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Review

The improbable targeted therapy: KRAS as an emerging target in non-small cell lung cancer (NSCLC)

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SUMMARY

KRAS is a frequent oncogenic driver in solid tumors, including non-small cell lung cancer (NSCLC). It was previously thought to be an "undruggable" target due to the lack of deep binding pockets for specific small-molecule inhibitors. A better understanding of the mechanisms that drive KRAS transformation, improved KRAS-targeted drugs, and immunological approaches that aim at yielding immune responses against KRAS neoantigens have sparked a race for approved therapies. Few treatments are available for KRAS mutant NSCLC patients, and several approaches are being tested in clinicals trials to fill this void. Here, we review promising therapeutics tested for KRAS mutant NSCLC.

INTRODUCTION

Mutations in Kirsten rat sarcoma viral oncogene homolog (KRAS) are one of the most common genomic alterations identified in solid tumors, especially lung cancer, colorectal cancer, and pancreatic ductal adenocarcinoma (Figure 1). These cancers rely on continued activation and signaling of KRAS, therefore making it an ideal therapeutic target. Unfortunately, therapeutic approaches specifically targeting KRAS, such as attempts to inhibit KRAS membrane localization with farnesyltransferase inhibitors, failed in clinical trials and have thus far not been successful. In non-small lung cancer (NSCLC), the current treatment for KRAS mutant patients relies on chemotherapy, with an average overall survival (OS) of 22 months, which is less than desired.[1] Since previous efforts failed to specifically target KRAS, researchers began to indirectly target KRAS through its role in various signaling pathways. Unfortunately, targeting KRAS dependencies in NSCLC has highlighted the difficulty in the effective inhibition of indirect targeting of KRAS.[2,3] In addition, downstream pathway blockade of rapidly accelerated fibrosarcoma kinase/mitogen-activated protein kinase/phosphatidylinositol 3-kinase (RAF/MEK/PI3K) with inhibitors has not yet been proven to be effective as shown by the negative results of the Selumetinib Evaluation as Combination Therapy-1 (SELECT-1) trial of selumetinib combined with docetaxel.[4–6] Small-molecule inhibitors have been challenging to develop since mutant KRAS has a very high affinity for guanosine triphosphate (GTP) and the catalytic sites are small and tough to target. Analyses of the KRAS subpopulation in landmark clinical trials in NSCLC have provided information on response (overall response rate of 17%–18%) and survival (median OS of 3 months) of this patient cohort for approved therapeutics,[7–9] but there is still a lack of effective treatments for mutant KRAS. Consequently, the search for therapies that successfully target frequent KRAS mutations has continued due to its large incidence in various cancers.

In <5 years, the availability of novel therapeutics has transformed the landscape of KRAS treatments, and a target that was once considered "undruggable" now has several therapeutic inhibitors undergoing clinical trials.[10–12] There are essentially four novel approaches to target KRAS that can be divided into targeted therapies and immunotherapies, potentially in combination with one another:[1] a novel class of direct KRAS inhibitors that specifically inhibit the G12C mutations through direct interaction with the cysteine residue, as well as a somewhat broader approach using a pan-KRAS inhibitor that does not attempt to inhibit specific mutations;[2] novel signaling inhibitors that can inhibit chronic activation of KRAS-dependent pathways;[3] immune checkpoint inhibitors (ICIs) that can be used in combination with other approaches;[4] and approaches that take advantage of the neoantigen feature of mutated KRAS protein and attempt to improve the T cell response. Therefore, we present in this review recent advances in therapeutic options from a clinical standpoint, to understand the mechanisms of these inhibitors and potential future considerations regarding KRAS and therapeutic efficacy in NSCLC.

