DIRECT TARGETING OF KRAS MUTANT CANCERS
WITH A KRAS G12C MUTATION-SELECTIVE INHIBITOR

THE KRAS REVIVAL

KRAS is the single most frequently mutated oncogene and—as the first of more than 700 genes to be causally implicated in human cancer (COSMIC) [1]. Mutations in *KRAS* are prevalent amongst the top three most deadly cancer types in the United States: pancreatic (95%), colorectal (45%), and lung (35%) [2]. Its frequent mutation across a spectrum of aggressive cancers has stimulated an intensive drug discovery effort to develop therapeutic strategies that block KRAS function for cancer treatment. However, despite nearly four decades of research, a clinically viable KRAS cancer therapy has remained elusive. Nonetheless, recent research findings have stimulated a new wave of activities to develop KRAS targeted therapies.

Capping off an era marred by drug development failures and punctuated by waning interest in and presumed intractability of direct targeting of KRAS, new technologies and strategies now are aiding in the target's resurgence [3,4]. Central to this renewal is a single mutation: KRAS G12C, a well-validated driver mutation and the most frequent individual KRAS mutation in lung cancer [5]. Associated with poor prognosis and resistance to treatment, KRAS-G12C, a mutation that causes a switch from glycine to cysteine at position 12 in the protein, represents both an extraordinary unmet clinical need and opportunity. This mutation has a causal role in 14% of lung adenocarcinomas (~14,000 new US cases annually), 5% of colorectal adenocarcinomas (~ 5,000 new US cases annually) and smaller fractions of other cancers. Collectively, KRAS G12C mutations comprise a patient population with a worldwide annual incidence of greater than 100,000 individuals. The scientific basis for targeting the KRAS G12C mutant variant was originally described in a breakthrough article by Shokat and colleagues, in which they reported the identification of small molecules that irreversibly bound to the mutant reactive cysteine at codon/residue 12 and identified a previously unappreciated binding pocket near the KRAS effector region. Small molecules binding to this pocket can inhibit KRAS by locking the protein in its GDP-bound and inactive state [6,7]. Although irreversibly targeting this site with small molecules was a clear advancement, it was also evident that the molecules identified required significant optimization to achieve drug-like potency and pharmaceutical properties. To this end, Mirati established a Drug-Discovery Partnership with Array Biopharma in 2014. Through an intensive structure-based drug design effort, MRTX849 was identified as an IND track clinical candidate in 2017. MRTX849 demonstrates drug-like cellular potency of ~10 nM, selectivity of >1000X for KRAS G12C compared with KRAS wild-type, broad-spectrum antitumor activity in nonclinical models, predicted human oral bioavailability of >30% and half-life of ~20 hours, and up to a 10-fold therapeutic index in nonclinical studies. The Investigational New Drug (IND) application supporting MRTX849 clinical development was submitted to the U.S. Food and Drug Administration (FDA) on October 29, 2018 with first patient dosing to begin soon after FDA approval of the IND application. Early proof of concept data is expected in 2019.

WHAT IS RAS?

RAS proteins are part of the family of small GTPases and are activated in response to growth factor stimulation and various other extracellular stimuli [8]. The RAS family regulates intercellular signaling pathways responsible for growth, migration, survival and differentiation of cells. The activation of RAS proteins at the cell membrane by growth factors results in the binding of key effector molecules, formation of signaling complexes, and the

initiation of a cascade of intracellular signaling pathways within the cell, including the RAF and PI3 kinase pathways. Since defects in RAS may result in aberrant cellular signaling and malignant transformation, the activation of RAS proteins is tightly controlled in normal cells [8].

