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KRAS G12C Game of Thrones, which direct KRAS inhibitor will claim the iron throne?

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Abstract

Mutations in Kirsten rat sarcoma viral oncogene homolog (KRAS) are among the most common aberrations in cancer, including non-small cell lung cancer (NSCLC). The lack of an ideal small molecule binding pocket in KRAS protein and its high affinity towards the abundance of cellular guanosine triphosphate (GTP) renders the design of specific small molecule drugs challenging. Despite efforts, KRAS remains a challenging therapeutic target.

Among the different known mutations; the $KRAS^{G12C}$ (glycine 12 to cysteine) mutation has been considered potentially druggable. Several novel covalent direct inhibitors targeting $KRAS^{G12C}$ with similar covalent binding mechanisms are now in clinical trials. Both AMG 510 from Amgen and MRTX849 from Mirati Therapeutics covalently binds to $KRAS^{G12C}$ at the cysteine at residue 12, keeping $KRAS^{G12C}$ in its inactive GDP-bound state and inhibiting KRAS-dependent signaling. Both inhibitors are being studied as single agent or as combination with immunotherapy phase 2 trials. In addition, two novel KRAS G12C inhibitors JNJ-74699157 and LY3499446 will have entered phase 1 studies by the end of 2019.

Given the rapid clinical development of 4 direct covalent KRAS G12C inhibitors within a short period of time, understanding the similarities and differences among these will be important to determine the best treatment option based on tumor specific response (NSCLC versus colorectal carcinoma), potential resistance mechanisms (i.e. anticipated acquired mutation at the cysteine 12 residue) and central nervous system (CNS) activity. Additionally, further investigation evaluating the efficacy and safety of combination therapies with agents such as immune checkpoint inhibitors will be important next steps.

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Keywords

Kirsten rat sarcoma viral oncogene homolog; non-small cell lung cancer; targeted therapy; AMG 510; MRTX 849; ARS 3248

Introduction

Mutations in Kirsten rat sarcoma viral oncogene homolog (KRAS) are among the most common aberrations in cancer. Approximately 30% of lung adenocarcinomas are known to harbor various KRAS mutations. While those patients harboring actionable mutations such as EGFR or ALK have multiple tyrosine kinase inhibitors as options of treatment, until recently, patients with KRAS mutant NSCLC had lacked specific inhibitors and tend to exhibit poor prognosis [1].

$KRAS^{G12C}$ (glycine 12 to cysteine) mutation has been identified as an oncogenic driver of tumorigenesis and is found in approximately 13% of lung cancer [2] and 3% of colorectal cancers [3]. $KRAS^{G12D}$ is the most common mutation in pancreatic (2/3 of KRAS mutations) and colorectal (almost 50% of KRAS mutations) [4]. KRAS is a GTP-binding protein that links receptor tyrosine kinase activation to intracellular signaling [5, 6]. KRAS mutation favors the GTP-bound active state and activates its downstream effects such as differentiation, proliferation and survival [7].

The lack of an ideal small molecule binding pocket in KRAS protein and its high affinity towards the abundance of guanosine triphosphate (GTP) renders the design of specific competitive small molecule drugs challenging. Despite efforts, KRAS remains a challenging therapeutic target. In recent years, there has been a drive to develop mutation specific approaches and several novel classes of compounds against individual KRAS alterations have emerged. Among the different known mutations; the $KRAS^{G12C}$ (glycine 12 to cysteine) mutation has been considered potentially Druggable; not by competing with GTP for binding to KRAS; however, by binding to a pocket nearby the nucleotide binding site and locking it in an inactive GDP-bound state.

Molecular structure

K-Ras (Kirsten Rat Sarcoma Viral Proto-Oncogene; KRAS) protein is a GTP/GDP-binding protein and belongs to a small GTPase family, Ras family. Crystal structure analysis of KRAS protein shows that KRAS protein consists of six beta strands and five alpha helices, which form two major domains: a G domain and a C terminal membrane targeting region [8, 9]. G domain binds to guanosine nucleotides while C terminal is lipid modified to attach to membrane. The size of KRAS protein is about 20 kDa. KRAS protein functions as a molecular switch to turn on or off the signal transduction in the receptor tyrosine kinase signaling and related pathways [8, 9]. The major molecules involved in KRAS signaling include EGFR, Raf, MEK, MAPK (Erk), PI3K and Akt, all of them are known to be activated in cancers. KRAS can be active by GTP-binding or inactive by GDP-binding. When GTP Binds to KRAS, the conformation of KRAS changes, which promotes the interaction of KRAS with its effectors such as Raf, PI3K and Ral-GDS, leading to cell

proliferation and survival. In contrast, when GDP binds to KRAS, KRAS protein is inactivated by GAPs (GTPase activating proteins), which increase the GTPase activity of KRAS protein, reducing cell growth. In this way, KRAS plays an important role in the control of cellular signaling transduction and regulation of cell proliferation.

In 1982, several groups of investigators reported a single missense mutation found in codon 12 of RAS gene in bladder cancer cell line [10–12]. Afterward, the mutations of RAS gene including KRAS, HRAS and NRAS have been found in various types of cancers including lung, colorectal, pancreatic and other cancers. Among them, KRAS mutation is the most frequent mutation [5]. KRAS mutation favors GTP-binding and the mutated KRAS becomes activated, leading to the increased downstream effects such as cell proliferation and survival [7].

