

# Targeting Kras<sup>G12C</sup>-mutant cancer with a mutation-specific inhibitor

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**Abstract.** Christensen JG, Olson P, Briere T, Wiel C, Bergo MO (Mirati Therapeutics, Inc., San Diego, CA, USA; and Karolinska Institutet, Huddinge, Sweden). Targeting KRAS<sup>G12C</sup>-mutant cancer with a mutation-specific inhibitor (Review). *J Intern Med.* 2020; **288**: 183–191.

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 signalling pathways. Most of these strategies have failed and no *KRAS*-specific drugs have yet been approved. However, for one specific

mutation, KRAS<sup>G12C</sup>, there is light on the horizon. MRTX849 was recently identified as a potent, selective and covalent KRAS<sup>G12C</sup> inhibitor that possesses favourable drug-like properties. MRTX849 selectively modifies the mutant cysteine residue in GDP-bound KRAS<sup>G12C</sup> and inhibits GTP-loading and downstream KRAS-dependent signalling. The drug inhibits the in vivo growth of multiple KRAS<sup>G12C</sup>-mutant cell line xenografts, causes tumour 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.

**Keywords:** KRAS, cancer, targeting, mutation-specific inhibitor, G12C mutation.

## 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 amongst 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<sup>G12C</sup> mutation which is a well-validated driver mutation and the most common individual *KRAS* mutation in lung cancer [5]. The KRAS<sup>G12C</sup> 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<sup>G12C</sup> mutation is causally linked to 14% of lung adenocarcinomas (~14 000 new US cases/year), 5% of colorectal adenocarcinomas

(~5000 new US cases/year) and smaller fractions of other cancer forms. All-in-all, KRAS<sup>G12C</sup> mutations comprise a patient population with a worldwide yearly incidence of over 100 000 individuals. The rationale for targeting the KRAS<sup>G12C</sup> 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 led 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<sup>G12C</sup> compared to the wild-type form; broad-spectrum antitumour activity in nonclinical models; predicted oral bioavailability in humans of ~50%; and ~20 h half-life and a 10-fold therapeutic index in nonclinical studies. On 29 October 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 nonclinical and clinical proof-of-concept data were 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 signalling through multiple intercellular pathways responsible for cell survival, growth, proliferation, migration and differentiation. Activation of the RAS proteins occurs at the inner surface of the cell membrane and results in the binding of key effector molecules, generation of signalling complexes, and initiation and propagation of intracellular signalling cascades including the RAF and PI3 kinase pathways. Because aberrant RAS function may result in enhanced or

sustained signalling 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 signalling complexes with the RAF family and PI3 kinase family proteins which in turn trigger the downstream signalling 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 cannot be turned off. This sustained RAS signalling 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 hydrolyse 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 amongst 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 nonsmall cell lung cancer (NSCLC) is KRAS<sup>G12C</sup> (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<sup>G12C</sup> 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 tumour [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 tumour 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 (Figures 1 and 2).