Mutated KRAS is a prominent oncogene in lung cancer

The intracellular guanine nucleotide-binding protein (G protein) KRAS is a member of the small GTPases *RAS* gene family that

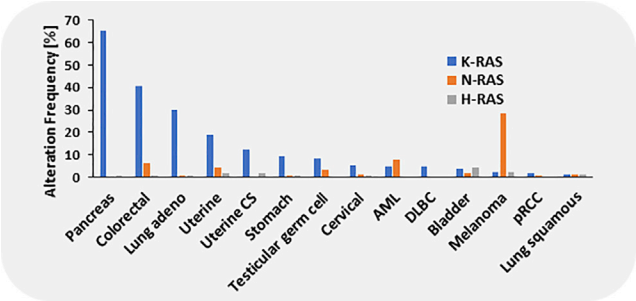

Figure 1. Frequency of KRAS mutations in relation to NRAS and HRAS mutation in various malignancies. Uterine CS, uterine carcinosarcoma; AML, acute lymphoblastic leukemia; DLBC, diffuse B cell lymphoma; pRCC, papillary renal cell carcinoma.

also includes *NRAS* and *HRAS*. *KRAS* encodes for six exons, resulting in two major splice variants, KRAS4A and KRAS4B, which differ in the C-terminal sequence. This region is required for membrane localization and contains a dual membrane-targeting motif in KRAS4A, in contrast to KRAS4B, which only contains one farnelysation site (Figure 2A). Since KRAS4B is the dominant form and KRAS4A is only marginally expressed in normal cells, it was long thought that the splicing of KRAS was of little consequence. In tumors, the overall expression of KRAS4A can be greatly elevated.[13] This is of interest since KRAS4A has been reported to be required for the formation of chemically induced lung cancer in mice.[14] Also, active KRAS4A functions as a regulator of hexokinase 1, which depends on its palmitoylation and colocalization with the metabolic enzyme on the outer mitochondrial membrane, thus effectively modulating glucose metabolism.[15] Most mutations in KRAS affect codons 12, 13, 61, and 146; however, codon 146 is rarely altered in some cancers, such as NSCLC. Oncogenic KRAS missense mutations result in the high-affinity binding of GTP and loss of GTPase activity, thereby leaving KRAS in an "on" state and deregulating various signaling pathways that rely on active RAS (Figure 2B).[16,17] More recent work suggests that another potential mechanism of oncogenic KRAS activation (A146T mutant and possibly also V14I and G12D) involves the acceleration of intrinsic and guanine nucleotide exchange factor-induced nucleotide exchange.[18] The most frequent KRAS mutations in NSCLC are G12C, with almost half of all cases, followed by G12V and G12D (Figure 2C).[19,20] Thus, lung cancer cells express mutations in both KRAS4A and KRAS4B.

Lung cancer is the number one cause of cancer-related death worldwide and accounts for ~228,820 new cases and 140,730 deaths in 2020.[21] NSCLC is the most frequent lung cancer subtype, and patients who are diagnosed with metastatic disease have reported 5-year OS rates of only 6%.[22] With ~25% to 30% of cases, KRAS mutations are represented in a large portion of NSCLC patients,[23] but effective therapies against KRAS have yet to be approved by the US Food and Drug Administration (FDA). The oncogenic mutations that drive NSCLC include not only KRAS but also epidermal growth factor receptor (EGFR) (17% in NSCLC), anaplastic lymphoma kinase (ALK) (7% in NSCLC), mesenchymal epithelial transition kinase (MET) (3% in NSCLC), and others, which are generally mutually exclusive.[24–27]

It should be noted that there are ethnic differences in the frequency of KRAS mutations in NSCLC, with a higher incidence rate of KRAS mutations in White NSCLC patients as compared to Black or east Asian patients.[28–31] However, these differences are not known to affect the potential efficacy of novel KRAS therapeutics. Numerous targeted therapies against these actionable mutations have been developed and tyrosine kinase inhibitors (TKIs) targeting EGFR, ALK, or MET are already standard of care. In fact, a poor prognosis and a shorter median survival are reported for patients with KRAS mutant NSCLC versus patients with actionable mutations coupled with approved personalized therapies (2.41 versus 3.5 years, $p < 0.001$).[32] These data demonstrate the impact of targeted therapies on response and OS in NSCLC cases caused by a specific driver mutation. Fortunately, several KRAS-targeted therapies are under investigation in clinical trials. Interestingly, many of the oncogenes in NSCLC signal through KRAS, and some of the approaches developed against active KRAS may also be applicable in these patients.