To initiate intracellular signaling, RAS proteins must be activated or turned "on" by extracellular stimuli, such as peptide growth factors. RAS proteins normally alternate between GTP- and GDP-bound conformations, where the GTP-bound conformation represents the "on" state and the GDP-bound the "off" state [8]. When RAS proteins are in the "on" GTP-bound state, two regions of the protein called Switch I and Switch II undergo marked structural changes, coalescing to form an interaction surface. This interaction surface is required for the formation of signaling complexes, including RAF family and PI3 kinase family proteins that initiate the cellular signaling cascades. The association of both GDP and GTP with RAS protein is extremely tight, with dissociation constants in the picomolar range; thus, RAS is almost always complexed with either GTP or GDP. In addition, RAS proteins have a slow dissociation rate for GDP and low intrinsic enzymatic GTPase activity. As a result, the conversion of inactive GDP-bound RAS to the active GTP-bound form requires stimulation of the nucleotide exchange rate by several orders of magnitude through interaction with guanine nucleotide exchange-factors (GEFs), while conversion back to the inactive form requires GTPase-activating proteins (GAPs). Dependence of RAS and other GTPases on GEFs and GAPs to switch them on and off allows both processes to be highly regulated and responsive to multiple signal inputs. In contrast, oncogenic mutants of RAS generally function by preventing hydrolysis of GTP, thereby generating highly active and GTP-bound RAS molecules with potentially severe consequences for the cell, including uncontrolled cellular growth and malignant transformation. The picomolar affinity of RAS for GTP, the presence of high levels of cellular GTP (> 500 ⊠M), and impaired ability of oncogenic RAS to hydrolyze GTP create a strong challenge for traditional drug discovery approaches, as highlighted below [4,7,9].

KRAS MUTANT CANCER

The RAS family is comprised of three members; KRAS, NRAS, and HRAS. RAS mutant cancers account for nearly 25% of all human cancers and one million deaths per year worldwide. Among the RAS family members, missense mutations most frequently occur in KRAS (85%), and less frequently in NRAS (12%) and HRAS (3%) [8]. The majority of RAS family mutations occur at amino acid residues/codons 12, 13, and 61; the frequency of mutation at each of these residues, and the isoform mutated, varies across different cancer types [8]. The vast majority of KRAS mutations occur at residue 12. The mutation of the glycine at residue 12 to anything other than proline produces a steric block that prevents GAP proteins from accessing KRAS, thereby inhibiting GTP hydrolysis and resulting in a dramatic increase in the GTP-bound form of KRAS [8].

In non-small cell lung cancer (NSCLC), KRAS G12C is the most common mutation, comprising nearly half of all KRAS mutations, followed by G12V (glycine to valine) and G12D (glycine to lysine) [5]. As noted above, the reactive cysteine inherent to the KRAS G12C mutant enables the irreversible targeting of this variant with small molecule inhibitors. Large genomics studies characterizing lung cancers have indicated that KRAS mutations, including G12C, are mutually exclusive with other known oncogenic driver mutations in NSCLC, including EGFR, ALK, ROS1, RET, and BRAF indicating that KRAS mutations define a unique segment of lung cancer without a current targeted therapy option [10,11]. KRAS mutant NSCLC is frequently co-incident with certain co-mutations such as STK11, KEAP1, and TP53 that cooperate with mutated RAS to transform cells into highly malignant and aggressive tumor cells [12,13]. Large scale functional genomics studies utilizing shRNA knockdown technology

to block the function of thousands of gene products across hundreds of cancer cell lines have demonstrated that cancer cells exhibiting KRAS mutations are highly dependent on KRAS function for cell growth and survival [12,13]. Together, these findings illustrate a critical role for KRAS mutations as causative factors in a significant number of human tumors and highlight a compelling opportunity for therapeutic agents targeting KRAS to make a profound impact in treating these cancers.