RAS mutation in various human malignancies

Most KRAS missense mutations occur in codon 12, leading to the amino acid changes from glycine to other amino acid. $KRAS^{G12D}$ (glycine 12 to aspartic acid) and $KRAS^{G12V}$ (glycine 12 to valine) mutations have been found in 90% of pancreatic cancers [13]. However, the $KRAS^{G12D}$ and $KRAS^{G12V}$ are so far undruggable because the substituted amid acids by mutation are currently chemically intractable [14].

More than 40% of cases of human colon cancer are reported to have KRAS mutations at codons 12, 13 and 61 is considered pathogenic. $KRAS^{G12D}$ (glycine 12 to aspartic acid) is the most common KRAS mutation in colorectal cancer and can be identified in both early stage and late stage. KRAS mutation in colorectal cancer is considered to be associated with a strong correlation to poor prognosis [15].

Another major mutation $KRAS^{G12C}$ (glycine 12 to cysteine) has also been identified as an oncogenic driver of tumorigenesis and is found in approximately 13% of lung cancer [2] and 3% of colorectal cancers [3]. Adenocarcinoma, the most common type of non-small cell lung cancer carries the KRAS mutation with a frequency of 20–50% [16–17] and is considered the most commonly detected oncogenic driver detected in lung cancer patients of non-Asian origin [18]. Recently, the $KRAS^{G12C}$ mutation has been considered potentially druggable due to the presence of substituted cysteine for drug binding [14]. However, there are currently no FDA approved drugs targeting the $KRAS^{G12C}$ mutation.

Biochemical and biophysical properties

Hunter et al., profiled the biochemical and biophysical properties of commonly occurring mutant forms of KRAS (G12A, G12C, G12D, G12R, G12V, G13D, Q61L, and Q61H), including the intrinsic and GAP-stimulated GTP hydrolysis rates, GTP and GDP-binding kinetics measurements, relative affinities for RAF kinase, and high resolution crystal structures [19]. The different mutant forms of KRAS were classified into the following broad categories: those with a high (WT, G12C, G12D, G13D) and low (G12A, G12R, G12V, Q61L, and Q61H) level of intrinsic GTPase activity. The mutants are further divided into as high RAF affinity (WT, G12A, G12C, G13D, and Q61L) or low RAF affinity (G12R, G12V, and G12D) based on their relative affinity for RAF kinase Ras-binding domain

(RBD) [19]. Combining these criteria, Hunter et al., proposed a prediction model of the relative dependence on or activation of the RAF kinase pathway compared with other pathways such as PI3K in tumors harboring specific KRAS mutations. For example, G12A- and Q61L-bearing tumors preferentially signal through the RAF kinase pathway due to their high affinity for RAF kinase and relatively lower rates of intrinsic hydrolysis. In contrast, G12D with its low affinity for RAF and faster hydrolysis rate would be predicted to show the lowest levels of RAF activation. The model further predicts that G12V and G12R would show moderate activation of RAF kinase due to their slow intrinsic hydrolysis rate coupled with a low RAF affinity. Likewise, G12C and G13D would be predicted to moderately activate RAF kinase due to their high affinity, but because they have a more rapid intrinsic GTPase activity, the duration of the activation is likely attenuated compared with G12A and Q61 [19].

Why targeting KRAS has been difficult

Direct targeting KRAS has remained challenging. Due to the specific features of KRAS molecular structure, KRAS protein has shown its high resistance to small molecule modulation. In the design of small molecule inhibitor therapy, the most appreciated approach to target a protein is to identify pockets in its structure where a small molecule inhibitor can bind to. However, KRAS protein is a small protein with relatively smooth surface. Besides the GTP/GDP-binding pocket, KRAS protein does not have other suitable pockets for small molecule inhibitor binding. In addition, under the physiological condition *in vivo*, GTP almost exclusively occupies the pocket with extremely high affinity that falls in the picomolar range [14]. This makes the development of a competing small molecule inhibitor an almost improbable task, as the chances of such inhibitor achieving adequate blood concentration enough to displace GTP would be exceedingly low. Moreover, the interactions of KRAS with other proteins makes surface of KRAS protein shallow, interfering small molecule inhibitor binding. Therefore, direct targeting KRAS by small molecule inhibitor is a difficult approach.

Similarly, indirect targeting the molecules within the KRAS signaling pathway (upstream or downstream of KRAS) to regulate KRAS signaling has also been known to be not very effective clinically. KRAS signaling pathway is a complex and highly interconnected signaling network. Although the mechanisms underlying the molecular regulation of KRAS signaling have been widely investigated, more work is needed to fully understand the complexity of KRAS signaling. Moreover, many positive and negative regulatory feedback loops intertwine in the highly interconnected KRAS signaling network. These features of KRAS signaling also make the indirect targeting KRAS by disrupting KRAS upstream regulators and downstream effectors ineffective. Furthermore, the mutant KRAS proteins could bypass the specific molecules that are inhibited by indirect KRAS inhibitors, leading to the low or no inhibitory effects of the drugs on KRAS signaling [20]. These issues have rendered KRAS as undruggable [21].