#### A historical perspective on RAS targeting efforts

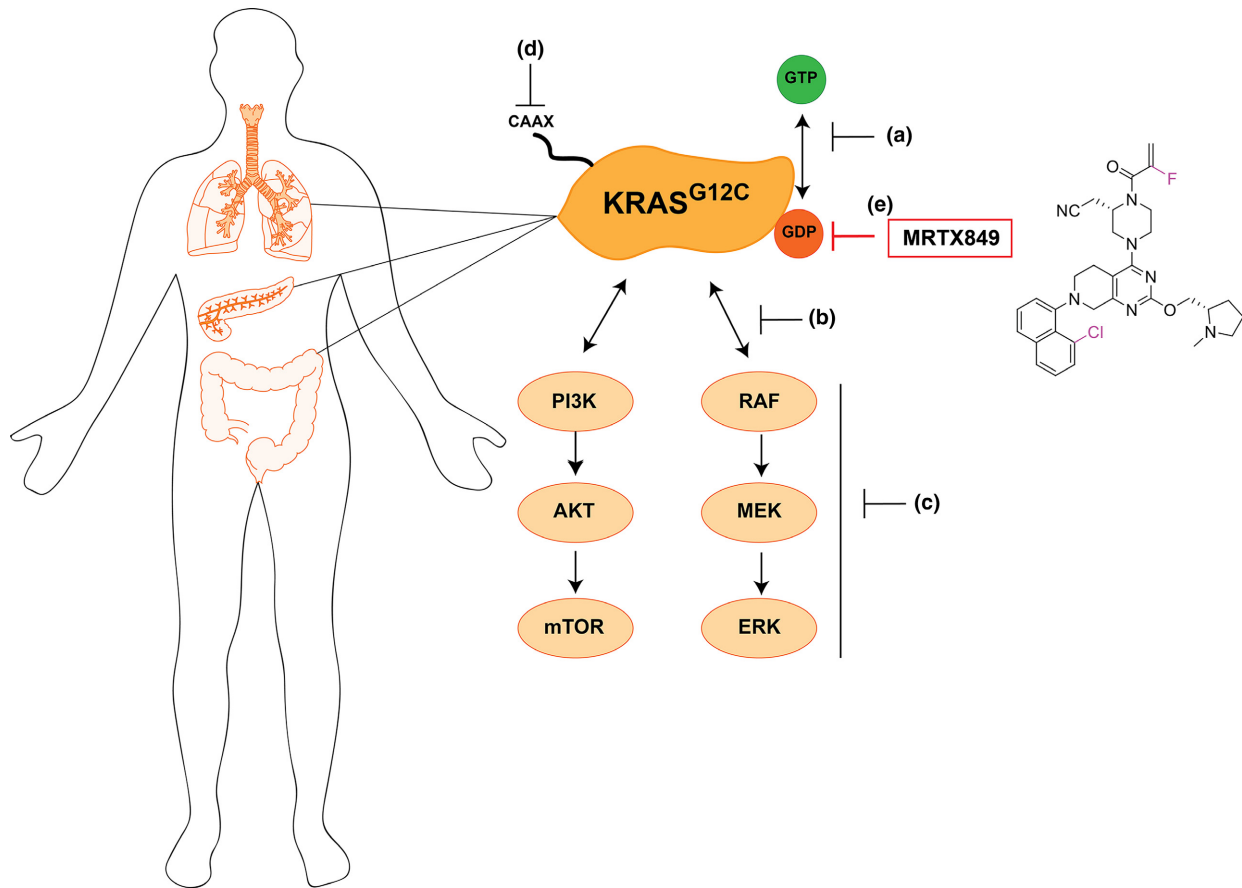
Therapeutic strategies that directly target oncogene products have produced substantial benefits for a variety of cancer patients, including patients

with NSCLC, breast cancer, melanoma and some types of leukaemia. 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 (i) lack of pockets large enough for small molecules to bind; (ii) high affinity for GTP which prevents direct targeting of the nucleotide-binding pocket; (iii) high intracellular GTP concentrations and fast nucleotide exchange cycles that hinders competition; and (iv) 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 signalling molecules including RAF, MEK, ERK and PI3K family proteins. These indirect targeting strategies have also faced severe challenges including (i) low therapeutic index which arises from the targeting of essential cell growth and survival pathways; (ii) compensatory escape mechanisms; and (iii) feedback regulation of signalling 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: (i) *reducing the proportion of active RAS-GTP (direct)*; (ii) *disrupting protein-protein interactions (direct)*; (iii) *reducing RAS plasma membrane targeting (indirect)*; (iv) *targeting downstream effector signalling*; and (v) *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



**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 signalling 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.

turned out to be the least clinically relevant; the most clinically relevant isoform is KRAS.

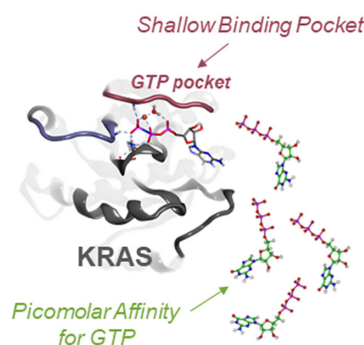
As outlined earlier, RAS GTPases exist in a GDP-bound 'off' state and a GTP-bound 'on' state. Due to its GTP-dependent activation, the earliest ideas on how to target mutant RAS focused on reducing the levels of active RAS-GTP. It was reasoned that if a small-molecule inhibitor could preferentially bind 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  $\mu$ M), 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.

