenocarcinoma, as judged by response evaluation criteria in solid tumors (RECIST) protocols [8], [Janne et al, unpublished data]. The ongoing Phase 1/2, first-in-human trial has enrolled 17 patients across five dose cohorts. Trial objectives include evaluation of safety, tolerability, pharmacodynamics (PD), pharmacokinetics (PK) and tumor response evaluated using RECIST v1.1 criteria. As of the data cut-off date of October 11, 2019, 12 patients across all dose levels were evaluable for response with at least one radiographic scan. At the highest dose (600 mg BID), three of five (3/5) evaluable patients with NSCLC and one of two (1/2) evaluable patients with CRC achieved a partial response (PR); the remaining patients experienced stable disease (SD). Clinical activity has been observed in patients with metastatic disease who have not responded to prior heavy treatment regimens. Two responding patients (1 NSCLC and 1 CRC) achieved confirmed PRs, both with continuing tumor shrinkage following their first scan. Clinical PK data demonstrated that the dose of 600 mg BID results in drug levels that meet or exceed those likely to lead to full inhibition of KRAS G12C signaling. Treatment-related adverse events (AEs) were primarily grade 1 events with two Gr3/4 dose limiting toxicities observed in the initial data set. The MTD has not yet been established and enrollment into dose expansion at the 600 mg BID dose is underway. It will be important to evaluate the drug in pancreatic cancer, where KRAS mutations are more prevalent and to evaluate combinations between MRTX849 and other agents including RTK, SHP2, CDK4/6, and mTOR inhibitors. Following the failures to develop anti-RAS therapies over the past decades, the identification of MRTX849 is a significant leap forward. The ability to treat KRAS-mutant cancer patients with MRTX849 represents a milestone in the history of cancer drug discovery and marks the beginning of a new era for patients with this disease.

REFERENCES

1. Bos JL. Ras Oncogenes in Human Cancer: A Review. *Cancer Res*. 1989;49(17):4682-4689.

2. Jarvis LM. Have drug hunters finally cracked KRas? | June 6, 2016 Issue - Vol. 94 Issue 23 | Chemical & Engineering News. Chemical & Engineering News. https://cen.acs.org/articles/94/i23/drug-hunters-finally-cracked-KRas.html. Published 2016. Accessed November 19, 2019.

3. Ledford H. Cancer: The Ras renaissance. *Nature*. 2015;520(7547):278-280. doi:10.1038/520278a

4. Papke B, Der CJ. Drugging RAS: Know the enemy. *Science (80-)*. 2017;355(6330):1158-1163. doi:10.1126/science.aam7622

5. Matikas A, Mistriotis D, Georgoulias V, Kotsakis A. Targeting KRAS mutated non-small cell lung cancer: A history of failures and a future of hope for a diverse entity. *Crit Rev Oncol Hematol*. 2017;110:1-12. doi:10.1016/j.critrevonc.2016.12.005

6. Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature*. 2013;503(7477):548-551. doi:10.1038/nature12796

7. Ostrem JML, Shokat KM. Direct small-molecule inhibitors of KRAS: From structural insights to mechanism-based design. *Nat Rev Drug Discov*. 2016;15(11):771-785. doi:10.1038/nrd.2016.139

8. Hallin J, Engstrom LD, Hargis L, et al. The KRASG12C Inhibitor, MRTX849, Provides Insight Toward Therapeutic Susceptibility of KRAS Mutant Cancers in Mouse Models and Patients. *Cancer Discov*. 2020;10(1):54-71. doi:10.1158/2159-8290.CD-19-1167

9. Simanshu DK, Nissley D V., McCormick F. RAS Proteins and Their Regulators in Human Disease. *Cell*. 2017;170(1):17-33. doi:10.1016/j.cell.2017.06.009

10. Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS: Mission Possible? *Nat Rev Drug Discov*. 2014;13(11):828-851. doi:10.1038/nrd4389

11. Campbell JD, Alexandrov A, Kim J, et al. Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. *Nat Genet*. 2016;48(6):607-616. doi:10.1038/ng.3564

12. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med*. 2017;23(6):703-713. doi:10.1038/nm.4333

13. Cisowski J, Sayin VI, Liu M, Karlsson C, Bergo MO. Oncogene-induced senescence underlies the mutual exclusive nature of oncogenic KRAS and BRAF. *Oncogene*. 2015;35(April):1328-1333. doi:10.1038/onc.2015.186