Various attempts to target KRAS

Once KRASG12D was deemed undruggable, researchers shifted their attention towards targets upstream or downstream of KRAS (Table 1). One of the attempts was made to target KRAS membrane anchoring which is necessary for the protein to exert its functions [22]. Membrane anchoring of KRAS is dependent on its farnesylation that is facilitated by the enzymes farnesyl transferase [23]. The farnesyl transferase promotes the posttranslational modification of both normal and mutated RAS, thus facilitating its anchoring to the cell membrane and activating various cell proliferation pathways. Several farnesyl transferase inhibitors (FTIs) were developed and they showed remarkable activity in pre-clinical models [24–26]. These FTIs combined with other inhibitors exerted potent anti-cancer activities in KRAS driven tumors. However, these FTIs were not translated in the clinic.

Similarly, RAS Converting CAAX Endopeptidase 1 (Rce1) and Isoprenylcysteine Carboxyl Methyltransferase (ICMT) are two CAAX-signaled RAS processing enzymes [27, 28] and the inhibition of these enzymes could disrupt RAS membrane localization, thus inhibiting RAS-driven tumorigenesis. Several inhibitors of Rce1 and ICMT have been designed and synthesized for the suppression of RAS-driven tumors [29–32]. It has been reported that these inhibitors suppressed the enzyme activities of Rce1 or ICMT, induced mislocalization of EGFP-RAS from the plasma membrane, caused cell cycle arrest and induced apoptosis *in vitro* [29–32]. However, Rce1 and ICMT are also required for the function of other proteins and the inhibition of Rce1 or ICMT could impact the normal function of other proteins, raising the questions about normal tissue toxicity of the inhibitors [33]. Moreover, it was found that loss of these enzymes could be concurrent with KRASG12D activation, causing enhanced cell proliferation and increased PanIN formation [34, 35]. Therefore, the inhibitors of Rce1 or ICMT are not good candidates of drug for the treatment of KRAS-driven tumors *in vivo*.

In addition, targeting RAS GTP/GDP cycle is another direction for the treatment of RAS-driven tumors. RAS GTP/GDP cycle is positively regulated by guanine nucleotide exchange factors (GEFs) which promote the binding of GTP and activate RAS. The known most prominent RasGEF is Sos1; therefore, the attempts have been made to design and synthesize Sos1 inhibitors to block Ras-Sos1 interaction [36–39]. It was found that these inhibitors suppressed Sos-mediated nucleotide exchange by blocking the interaction between RAS and Sos, inhibiting RAS activation [38, 39]. By inhibiting formation of the KRAS-Sos1 complex, these inhibitors blocked reloading of KRAS with GTP, leading to the inhibition of cell proliferation [36] and downregulation of RAS signaling in response to receptor tyrosine kinase activation [37]. However, the binding activity of these inhibitors to RAS is weak. In addition, it is unknown whether the Sos1 inhibitors have similar effects on the KRAS mutational setting. Therefore. These inhibitors have not translated into clinical use.

In addition to the inhibitors targeting RAS protein interaction and membrane localization, other inhibitors targeting KRAS downstream effectors have also been synthesized and used for the inhibition of KRAS induced signaling. Since no specific Inhibitor for mutant RAS was developed, targeting RAS effectors could be a useful therapeutic strategy. The inhibitors of RAF-MEK-ERK and Akt-mTOR signaling have been tested in RAS-driven tumors *in*

vitro. Both RAF-MEK-ERK and PI3K-AKT-mTOR pathways are important intracellular signal transduction cascades which are also activated by RAS signal and the activation of these signaling promotes cell proliferation, survival, mobility and invasion [40–42]. It has been reported that Akt inhibitor significantly suppressed RAS-induced Akt signaling whereas ERK inhibitor downregulated RAS-mediated RAF-MEK-ERK signaling *in vitro* [43–47]. However, clinical benefits are limited, which could be because of the significant crosstalk between these important pathways and the drug resistance [48, 49].

Strategies to directly target RAS

The strategies to target KRAS could be designed in different directions. One strategy is to prevent formation of Ras–GTP complex so that KRAS cannot be activated. In earlier investigation, competing GTP analogs had been synthesized [50]. These analogs could directly compete with nucleotide binding to RAS. It has been reported that the GTP analogs with alternations at the ribose or nucleotide moiety had moderately higher affinity with RAS compared to GDP [50]. However, the actual inhibition of KRAS activation by these GTP competitors was found to be low. The reasons for the low inhibitory effects of GTP analogs on KRAS activation are high affinity of GTP with KRAS protein, high cellular GTP concentrations *in vivo* and low specificity of GTP competitors for binding to KRAS protein [51]. Therefore, using GTP competitors to inhibit GTP binding to KRAS protein has been believed as an unlikely strategy to inhibit the activity of KRAS for therapeutic purposes.

Another strategy to prevent Ras-GTP complex formation is to inhibit the interaction of KRAS with guanine nucleotide exchange factors (GEFs). In the event of GTP binding to KRAS, nucleotide exchange occurs, and it requires the interaction of KRAS with GEFs. Therefore, inhibition of interaction between KRAS and GEFs could interfere the formation of KRAS-GTP complex, leading to the inhibition of KRAS activation. Several studies have screened a number of small molecule inhibitors for inhibition of RAS-GEF interaction [39, 52]. It has been found that these small molecule inhibitors could bind to a unique ligand-binding pocket on the RAS protein or RAS-GEFs-RAS complex to inhibit the interaction of RAS with GEFs, causing the inhibition of RAS activation [39, 52]. However, the inhibitors of RAS-GEF interaction exert their effects on both wild-type and mutant RAS. This feature of the inhibitors make some limitations for the inhibitors to be used in clinic because the RAS-GEF inhibitor would be quite toxic to normal cells with wild type KRAS.