TARGETING RAS – HISTORICAL PERSPECTIVE

The development of therapeutic strategies directly targeting oncogenic genomic alterations has resulted in substantial benefits for patients with a variety of cancers, including NSCLC, melanoma, breast cancer, and certain types of leukemia. Current therapies in development include selective small molecule inhibitors of oncogenic drug targets such as EGFR mutations, BRAF mutations, HER2 gene amplification, and rearrangements involving the ALK or ROS1 genes. One of the most attractive oncogenic targets in drug discovery, mutant KRAS, is also one of the most challenging to inhibit. Historically, direct targeting of mutant KRAS has been met with challenges that include: 1) a lack of tractable pockets that are large enough to enable small molecule binding; 2) a high affinity for GTP that precludes direct targeting of the nucleotide-binding pocket; 3) high intracellular concentrations of GTP and a rapid nucleotide exchange cycle that stymies competition; and 4) possible toxicity resulting from indiscriminate inhibition of the wild-type target [4,7,9]. Indirect KRAS targeting strategies have also been evaluated and include blockade of KRAS cell membrane localization and targeting signaling molecules downstream of KRAS including RAF, MEK, ERK, and PI3K family members. These indirect targeting strategies have also been met with challenges--specifically, 1) a low therapeutic index associated with targeting essential pathways involved in human cell growth and survival, 2) compensatory escape mechanisms, and 3) signaling feedback and redundancy due to tight regulation of these cell-essential pathways [4]. Organized here are the primary categories of hurdles to KRAS inhibition strategies: **1) decreasing the proportion of active RAS-GTP (direct); 2) disrupting protein-protein interactions (direct); 3) decreasing the population of RAS at the plasma membrane (indirect); 4) targeting downstream effector signaling; and 5) synthetic lethality.**

DIRECT TARGETING: IN THEORY

Decreasing the Proportion of Active RAS-GTP   Early attempts at drugging RAS hinged on misconceptions, the most disadvantageous of which was the hypothesis that all RAS proteins function identically. The three RAS oncogenes (*HRAS*, *NRAS*, and *KRAS*) encode, among minor isoforms, four major RAS proteins (HRAS, NRAS, KRAS4A, and KRAS4B). Although the oncogenic potential of all four proteins had been recognized, disproportionate attention was paid to HRAS, the isoform to which an abundance of reagents was readily available (antibodies, expression vectors, cell cultures, and transgenic mice). An ostensible representative of its protein family, HRAS, it turned out, is the least clinically relevant of the RAS proteins; the most clinically relevant is KRAS.

As noted above, RAS GTPases exist in two functional states: the inactive, GDP-bound "off" state and the active, GTP-bound "on" state. Owing to its GTP-dependent activation, the earliest ideas about mutant RAS inhibition centered on decreasing the active RAS-GTP population. A small molecule antagonist preferentially bound to the RAS-GTP pocket, it was reasoned, would likely prove an effective inhibitor of mutant RAS. Unfortunately, this was not a tenable strategy as further biochemical analysis demonstrated that the high picomolar affinity of RAS for GTP, combined with high cellular concentrations of GTP (~500 ⊠M), make successful competition for the

nucleotide-binding pocket highly improbable [4,9]. In contrast, the binding affinity for association of the "druggable" protein kinase families with their natural co-substrate, ATP, are in the micromolar range (a ~million-fold difference in binding affinity between druggable kinases and RAS GTPases) and concentrations of ATP inside the cells range from 1-10 mM [14]. Furthermore, the nondescript surface of RAS, as recently visualized for the first time through crystallography, did little to restore confidence in the target; if anything, the relatively smooth surface, otherwise lacking in pronounced hydrophobic pockets into which a drug can securely bind, only supported notions of its intractability.

Disrupting Protein-Protein Interactions   With greater understanding of the extraordinary RAS-GTP binding kinetics, efforts turned away from the RAS nucleotide-binding pocket to other surface regions—namely, sites of effector protein-protein interaction. In an oncogenic state, disruption of mutant RAS-GTP-effector protein interactions could potentially attenuate hyperactivation of downstream pathways. Development of such a clinically effective inhibitor, however, has proven challenging, as small molecules cannot cover a large enough portion of the protein-protein interaction and are chemically too weak to sufficiently mask protein-protein interaction sites. Two separate groups have reported the discovery of small molecules that bind to RAS and disrupt its interaction with SOS, a GEF catalyst of nucleotide exchange [15,16]. Molecules that block GEF binding to mutant RAS, inhibit hyperactivation of the GTPase by shifting cycle equilibrium in favor of the inactive GDP-bound state. However, this class of molecules has not proven active enough to advance toward clinical development.