14. Cisowski J, Bergo MO. What makes oncogenes mutually exclusive? *Small GTPases*. 2017;8(3):187-192. doi:10.1080/21541248.2016.1212689

15. McDonald ER, de Weck A, Schlabach MR, et al. Project DRIVE: A Compendium of Cancer Dependencies and Synthetic Lethal Relationships Uncovered by Large-Scale, Deep RNAi Screening. *Cell*. 2017;170(3):577-592.e10. doi:10.1016/j.cell.2017.07.005

16. Tsherniak A, Vazquez F, Montgomery PG, et al. Defining a Cancer Dependency Map. *Cell*. 2017;170(3):564-576.e16. doi:10.1016/j.cell.2017.06.010

17. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature*. 2001;411(6835):355-365. doi:10.1038/35077225

18. Burns MC, Sun Q, Daniels RN, et al. Approach for targeting Ras with small molecules that activate SOS-mediated nucleotide exchange. *Proc Natl Acad Sci U S A*. 2014;111(9):3401-3406. doi:10.1073/pnas.1315798111

19. Maurer T, Garrenton LS, Oh A, et al. Small-molecule ligands bind to a distinct pocket in Ras and inhibit SOS-mediated nucleotide exchange activity. *Proc Natl Acad Sci U S A*. 2012;109(14):5299-5304. doi:10.1073/pnas.1116510109

20. Athuluri-Divakar SK, Vasquez-Del Carpio R, Dutta K, et al. A Small Molecule RAS-Mimetic Disrupts RAS Association with Effector Proteins to Block Signaling. *Cell.* 2016;165(3):643-655. doi:10.1016/j.cell.2016.03.045

21. BM W, RW E, EM S, DR L. Further Genetic Localization of the Transforming Sequences of the p21 V-Ras Gene of Harvey Murine Sarcoma Virus. *J Virol*. 1984;49(2).

22. Willumsen BM, Norris K, Papageorge AG, Hubbert NL, Lowy DR. Harvey murine sarcoma virus p21 ras protein: biological and biochemical significance of the cysteine nearest the carboxy terminus. *EMBO J*. 1984;3(11):2581-2585. doi:10.1002/j.1460-2075.1984.tb02177.x

23. Kohl NE, Omer CA, Davide JP, et al. Inhibition of farnesyltransferase induces regression of mammary and salivary carcinomas in ras transgenic mice. *Nat Med*. 1995;1(8):792-797. doi:10.1038/nm0895-792

24. Lobell RB, Omer CA, Abrams MT, et al. Evaluation of farnesyl:protein transferase and geranylgeranyl:protein transferase inhibitor combinations in preclinical models. *Cancer Res*. 2001;61(24):8758-8768. http://www.ncbi.nlm.nih.gov/pubmed/11751396. Accessed November 27, 2019.

25. DeSolms SJ, Ciccarone TM, MacTough SC, et al. Dual protein farnesyltransferase-geranylgeranyltransferase-I inhibitors as potential cancer chemotherapeutic agents. *J Med Chem*. 2003;46(14):2973-2984. doi:10.1021/jm020587n

26. Sjogren AM, Andersson KME, Liu M, et al. GGTase-I deficiency reduces tumor formation and improves survival in mice with K-RAS – induced lung cancer. 2007;117(5). doi:10.1172/JCI30868.1294

27. Liu M, Sjogren A-KM, Karlsson C, et al. Targeting the protein prenyltransferases efficiently reduces tumor development in mice with K-RAS-induced lung cancer. *Proc Natl Acad Sci*. 2010;107(14):6471-6476. doi:10.1073/pnas.0908396107

28. Mijimolle N, Velasco J, Dubus P, et al. Protein farnesyltransferase in embryogenesis, adult homeostasis, and tumor development. *Cancer Cell*. 2005;7(4):313-324. doi:10.1016/j.ccr.2005.03.004

29. Yang SH, Bergo MO, Farber E, Qiao X, Fong LG, Young SG. Caution! Analyze transcripts from conditional knockout alleles. *Transgenic Res*. 2009;18(3):483-489. doi:10.1007/s11248-008-9237-9

30. Liu M, Bryant MS, Chen J, et al. Effects of SCH 59228, an orally bioavailable farnesyl protein transferase inhibitor, on the growth of oncogene-transformed fibroblasts and a human colon carcinoma xenograft in nude mice. *Cancer Chemother Pharmacol*. 1999;43(1):50-58. doi:10.1007/s002800050862

31. Feldkamp MM, Lau N, Roncari L, Guha A. Isotype-specific Ras.GTP-levels predict the efficacy of farnesyl transferase inhibitors against human astrocytomas regardless of Ras mutational status. *Cancer Res*. 2001;61(11):4425-4431. http://www.ncbi.nlm.nih.gov/pubmed/11389071. Accessed November 27, 2019.

32. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417(6892):949-954. doi:10.1038/nature00766

33. Karoulia Z, Gavathiotis E, Poulikakos PI. New perspectives for targeting RAF kinase in human cancer. *Nat Rev Cancer*. 2017;17(11):676-691. doi:10.1038/nrc.2017.79

34. Zhao Y, Adjei AA. The clinical development of MEK inhibitors. *Nat Rev Clin Oncol*. 2014;11(7):385-400. doi:10.1038/nrclinonc.2014.83

35. Poulikakos PI, Zhang C, Bollag G, Shokat KM, Rosen N. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature*. 2010;464(7287):427-430. doi:10.1038/nature08902

36. Lito P, Solomon M, Li LS, Hansen R, Rosen N. Cancer therapeutics: Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. *Science (80-)*. 2016;351(6273):604-608. doi:10.1126/science.aad6204

37. Patricelli MP, Janes MR, Li LS, et al. Selective inhibition of oncogenic KRAS output with small molecules targeting the inactive state. *Cancer Discov*. 2016;6(3):316-329. doi:10.1158/2159-8290.CD-15-1105

38. Janes MR, Zhang J, Li LS, et al. Targeting KRAS Mutant Cancers with a Covalent G12C-Specific Inhibitor. *Cell*. 2018;172(3):578-589.e17. doi:10.1016/j.cell.2018.01.006

CONFLICT OF INTEREST STATEMENT

JGC, PO, and TB are employees and stockholders of Mirati Therapeutics Inc.
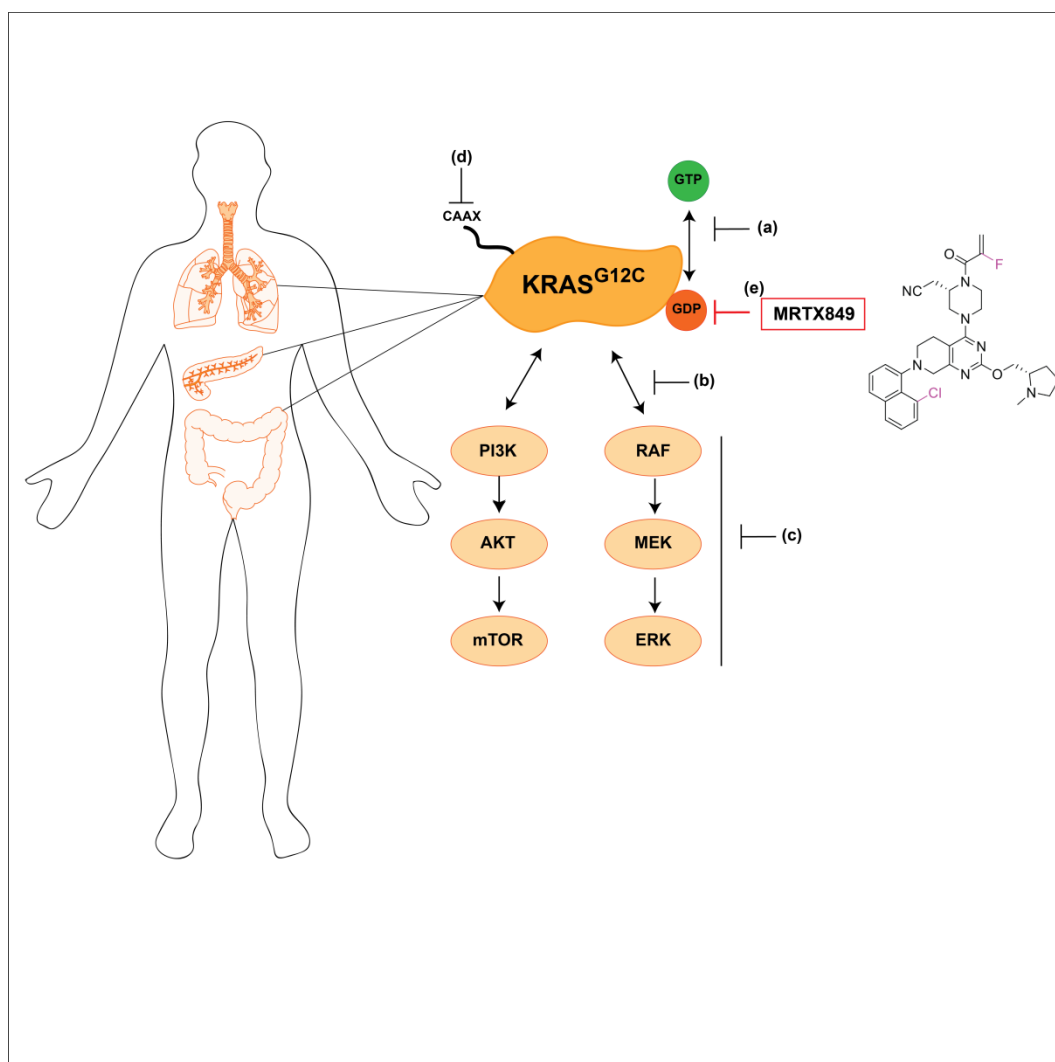
CW and MB have no conflicts of interest to report.