One more strategy targeting KRAS is to change the correct localization of KRAS so that the oncogenic signal transduction can be prevented or inhibited. During the activation of KRAS signaling, the intracellular localization of KRAS protein should be on the inner side of cellular plasma membrane to which the lipid residues at the C terminus of KRAS attach. It has been found that prenyl-binding protein PDEδ maintains the correct intracellular localization of KRAS. Downregulation of PDEδ gene suppressed transduction of KRAS signal and activation of Erk which is a KRAS downstream effector [53]. Several inhibitors of KRAS–PDEδ interaction were developed to target KRAS activation [54]. It was found that one of the inhibitors, deltarasin, significantly inhibited the interaction between KRAS and PDEδ, relocating KRAS to endomembranes at a nanomolar concentration. By relocating KRAS, deltarasin inhibited activation of Erk and suppressed proliferation of KRAS–

transformed pancreatic cancer cells *in vitro* and *in vivo*. However, much higher concentration (micromolar range) of deltarasin was needed for inhibition of Erk activation and cancer cell proliferation. In addition, similar as RASGEF inhibitors, the RAS-PDEδ inhibitors also exert inhibitory effect on both wild-type and mutant KRAS. Therefore, the RAS-PDEδ inhibitor would be also quite toxic to normal cells.

In order to develop specific inhibitors for mutant $KRAS^{G12C}$ cells, several GDP-derived inhibitors have been synthesized to covalently lock GDP-bound state to keep the $KRAS^{G12C}$ inactivated [55, 56].The thiol function of substituted cysteine 12 caused by mutation is used to covalently trap the inhibitors, keeping the mutant KRAS in inactivated state. These GDP-derived inhibitors can covalently bind to $KRAS^{G12C}$ in the presence of very high concentration of GTP, even in millimolar range, locking the KRAS-GDP state and inhibiting proliferative activity of the KRAS mutant cells. First generation of the inhibitor (SML-8-73-1) has low cell permeability whereas the second generation of the inhibitor (SML-10-70-1) has shown increased stability, significantly improved cell membrane permeability and partially inhibited activation of ERK and AKT which are downstream effectors of KRAS [55, 57]. Experiments have shown that these inhibitors had effects on $KRAS^{G12C}$ and no effect on wild-type KRAS. Studies also showed that these inhibitors increased the accumulation of GDP-bound KRAS and decreased GTP-bound KRAS, leading to KRAS mutant cell apoptotic death. However, these inhibitors also exerted their effects on $KRAS^{G12S}$ cells [55]. Therefore, the specificity of these inhibitors is somewhat low and may have off-target effects when used in clinic.

To develop more promising inhibitors targeting specific KRAS as mutants, investigators have found new approach to design and synthesize new inhibitors with high specificity for specific KRAS mutants. Further modification of the inhibitors with altered electrophilic groups has been utilized to create new derivatives such as vinyl sulphonamide analogues and acrylamide analogues [56]. These analogs do not compete with GTP for binding to KRAS; however, they can bind to a pocket nearby the nucleotide binding pocket [56]. Binding of these compounds to this pocket makes KRAS more preferential accept of GDP binding than GTP. Importantly, these compounds only bind to $KRAS^{G12C}$ and have no inhibitory effects on wild-type KRAS and other types of mutant KRAS such as $KRAS^{G12S}$. Moreover, another similar compound named ARS853 showed more potent inhibitory effects on $KRAS^{K12C}$ cells than acrylamide analogues [58, 59]. ARS853 also specifically binds to $KRAS^{G12C}$, locking the KRAS in the GDP-bound state. Because additional signals such as EGFR and GEFs are required to activate $KRAS^{G12C}$, combination treatment of $KRAS^{G12C}$ cells with ARS853 and EGFR inhibitors significantly suppressed the proliferation of $KRAS^{G12C}$ cancer cells [58, 59]. These results demonstrate that this class of inhibitors exerts their effects through the existence of cysteine obtained from $KRAS^{G12C}$ mutation, suggesting their specificity of inhibitory effects on $KRAS^{G12C}$ without off-target effects [60]. Therefore, they could be more promising therapeutic agents used in clinic for the treatment of $KRAS^{G12C}$ mutant cancers.

Clinical studies of novel direct covalent KRAS G12C inhibitors

Recently, several novel inhibitors targeting $KRAS^{G12C}$ with similar covalent binding mechanisms have been developed and tested in clinical trials. AMG 510 from Amgen covalently binds to the cysteine amino acid of $KRAS^{G12C}$ mutant proteins, locking $KRAS^{G12C}$ in an inactive state [61–63]. Similarly, MRTX849 produced by Mirati Therapeutics also covalently binds to $KRAS^{G12C}$ at the cysteine at residue 12, keeping $KRAS^{G12C}$ in its inactive GDP-bound state and inhibiting KRAS-dependent signaling [64]. Both inhibitors have been used in early phase clinical trials. In addition, Wellspring Biosciences and Janssen recently received an investigational new drug (IND) approval for their $KRAS^{G12C}$ inhibitor ARS-3248, which is a significantly improved new version of the $KRAS^{G12C}$ inhibitor ARS-1620. Although ARS-1620 was one of the first compounds to be validated for its ability to directly inhibit $KRAS^{G12C}$, the challenge was its suboptimal potency owing to the small volume of the pocket being occupied by ARS-1620 [65]. With new development of novel small molecule inhibitors using novel molecular and chemical techniques, the mutant KRAS could finally become druggable.