A second approach to block KRAS function through disrupting protein interaction involved the blockade of RAS-RAF family dimerization. This was predicted to be an effective strategy as one of the primary effectors mediating RAS function is the RAF family of serine-threonine kinases (ARAF, BRAF, and CRAF). Upon activation, RAS-GTP recruits RAF to the plasma membrane, whereby it promotes RAF dimerization, activating downstream signal transduction [8]. If, as with RAF isoforms, RAS-GTP dimerization or multimerization is required for binding with downstream effectors, disrupting this complex could prove an effective means of inhibiting mutant RAS hyperactivation [4,8]. However, this concept has not advanced past academic proof of principle [17].

INDIRECT TARGETING STRATEGIES
Decreasing the Population of RAS at the Plasma Membrane   In light of the growing perception that RAS could not be inhibited directly, efforts shifted toward the pursuit of indirect strategies, the most promising of which hinged on the requirement of plasma membrane localization for RAS family function. The C-terminus of the four major RAS isoforms contains a CAAX (cysteine-aliphatic-aliphatic-X) motif that signals its post-translational modification to a fatty membrane anchor through the farnesyl transferase enzyme. Farnesyl transferases, unlike RAS itself, can be readily targeted. The fatty anchor introduced to the RAS family protein C-terminal tail by farnesyl transferase tethers RAS to the membrane. If membrane localization is a requirement of RAS function, it was reasoned, a farnesyl transferase inhibitor (FTI) should attenuate oncogenic activity as a consequence of RAS displacement [3,4].

This strategy yielded many FTIs that demonstrated potent inhibition of HRAS-driven cancer cell growth in both *in vitro* and *in vivo* models (and in recent clinical trials selectively enrolling HRAS mutated tumors). In contrast, 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, KRAS membrane anchors are also introduced

by an alternative enzyme, geranylgeranyl transferase, resulting in intrinsic resistance of KRAS mutated cancers to farnesyl transferase inhibition. These failed clinical trials 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 From its central role as a cellular signaling hub, RAS regulates at least 11 downstream effector families, the most critical of which to RAS-driven cancers are the RAS-RAF-MEK-ERK and, to a lesser extent, RAS-PI3K-AKT-mTOR pathways. In a large percentage of tumors, RAS-RAF-MEK-ERK (ERK) signaling is hyperactivated in response to RAS mutations. Interest in targeting RAF-MEK-ERK (ERK) signaling also increased when BRAF mutations were identified as causative factors in melanoma and other of cancer types suggesting that blockade of this pathway could have utility independent of RAS mutant cancers [18]. Indeed, subsequent clinical development of BRAF inhibitors and BRAF and MEK inhibitor combinations resulted in the approval of multiple drugs and combination regimens in BRAF-mutated melanoma and has demonstrated promise in other BRAF-mutant cancers including NSCLC [19]. In contrast, the activity of both BRAF and MEK inhibitors in KRAS mutated cancers has been generally disappointing. Inhibition of MEK1 and -2, once considered a promising strategy in the treatment of KRAS mutant cancers, failed to demonstrate sufficient activity in clinical trials [20]. Failure of MEK inhibitors in clinical trials has been attributed to both the inability to achieve a therapeutic index that would enable robust pathway inhibition (owing to the importance of MEK enzymes in normal cells), as well as the compensatory pathway activation through which cancer cells escape inhibition. The blockade of BRAF in KRAS mutated cells is actually contraindicated due to the observation of paradoxical ERK signaling activation due to compensatory feedback activation of the closely related RAF1 enzyme in the setting of activated KRAS [19]. A new generation of RAF-MEK-ERK (ERK) pathway inhibition strategies have entered clinical trials more recently and include highly selective dual BRAF/RAF1 inhibitors and ERK1/2 inhibitors. These recent agents have demonstrated less susceptibility to compensatory pathway activation and have shown some evidence of clinical activity; however, it is still not clear that these agents can achieve an optimal level of target inhibition and single-agent antitumor activity due to the importance of these targets in normal cell types [19,21]. Thus, it is also likely that these classes of drugs will need to be combined to effectively treat KRAS mutant cancers, and it is still too early to determine if this approach will be effective.