KRAS mutations and clinical characteristics

In past studies, KRAS mutations have been reported as a prognostic indicator of poor OS in NSCLC,[33–35] while other studies have demonstrated no difference in response to chemotherapy/targeted therapy or OS.[36–38] Although its true prognostication is somewhat unclear due to the complexity of KRAS-driven tumors, several analyses have noted a predictive value of KRAS, indicating poor prognosis.[39,40] For example, a retrospective analysis of 482 lung adenocarcinoma cases at a single institution characterized the incidence of KRAS and its association with age, gender, race, and smoking history.[41] The study determined that NSCLC patients who are positive for KRAS mutations are typically White and have a history of cigarette smoking. They also reported that age, gender, or the number of pack-years was not associated with KRAS mutation incidence. Interestingly, never smokers were more likely to have G12A transition mutations, while former or current smokers were more likely to report G12T or G12C transversion mutations,[41] which was recapitulated in other studies.[19,42,43] Another study by Dogan et al.[19] examined 3,026 lung adenocarcinoma cases for clinical correlations of common driver mutations EGFR and KRAS. They reported a high frequency of KRAS G12C in women compared to men ($p = 0.007$).

Reports have been inconsistent in determining whether treatment response, progression-free survival (PFS), and OS differ based on the KRAS mutation subtype. KRAS mutation subtypes have been reported to rely on different signaling pathways, with G12C and G12V activating the Ral signaling pathway, while KRAS G12C tumors use PI3K and MEK signaling pathways.[44] However, the putative molecular mechanisms that regulate these pathway switches are unclear, and the results have not been independently validated. Similarly, *in vitro* studies have demonstrated that KRAS point mutations have variable responses to chemotherapy,[45] but other analyses found no significant differences in response to or survival in chemotherapy or immunotherapy.[7] In an analysis of 179 surgically resected lung adenocarcinoma patients, KRAS G12C was found to be associated with worse disease-free survival (DFS) and OS as compared to G12D (hazard ratio [HR] 2.81, $p = 0.035$) or G12A (HR 5.99, $p = 0.016$).[46] However, this was not