AMG 510

AMG 510 is a novel small molecule that specifically and irreversibly inhibits $KRAS^{G12C}$ by locking it in an inactive guanosine diphosphate (GDP)-bound state. This covalent inhibitor slowly switches the concentration of KRAS to KRAS-GDP with a half-life of 30 minutes (as compared to seconds with KRAS-GTP form).

Researchers of Amgen, in collaboration with Carmot Therapeutics, screened for potential inhibitors to $KRAS^{G12C}$ and found many molecules that bound within the pocket in different ways. For some of these, crystallographic data showed that a histidine residue could flip up to reveal a hidden groove. The key breakthrough that led to AMG 510 was the discovery that this surface groove, created by an alternative orientation of His95, could be occupied by aromatic rings, which enhanced interactions with the of $KRAS^{G12C}$ protein [66]. As the researchers further explored the mutant-binding compounds, they found that the best performers were also able to wiggle into this pocket by flipping out the histidine and led to the development of AMG 510. Although AMG 510 and ARS-1620 are structurally related and overlap, the His95 groove is a novel feature of the binding of AMG 510 and the enhanced interactions improved the potency of AMG 510 approximately 10-fold, as compared to ARS-1620 in a nucleotide-exchange assay with recombinant GDP-bound $KRAS^{G12C}$ [67]. This drugs methyl-, isopropyl-substituted pyridine ring gets locked in one of the two conformations, making AMG 510 an atropisomer [66, 67]. The molecule structure is shown in Table 2.

Early efforts to evaluate the potential for $KRAS^{G12C}$ inhibitors in combination with other agents such PD-1 immuno-oncology agents are ongoing. Amgen presented preclinical data on AMG 510 at the American Association for Cancer Research (AACR) 2019 meeting reporting the impact of $KRAS^{G12C}$ inhibition on immune surveillance *in vivo*. They generated a syngeneic tumor cell line suitable for testing AMG 510 in combination with checkpoint inhibitor therapies and characterized this line *in vitro*; AMG 510 was able to

clear colon cancer from mice when given in combination with checkpoint inhibitors [68]. Preclinical data have revealed an increased number of total and proliferating CD3+ T cells and total CD8+ T cells after AMG 510 treatment, which were further increased after the combination with a PD-1 immune checkpoint inhibitor. In pre-clinical models, AMG 510 also induced a pro-inflammatory microenvironment characterized by increased interferon signaling, chemokine production, antigen processing, cytotoxic and natural killer cell activity, as well as markers of innate immune system stimulation, that were significantly higher compared to the effects induced by MEK inhibition [67]. The current phase 1/2 study of AMG 510 is planned to utilize its combination with PD1/L1 inhibitors (NCT# 03600883). Just like BRAF and MEK inhibition [69], AMG 510 and other inhibitor of MAPK signaling pathways are under investigation.

In the first in human study (NCT 03600883) evaluating AMG 510 in adult patients with locally advanced or metastatic $KRAS^{G12C}$ mutant solid tumors, Govindan et al. presented their data where 11 out of 23 patients (48%) with NSCLC had partial response (PR) [63]. Fakih et al showed that in patients with colorectal and other solid tumors, 14 out of 19 achieved stable disease as their best response although there were no PR that were reported [62]. Patients with active brain metastases were ineligible. Most common adverse events related to AMG 510 were gastrointestinal side effects such as diarrhea and nausea. Data from the 35 patients in the dose exploration portion showed no DLTs with AMG 510 and no cumulative toxicities were noted with extended treatment [62, 63]. The data in colorectal cancer were less promising compared to NSCLC, but caution is needed to interpret data in such a small sample size and it should be noted that only one colorectal cancer patient had so far received the 960mg dose. More data is necessary to determine if there is a difference in biology; as was in the case with Braf/Mek inhibition which produced lower efficacy in colorectal cancer versus melanoma [69, 70].

MRTX 849

MRTX 849 is an orally available, mutation-selective small molecule inhibitor of $KRAS^{G12C}$. It 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. It irreversibly binds to Cysteine 12 in the inducible Switch II pocket of $KRAS^{G12C}$ and locks it in an inactive GDP-bound state, inhibiting the RAS/MAP kinase pathway. MRTX 849 is orally bioavailable and demonstrates linear pharmacokinetics with extensive tissue distribution. The half-life was approximately 25 hours after a single dose. In preclinical studies, MRTX 849 demonstrated that it was highly potent in blocking KRAS-dependent signal transduction and cancer cell viability (EC_{50} ~10nM). It also showed >1,000-fold selectivity inhibition of $KRAS^{G12C}$-compared with other cellular proteins. In *in vivo* models, MRTX 849 has displayed broad-spectrum antitumor activity ($KRAS^{G12C}$ mutant pancreatic, lung and colon) across panels of $KRAS^{G12C}$-positive patient- and cell-derived tumors, achieving reasonable tumor regression in most models and subset of models showing complete tumor regression. The activity was most pronounced in pancreatic and lung cancer patient derived models. Deep responses were also observed in KRAS mutant tumor models that exhibited co-mutations including STK11, KEAP1, and TP53 [71–73].