Synthetic Lethality Synthetic lethal interaction partners of RAS are those genes whose products are essential for survival of RAS-mutant but not wild-type enzyme in normal cells. Large-scale screens designed to identify RAS synthetic lethal partners earned a great deal of attention in the last decade, the results of which offered up new potential targets for mutant RAS inhibition strategies. The most critical of these hits were irreproducible under varied conditions, issuing another blow to drug discovery efforts [3,4]. In the most recent applications of synthetic lethality, two independent large-scale deep RNAi screens, Project DRIVE and Project Achilles, have improved upon the strategies, reagents, and computational approaches of past screens and enhanced the ability to map cancer gene dependencies. In the Project DRIVE screen, a high-density shRNA library was used to knock down 7,837 genes across 398 cell lines to assess and classify genetic, expression, metabolic, and synthetic lethal relationships amongst cancer genes [12,13]. Alongside mutated NRAS and BRAF, data from Project DRIVE confirmed that cells exhibiting mutated KRAS were highly dependent on KRAS for their growth and viability. However, the screen failed to identify any independent synthetic lethal genes that interact with KRAS and that could represent novel drug targets modifiers of KRAS function, illustrating the likely requirement for direct inhibition of KRAS to block its oncogenic properties.

DIRECT TARGETING: IN ACTION

Covalent Irreversible KRAS-G12C Inhibition   The lack of defined, tractable pockets stood in the way of KRAS mutant inhibitor identification for decades. In recent years, however, improvements in computer modeling software helped enable better prediction of small molecule affinities for proteins with relatively smooth surface topology without a pocket to allow drug binding. Virtual modeling allowed for the design of the fragment-based libraries screens through which the allosteric Switch II Pocket (S-IIP) region was discovered [6]. It is also notable that the S-IIP is proximal to the commonly mutated glycine at residue 12. Based on these observations, Shokat and colleagues developed a screening approach against inactive GDP-bound KRAS-G12C via evaluation of a library of small molecule electrophilic fragments intended to exploit the reactive cysteine inherent to the G12C mutation. The small molecules identified induced significant structural modifications. Crystallography of KRAS-G12C bound to a fragment hit revealed the nature of a previously undetected S-IIP binding pocket adjacent to the reactive cysteine and the observation that the compound formed key hydrophobic contacts and direct hydrogen bonds to ensure its orientation in the S-II pocket [6]. Optimized compounds were subsequently developed based on the initial fragment hits that also form covalent bonds with the KRAS-G12C reactive cysteine, lock KRAS in its GDP-bound inactive form, and exhibit improved potency [6,22-24]. However, to date, there are no reports of these molecules having advanced to IND-track development.

IND-Track Mutant-Selective KRAS Inhibitor MRTX849   MRTX849 was identified through intensive structure-based drug design effort involving more than 150 unique co-crystal structures along with synthesis and evaluation of ~2000 discrete small molecules. The clinical candidate, MRTX849, is a potent and orally available small molecule inhibitor of KRAS G12C and is advancing to an IND application in Q4, 2018. MRTX849 demonstrates drug-like potency with EC_{50} values of ~10 nM in blocking KRAS-dependent signal transduction and cancer cell viability, as well as selectivity of >1000X for KRAS G12C compared with KRAS wild-type as determined by cellular and whole-cell proteomic screening approaches. In addition, MRTX849 demonstrates broad-spectrum antitumor activity 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 a subset of models. Deep responses were also observed in KRAS mutant tumor models that exhibited co-mutations including STK11, KEAP1, and TP53. MRTX849 exhibited predicted human oral bioavailability of >30% and a half-life of ~20 hours and a therapeutic index of up to 10-fold in rodent and nonrodent repeat-administration toxicology studies. Thus, MRTX-849 appears to exhibit significantly improved potency and a higher degree of antitumor activity than reported previously for KRAS mutant-selective inhibitors. MRTX849 entered the clinic in January 2019 thus providing a long-awaited targeted therapy option for patients exhibiting a KRAS G12C driver mutation.