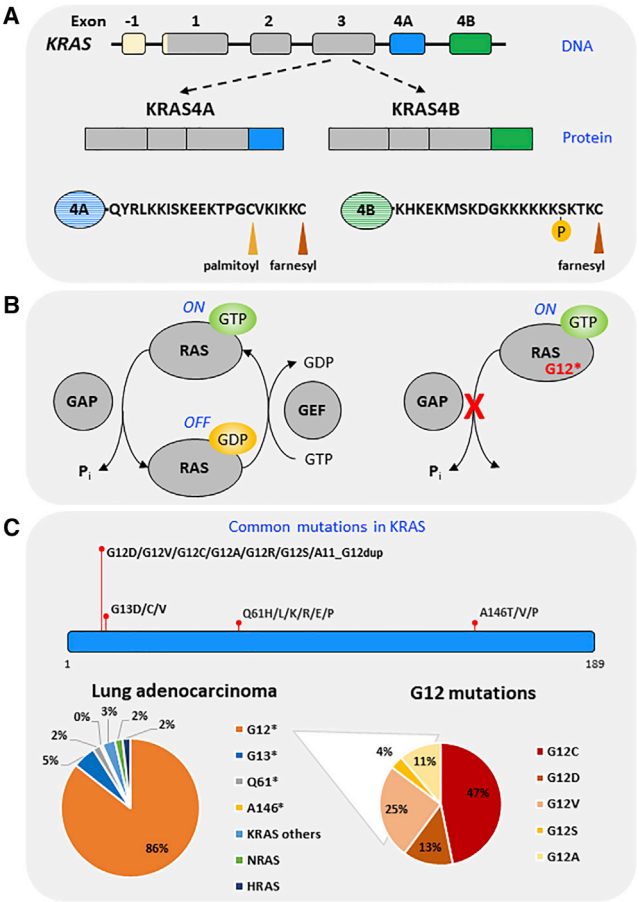

Figure 2. Structure and function of KRAS

(A) The *KRAS* gene consists of 6 exons, wherein exon −1 and part of exon 1 are non-coding. The mature protein exists in 2 major splice variants, KRAS4A and KRAS4B (top). KRAS4A and KRAS4B differ in the C-terminal membrane-targeting region. KRAS4A contains a single palmitoylation and farnesylation site, while KRAS4B contains 1 farnesylation and a putative serine phosphorylation site (bottom).

(B) RAS proteins are cycled from an active form (ON) through GTP binding facilitated by a guanine nucleotide-exchange factor (GEF) to an inactive form (OFF) by GTP hydrolysis through RAS itself, catalyzed by a GTPase-activating protein (GAP). In oncogenic RAS (e.g., with a G12 mutation, G12*), this process is disrupted, and RAS remains active.

(C) Common KRAS mutations in lung adenocarcinoma by specific subtypes.

true when compared to G12V (HR 1.62, p = 0.259). An analysis of 43 KRAS mutant NSCLC patients who were enrolled in the BATTLE-1 (Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination) clinical trial determined that KRAS mutation variants G12C and G12V were significantly associated with worse median PFS compared to other KRAS variants or wild-type KRAS (1.84 versus 3.35 versus 1.95 months, respectively, p = 0.046).[44] In contrast to these findings, a large retrospective study evaluated 677 patients with KRAS-mutated metastatic NSCLC and discovered no significant difference between KRAS mutation subtypes.[47] The overall data suggest that differences among mutation subtypes are rather small.

KRAS and other transforming pathways in NSCLC

The mutational landscape in NSCLC is complex, and despite the mutational activation of KRAS, one can distinguish KRAS-dependent or KRAS-independent groups of KRAS mutant NSCLC through their gene expression signatures. KRAS-dependent tumors, which rely on the continued activation of mutant KRAS through downstream signaling pathways, are correlated with a well-differentiated epithelial signature. Meanwhile, KRAS-independent tumors, which have decreased KRAS coupling to downstream signaling pathways, are correlated with an epithelial-to-mesenchymal transition (EMT) signature, a key process in cancer cell invasion, migration, and drug resistance.[48,49] These findings suggest that KRAS-dependent and -independent NSCLCs should be therapeutically approached as distinct subtypes since they are driven by different signaling cascades.[50] Although KRAS amplification can occur concurrently with EGFR amplification,[51] KRAS mutations tend to occur independently of EGFR mutations in NSCLC and do not respond to EGFR TKIs, as highlighted by their contrasting clinical characteristics.[52–55] Like other important NSCLC driver oncogenes such as EGFR and ALK, co-mutations with KRAS are quite frequent.[56] Research has established that the existence of KRAS and its specific co-mutation partner signifies distinct biological subtypes and variable responses to therapeutic treatments.[57] For example, KRAS was found to occur often with other important oncogenes such as TP53 (40%), STK11/LKB1 (32%), and CDKN2A (19.8%).[58] The study demonstrated that with each of these common co-mutation partners, the downstream signaling effects diverge, leading to variances in genetic events, tumor differentiation, and gene expression, suggesting a difference in response to therapies. It also indicated a worse prognosis in KRAS mutant NSCLC patients with STK11/LKB1 or CDKN2A alterations compared to patients with TP53 alterations. The prognostic role of KRAS and TP53 co-occurrence as an indicator of worse PFS remains controversial.[59,60] A study on co-occurring mutations in KRAS mutant NSCLC confirmed a high prevalence of TP53 and STK11 but also noted KEAP1/NFE2L2 incidence (27%).[61] KEAP1 and KRAS co-mutated tumors were associated with shorter OS after the initiation of immunotherapy. Skoulidis et al.[62] found that KRAS mutant NSCLC patients with STK11/LKB1 co-mutations stimulated resistance to anti-programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) antibodies. Of 174 KRAS mutant lung adenocarcinomas, most of which were treated with immunotherapy, 31% expressed STK11/LKB1 alterations, while the rest either expressed TP53 or no major co-mutation. Objective response rates (ORRs) were calculated, and patients expressing both KRAS and STK11/LKB1 alterations demonstrated an ORR of 7.4%. This was significant when compared with KRAS/TP53 or KRAS alone cohorts (7.4% versus 35.7% versus 28.6%, respectively; p < 0.001). The authors confirmed this finding by analyzing the KRAS subgroup ORR to nivolumab in the CheckMate-057 trial (0% in KRAS/STK11 patients versus 57.1% in KRAS/TP53 patients versus 18.2% in KRAS alone; p = 0.047).[63] The median PFS (1.8 versus 3.0 versus 2.7 months; p = 0.0018) and OS (6.4 versus 16.0 versus 16.1 months; p = 0.0045) of these patients were also similarly affected.