MRTX 849 exhibited predicted human oral bioavailability of >30% and a half-life of ~20 hours, as well as therapeutic index of up to 10-fold in repeat-administration toxicology studies. MRTX 849 appears to possess significantly improved potency and a higher degree of antitumor activity than reported previously for other KRAS mutant-selective inhibitors and is the first such molecule reported to advance to IND-track development. The multicenter phase I/II first-in-human started enrollment in January 2019 and is currently ongoing (NCT 03785249). The preliminary results of this study was first presented at the 2019 AACR-NCI-EORTC “triple meeting (International Conference on Molecular Targets and Cancer Therapeutics) [73]”. Out of 12 all evaluable patients (6 NSCLC, 4 colorectal: CRC and 2 appendiceal cancer) who were heavily treated with more than 70% having had more than 3 prior systemic regimens, 4/12 (33%) had confirmed or unconfirmed PR and the remainder 8/12 (66%) had confirmed or unconfirmed SD. Three out of the 4 responders had NSCLC and one response was seen in CRC. None of the patients had brain metastases and thus central nervous system (CNS) activity was not reported. MRTX 849 was associated with a favorable safety profile with the most common adverse events being grade 1 or 2 diarrhea or nausea. Clinical expansion is being pursued at 600mg po BID [73].

JNJ-74699157 (ARS-3248)

Wellspring Biosciences and Janssen recently received an investigational new drug (IND) approval for their $KRAS^{G12C}$ inhibitor ARS-3248, which is a new generation of $KRAS^{G12C}$ inhibitor ARS-1620.

Based upon pioneering research into $KRAS^{G12C}$ inhibitors conducted by Shokat et al., Wellspring discovered ARS-1620, the first small molecule inhibitor that induced tumor regression in patient-derived tumor xenografts that served as a valuable pharmacologic tool to interrogate KRAS biology *in vivo* [65]. Wellspring, through Araxes Pharma, entered into an exclusive arrangement with Janssen in February 2013 to develop small molecule inhibitors of the KRAS G12C oncoprotein for the treatment of cancer. ARS-3248 was discovered as part of an exclusive drug discovery and development agreement with Janssen, which will conduct the Phase 1 trial and have sole responsibility for clinical development. ARS-3248 is an investigational, orally available small molecule that is designed to potently and selectively inhibit $KRAS^{G12C}$.

LY3499446 and other drugs in development

New compounds under development as $KRAS^{G12C}$ inhibitors include the Eli Lilly drug LY3499446 (NCT #04165031), the Pfizer drug tetrahydroquinazoline derivatives (US 2019/0248767A1) and the AstraZeneca drug tetracyclic compounds (WO 2019/110751 A1). Out of these three, LY3499446 appears to be ahead of the game as its phase 1 study started recruitment in Australia and US sites are expected to open towards the end of 2019. In this study (NCT #04165031), LY3499446 will be evaluated as monotherapy and in combination with other agents including abemaciclib, cetuximab and erlotinib in advanced solid tumors including NSCLC and CRC (Table 3).

As shown in Table 3, further novel attempts to target KRAS are ongoing and these include anti-KRAS engineered T-cell receptor therapy (NCT# 03745326) and combination therapies with the upstream pathway of SHP2 inhibitors (NCT # 03989115, NCT # 03114319).

Interestingly, Revolution Medicine revealed a novel tri-complex inhibitors of the oncogenic, GTP-bound form of $KRAS^{G12C}$ overcome RTK-mediated escape mechanisms and drive tumor regressions in preclinical models of NSCLC which could now be categorized as the second generation $KRAS^{G12C}$ inhibitor [74].

Data on direct KRAS inhibitors and combination strategies

In vitro combination of experiments were conducted in several $KRAS^{G12C}$ cell lines with matrices of AMG 510 and inhibitors of HER kinase, EGFR, SHP2, PI3K< AKT and MEK. The combination of MEK inhibitor was synergistic in multiple settings and showed enhanced antitumor activity *in vivo* with a minimally efficacious dose of AMG 510 in combination with a MEK inhibitor, when compared to either of the single agents alone [67]. AMG 510 with MAPK inhibitors may eliminate bypass or residual signaling that could limit is efficacy or induce resistance and further studies are warranted.

Similarly, combination screening has been conducted *in vitro* using MRTX 849 and a focused library of approximately 70 compounds across a panel of sensitive and partially resistant non-clinical models in order to identify combinations that may enhance the response to MRTX 849 and overcome potential resistance. Promising combinations of MRTX and a small molecule inhibitor included the HER2 family inhibitor afatinib, the CD4/6 inhibitor palbociclib, the SHP2 inhibitor RMC-4450, and the mTOR pathway inhibitors [72]. Future studies should not only continue to evaluate the utility of covalent $KRAS^{G12C}$ inhibitors in the treatment of $KRAS^{G12C}$ mutated cancers, but also should focus on identifying those who are likely to derive adequate benefit from single agent use vs those who will likely benefit from rationally directed combination strategies. The current clinical data on AMG 510 and MRTX 849 both lack evaluation of CNS penetration. The AMG 510 study did not enroll those with active brain metastasis and the subjects treated with MRTX 849 did not have documented brain metastases [63, 73]. As CNS is a common site of metastasis in KRAS mutated cancer especially NSCLC, further evaluation of CNS activity of these compounds will need to be studied.