FIGURE LEGENDS

Fig. 1. Previous and the current RAS drug-targeting strategies. KRAS mutations are common in cancer of the pancreas, colon, and lung and have proven. Previous strategies to target oncogenic RAS were essentially unsuccessful. Those strategies included (a) efforts to reduce the levels of
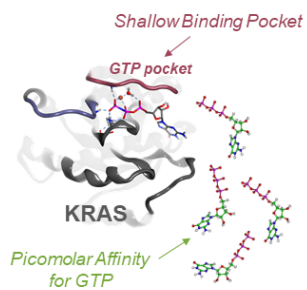
GTP-bound RAS; (b) target the binding of RAS to immediate downstream effectors (e.g., RAF); (c) targeting one or several enzymes of the canonical downstream signaling pathways (e.g., MEK, mTOR); and (d) reducing the ability of RAS to reach its site of action at the inner surface of the plasma membrane by inhibiting enzymes that modify the carboxyl-terminal CAAX-motif. In this review, we discuss the history of these RAS drug targeting efforts and how they led up to the discovery of MRTX849 (e)—a new drug that provides an exciting and long-awaited opportunity to selectively target mutant KRAS in patients. Left part of image made with Motifolio illustrations.

Fig 2. Covalent KRAS^{G12C} inhibition is a major breakthrough for a previously "undruggable" target. Left, the RAS GTPases switch between inactive GDP-bound and active GTP-bound states. Early ideas on how to target RAS focused on reducing levels of RAS-GTP using compounds that could preferentially bind to the RAS-GTP pocket. But this strategy was unsuccessful primarily because RAS has an extremely high affinity (picomolar) for GTP and the intracellular GTP concentrations are high (~500 μM), which makes it unlikely that any drug could compete for binding in the GTP pocket. Right, MRTX849 and other small molecules bind to KRAS^{G12C} mutants, open a previously unknown pocket called Switch II next to the reactive cysteine residue; and locks KRAS^{G12C} in its inactive GDP-bound state.
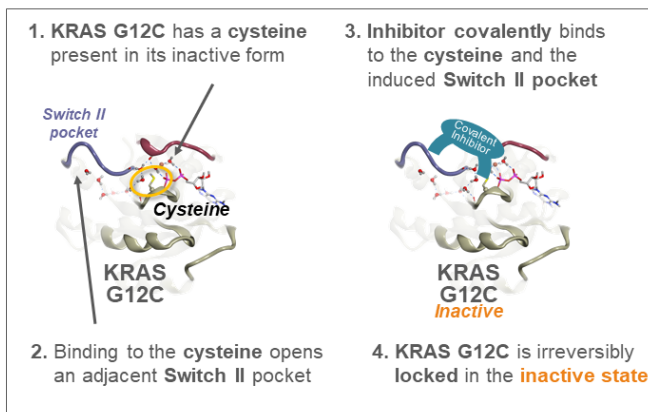
joim_13057_f1.png

joim_13057_f2.png