Glossary of terms
- KRAS – Kirsten Rat Sarcoma 2 Viral Oncogene Homolog.
- COSMIC – Catalogue of Somatic Mutations in Cancer.
- KRAS G12C – refers to the mutated version of the protein in which a DNA mutation results in a change in the amino acid glycine (G) at codon 12 to a cysteine (C). This mutation dramatically reduces the conversion of GTP-bound KRAS (active) to GDP-bound KRAS (inactive) and leads to hyperactivated signaling.
- IND – Investigational New Drug.
- GTPase – family of hydrolase enzymes that hydrolyze guanine triphosphate (GTP) and regulate signal transduction pathways based on binding to GTP or GDP.

- GTP – guanine triphosphate; binding to KRAS leads to a conformational change that activates signaling.
- GDP – guanine diphosphate; hydrolysis of GTP to GDP in KRAS leads to a conformational change that inactivates signaling.
- RAF – proto-oncogene serine/threonine-protein kinase downstream of KRAS.
- PI3 kinase – phosphoinositide 3-kinase downstream of KRAS.
- GEF – guanine nucleotide exchange factor – stimulates the exchange of GDP for GTP in RAS proteins leading to RAS activation.
- GAP – GTPase activating protein – stimulates the low intrinsic GTPase activity of RAS proteins leading to RAS inactivation.
- HRAS – Harvey Rat Sarcoma Viral Oncogene Homolog.
- NRAS – Neuroblastoma RAS Viral Oncogene Homolog.
- NSCLC – non-small cell lung cancer.
- EGFR, ALK, ROS1, RET, and BRAF – Epidermal Growth Factor Receptor, Anaplastic Lymphoma Kinase, ROS Proto-Oncogene 1, Rearranged during Transfection, and B-RAF Proto-Oncogene, Serine/Threonine Kinase are oncogenic drivers in NSCLC and other cancers that are hyperactivated via mutation or chromosomal rearrangement. Targeted therapies have been or are being developed to treat patients with cancers harboring these alterations.
- STK11, KEAP1, TP53 – Serine/Threonine Kinase 11, Kelch Like ECH Associated Protein 1, and Tumor Protein 53 are tumor suppressors commonly mutated in NSCLC. When co-mutated with KRAS, these mutations further enhance the malignant phenotype of KRAS mutant cancer.
- shRNA – short hairpin RNAs that can inhibit the protein expression of target genes. Genome-wide shRNA libraries have enabled the systematic interrogation of the role of most genes in the genome across panels of cell line cancer models revealing genetic dependencies of cancers including KRAS dependency in KRAS mutant cancers.
- RAF, MEK, ERK, and PI3K – RAF Kinase, Mitogen-activated Kinase, Extracellular Signal-regulated Kinase, Phosphoinositide 3-Kinase – Kinase enzymes downstream of RAS that transduce the activated signal from RAS to downstream effector proteins.
- KRAS4A / KRAS4B – Two KRAS protein products that result from alternative splicing of exon 4. These proteins have different amino acid sequences at their C-termini and can use different mechanisms to associate KRAS with the plasma membrane.
- SOS – Son of sevenless is a GEF that activates RAS.
- FTI – Farnesyl transferase inhibitor – targets the enzyme responsible for adding a lipid moiety to the C-terminus of KRAS, which was hypothesized to be required for association of KRAS with the plasma membrane and KRAS function.
- RAS-RAF-MEK-ERK – major serine/threonine kinase pathway that is hyperactivated in KRAS mutant cancers and responsible for increased cell proliferation, survival and motility.
- RAS-PI3K-AKT-mTOR – major kinase pathway that is hyperactivated in KRAS mutant cancers and responsible for increased proliferation, survival and protein translation.
- Covalent, irreversible inhibitor – class of drugs that forms a covalent bond with the target protein that does not break, thereby irreversibly inhibiting its target.
- SII-P – Switch II pocket – "pocket" in KRAS first identified by the Shokat team through structural studies. When occupied by KRAS G12C covalent inhibitors the protein is locked in the inactive state.
- EC_{50} – concentration of drug that inhibits half of the maximal activity.

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