Other strategies to tackle KRAS and related pathways

While direct $KRAS^{G12C}$ inhibitors have started to show promise in some solid tumors, there are many other KRAS mutations (such as $KRAS^{G12D}$ and $KRAS^{G12V}$) and related pathways that lack treatment options. Although the inhibitors to the downstream pathway of MEK lack single agent clinical efficacy in RAS mutant cancers, MEK inhibitors in combination with BCL-XL inhibitors, has shown promising activity of tumor regressions in mouse models of RAS mutant cancers. In a phase I/II study () reported by Corcoran et al, 43 patients received escalating doses of navitoclax (BCL-XL inhibitor) and trametinib (MEK inhibitor). 9/43 (20.9%) had colorectal cancer (CRC), 8/43 (18.6%) pancreatic, 9/43 (20.9%) NSCLC and 11/43 (25.6%) gynecologic cancers. 14/43 (32.6%) were KRAS G12D, 7/43 (16.3%) G12C,

7/43 (16.3%) G12V. Grade 3–4 treatment related AEs occurred in 40% with AST increase, diarrhea, decreased platelets most common. At RP2D, 2/13 evaluable pts had confirmed PR (15.4%) with disease control rate of 46.2%. Early potential disease-specific differences in efficacy were noted. Initial signs of efficacy were noted, with favorable DCR (63.6%) and durable PRs (18.1%) in RAS mutant gynecologic cancer patients. By contrast, no PRs were seen in 9 CRC pts, with overall DCR only 22%. Expansion cohorts are currently enrolling in GYN, NSCLC, pancreatic pts, and NRAS mutant cancers [75]. Similarly, Gershenson et al, also reported the improved PFS and ORR of trametinib 2mg daily in patients with heavily pre-treated low-grade serous ovarian or peritoneal cancer when compared to five standard of care options (including weekly paclitaxel, PLD, topotecan, letrozole, or tamoxifen) [76].

As shown in Table 3, compounds including mRNA-based cancer vaccine that targets four of the most commonly occurring KRAS mutations (G12D, G12V, G13D, and G12C) are also being developed; as clearly, KRAS G12C is only part of the problem; just the tip of the iceberg.

Future challenges and questions to be answered

1. Why is there tumor-based differential response to KRAS G12C in NSCLC versus KRAS G12C colon cancer with the same KRAS inhibitor? Is KRAS G12C mutation in colon cancer not a driver mutation? Understanding the downstream signaling pathways will also be of utmost importance.
2. CNS metastasis occur frequently in NSCLC. Which of the four direct covalent KRAS inhibitors will be able to penetrate the CNS will likely favorably differentiate the inhibitor?
3. It is likely that to maximize the clinical efficacy of these KRAS G12C inhibitors, evaluation of the clinical efficacy and safety of combination therapy with checkpoint inhibitors, anti-EGFR therapies (such as erlotinib or cetuximab) or other inhibitors geared toward the upstream or downstream KRAS pathway (such as SHP2 inhibitors, MEK inhibitors or SOS1 inhibitors) will be required. Documentation of synergy with reasonable tolerability of the combination in regards to toxicity and drug administration feasibility would be ideal and certain approach may open up treatment options for non G12C mutated patients.
4. It will be critical to describe the resistance mechanisms of the first generation KRAS G12C inhibitors to further develop a durable therapeutic strategy such as combination treatment from the beginning in those at high risk of resistance if that cohort of patients could be determined early on.

Conclusions

Early data on AMG 510 and MRTX 849 appear promising. KRAS, especially G12C, may no longer be an”undruggable target”. It has established itself as a valuable addition to the molecular alterations potentially targetable in NSCLC. The fierce competition to bring forward the most effective KRAS G12C inhibitor has just started. Further investigation is critical to better define sensitivity to select inhibitors and also to document the various on

target and off target resistance mechanisms and to capture treatment opportunities with potential combination therapies such as immune checkpoint inhibitors and also inhibitors of related pathways.

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Highlights

- Mutations in Kirsten rat sarcoma viral oncogene homolog (KRAS) are common in cancer.
- KRAS remains a challenging therapeutic target.
- Recently, several novel compounds against individual KRAS alterations have emerged.
- Initial activity of AMG 510 and MRTX 849 appears promising in KRAS G12C NSCLC.
- Single agent and combination studies are ongoing to evaluate their safety and efficacy.