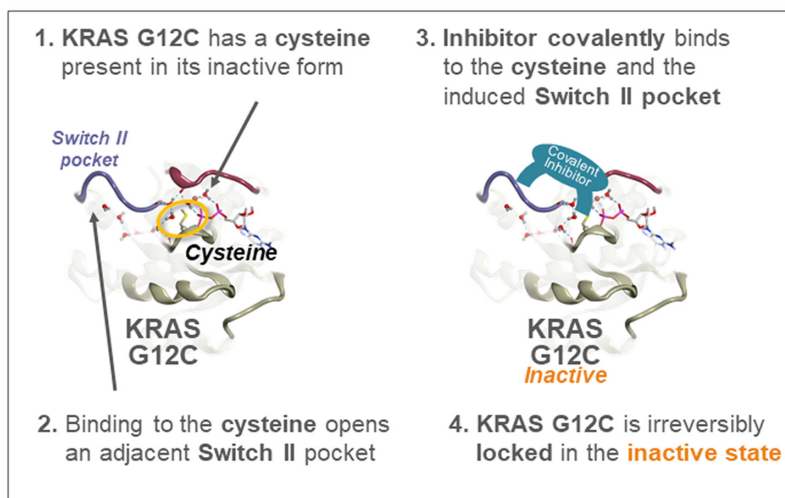


### Targeting KRAS

Historically “Undruggable”



### KRAS G12C Can Be Inhibited Covalently



KRAS tumor cell death

**Fig. 2** Covalent KRAS<sup>G12C</sup> 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  $\mu$ M), which makes it unlikely that any drug could compete for binding in the GTP pocket. Right, MRTX849 and other small molecules bind to KRAS<sup>G12C</sup> mutants, open a previously unknown pocket called Switch II next to the reactive cysteine residue; and locks KRAS<sup>G12C</sup> in its inactive GDP-bound state.

#### 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 signalling pathways. However, the development of clinically effective inhibitors that disrupt such interactions has been challenging, primarily because small molecules cannot 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 favour 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 signalling 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 signalling are 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 signalling 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 towards

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). Secondly, the last three amino acids (i.e. the -AAX) are cleaved off by RAS converting enzyme 1 (RCE1). Thirdly, 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 tumours). FTIs even caused regression of established tumours 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 catalysed 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 did not 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-tumour 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 tumour 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 towards targeting proteins that mediate signalling downstream of KRAS.

#### *Targeting downstream effector signalling*

RAS is a central hub for signalling 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 tumours, RAS mutations lead to hyperactivation of RAS-RAF-MEK-ERK signalling. The interest in targeting RAS-RAF-MEK-ERK signalling 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 MEK2 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 signalling 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 signalling 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 antitumour activity simply because the target signalling 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 amongst 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<sup>G12C</sup> inhibition*

For decades, the absence of defined pockets in the KRAS oncoprotein prevented the identification of effective inhibitors. Recently, however, software and computer modelling strategies have improved the prediction of affinities of small molecules for proteins harbouring a smooth surface topology without a pocket that facilitates drug binding. Virtual computer modelling 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<sup>G12C</sup>. 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<sup>G12C</sup> 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<sup>G12C</sup> 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<sup>G12C</sup> 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 EC50 values of ~5–10 nM, and selectivity of >1000× for KRAS<sup>G12C</sup> compared with wild-type KRAS as judged by cellular and whole-cell proteomic screening approaches. Moreover, MRTX849 has broad-spectrum antitumour activity (it showed >30% tumour reduction in 65% of 26 models tested) across a panel of KRAS<sup>G12C</sup>-positive patient- and cell-derived tumour models implanted in mice at well-tolerated dose levels, including complete tumour responses in some models. The drug showed deep responses in KRAS-mutant tumour models that exhibit co-occurring mutations including STK11, KEAP1 and TP53. Pharmacodynamic and pharmacogenomic analyses in sensitive and partially resistant nonclinical models led to the identification of mechanistic factors that underlie the drug's antitumour 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 tumour regression in multiple tumour models, including models that were refractory to MRTX849 single therapy [8]. Collectively, these results support the notion that KRAS-mutant tumours 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 antitumour activity. MRTX849 has a predicted oral bioavailability in humans of ~50%, a ~20-h half-life, and a therapeutic index of up to 10-fold in rodent and nonrodent repeat-administration toxicology studies. Consequently, MRTX849 exhibit significantly improved potency and antitumour 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 tumours are driven by the KRAS<sup>G12C</sup> mutation.

**Proof of principle in patients and conclusions**

Recently, MRTX849 was demonstrated to produce objective responses in patients with KRAS<sup>G12C</sup>-positive lung and colon adenocarcinoma, as judged by response evaluation criteria in solid tumours (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 tumour response evaluated using RECIST v1.1 criteria. As of the data cut-off date of 11 October 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 tumour 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 signalling. 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 enrolment 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.

**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.

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