Table 1

Inhibitors targeting RAS upstream and downstream

Inhibitors	Target	Specificity	Ref
Inhibitors of farnesyltransferase	Farnesyl transferase	With a zinc-site recognition moiety and a farnesyl/dodecyl group	[24]
FGTI-2734	Farnesyl transferase and geranylgeranyltransferase	Inhibiting membrane localization of KRAS	[25]
FTI-277	Farnesyl transferase	Blocking HRAS activation	[26]
Rce1 inhibitor	Rce1	Inducing mislocalization of EGFP-RAS from the plasma membrane	[29]
Rce1 inhibitor	Rce1	Inducing a Ras2p delocalization phenotype	[30]
UCM-1336	ICMT	Impairing the membrane association of the four RAS isoforms	[31]
Cysmethynil	ICMT	Inhibiting ICMT enzymatic activity	[32]
BAY-293	Sos1	Preventing formation of the KRAS-Sos1 and blocking reloading of KRAS with GTP	[36]
HBS 3	Sos1	Interfering with RAS-Sos interaction and downregulating RAS signaling	[37]
Small molecules binding to KRAS	RAS-Sos complex	Binding to KRAS and blocking binding to Sos	[38]
DCAI	RAS-Sos complex	Inhibiting SOS-mediated nucleotide exchange and preventing RAS activation	[39]
Trametinib	MEK 1/2	Significantly downregulating pERK and pS6	[48]
GSK2141795	Akt	Inhibiting Akt signaling	[48]
Sorafenib	RAS/MEK/ERK	Inhibiting RAS/MEK/ERK and PI3K/Akt/mTOR	[45]
Compound 8	MEK and PI3K	Significantly inhibiting MEK and PI3K signaling	[43]
U0126	MEK	Inhibiting p-ERK1/2 expression and its downstream target p-eIF4E	[46]
PD901	MEK	Efficiently inhibiting ERK activation in KRAS/NICD tumor cells	[46]
Selumetinib	MEK	Promoting growth suppressive effects	[46]
PD98059	MEK	Enhancing anti-tumor effects of Akt inhibitor in KRAS mutant cancers	[47]

Table 2

AMG 510 and MRTX 849.

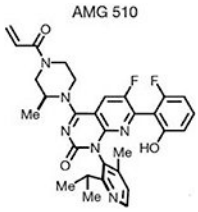
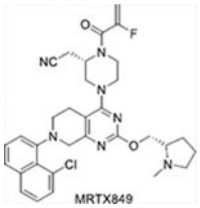
Compound	AMG 510	MRTX 849
MOA	Irreversible small molecule inhibitor of $KRAS^{G12C}$	Irreversible selective covalent $KRAS^{G12C}$ inhibitor
Structure		
Key Features	Small molecule that specifically and irreversibly inhibits $KRAS^{G12C}$ by permanently locking it in an inactive GDP-bound state.	Mutant-selective inhibitor of $KRAS^{G12C}$ that irreversibly binds to $KRAS^{G12C}$ and locks in its inactive, GDP-bound state.
	AMG510 binds to His95 groove in the P2 pocket of KRAS.	
RP2D	960mg daily	600mg BID
Half life	5.5 hours	24.7 hours
References	61, 62, 63, 67, 68	64, 71, 72, 73

Table 3

Novel KRAS^{G12C} inhibitors and US-based clinical trials

NCT Trial#	Agent(s) / Mechanism	Phase	Company	Setting	N of pts
03600883	AMG 510 (+/− PD1/L1) / KRAS^{G12} inhibitor	1 / 2	Amgen / Carmot Therapeutics	AMG 510 monotherapy in KRAS^{G12C} advanced solid tumors and in combination w/PD1/L1 in KRAS^{G12C} advanced NSCLC	158
037855249	MRTX 849 / KRAS^{G12} inhibitor	1 / 2	Mirati (ex Array)	MRTX 849 in KRAS^{G12C} advanced solid tumors	200
04006301	ARS-3248 (JNJ-74699157)/ KRAS^{G12} inhibitor	1	Wellspring Biosciences and Janssen	ARS-3248 (JNJ-74699157) in KRAS^{G12C} advanced solid tumors	140
04165031	LY3499446 / KRAS^{G12} inhibitor +/− abemaciclib, cetuximab, erlotinib vs docetaxel (phase 2)	1/2	Eli Lilly and Company	Advanced solid tumors including NSCLC and CRC	230
03114319	TNO155 / SHP2 inhibitor	1	Novartis	TNO155 in EGFR mutant NSCLC, KRAS^{G12C} mutant cancers (NSCLC, CRC, esophageal, HNSCC), RAS/RAF wild type other solid tumor	135
03745326	KRAS TCR/Anti- KRAS^{G12D} engineered T-cell receptor	1 / 2	Gilead (ex Kite/ NCI)	Peripheral Blood Lymphocytes Transduced w/a Murine T-Cell Receptor Recognizing the G12D Variant of Mutated RAS in HLA-A*11:01 pts	70
03989115	RMC-4630 + cobimetinib / SHP 2 inhibitor + MEK inhibitor	1 / 2	Revolution Medicine	RMC-4630 and cobimetinib in solid tumors w/ specific genomic abberations	144
04111458	BI 1701963 (pan-KRAS/SOS1 inhibitor) +/− MEK inhibitor	1	Boehringer Ingelheim	BI 1701963 +/− trametinib in advanced metastatic KRAS mutant solid tumors	140
03948763	mRNA-5671/V941 +/− pembrolizumab	1	Merck Sharp & Dohme Corp.	A mRNA vaccine targeting KRAS mutations (G12D, G12V, G13D, and G12C)-5671/V94 +/ − pembrolizumab in KRAS mutant advanced or metastatic NSCLC, CRC or pancreatic adenocarcinoma	100