Targeting KRAS dependencies in lung cancer

Since KRAS mutant tumors survive and proliferate via major signaling pathways often involving MEK, PI3K-AKT, and mammalian target of rapamycin (mTOR), inhibition of their signaling mechanisms should ideally result in response and tumor regression. In the search for signaling mechanisms that are required for mutant KRAS transformation in NSCLC, several genes have been identified via synthetic lethal screenings, including B-cell lymphoma-extra large (BCL-XL), TANK-binding kinase 1 (TBK1), and cyclin-dependent kinase 4 (CDK4), and targeting their expression resulted in the death of mutant KRAS cells *in vitro*.[64] Corcoran et al.[65] demonstrated that the dual inhibition of BCL-XL and MEK triggered significant apoptosis and tumor regression in KRAS mutant xenograft models. Based on preclinical data, an ongoing clinical trial is investigating the safety and tolerability of combining trametinib (MEK1/2 inhibitor) and navitoclax (BCL-XL/BCL-2 inhibitor) in advanced or metastatic solid tumors (NCT02079740). Barbie et al.[66] identified TBK1, a gene involved in the Ral-GEF signaling pathway essential for mutant KRAS tumors, as a synthetic lethal gene in preclinical models. Subsequent research revealed that TBK1 enhances tumor survival by activating CCL5 and interleukin-6 in aggressive KRAS-mutated lung adenocarcinoma mouse models.[67] A phase IB study of momelotinib (JAK2 and TBK1 inhibitor) combined with trametinib (MEK1/2 inhibitor) in previously treated, refractory, or metastatic KRAS mutant NSCLCs[2] of 21 patients showed no ORR in any patient, and 12 patients (57.1%) had a disease control rate (DCR) of 8 weeks (90% confidence interval [CI], 37.2%–75.5%). The median PFS and median OS were 3.6 months (90% CI, 2.2–5.6 months) and 7.4 months (90% CI, 4.0–15.3 months), respectively. However, the maximum plasma concentrations of momelotinib failed to inhibit TKB1 in patients, emphasizing the need for the development of more potent and selective inhibitors. Thus, whereas the addition of momelotinib demonstrated no clinical improvement over trametinib monotherapy in KRAS mutant NSCLC, these results do not answer the question of whether TKB1 targeting could have therapeutic benefits *in vivo*.

Another synthetic lethal screening revealed that loss of the CDK4, involved in cell-cycle progression, resulted in cell deterioration and tumor inhibition of mutant KRAS cells in mouse models.[68] Although CDK inhibitors demonstrate major drug toxicities and limited clinical activity as monotherapy, several clinical trials have been conducted to elucidate the role of CDK inhibitors in KRAS mutant lung cancer. A phase I trial examining abemaciclib (CDK4/6 inhibitor) confirmed encouraging clinical activity in patients with KRAS mutant versus KRAS wild-type NSCLC tumors.[69] Recently, the team conducting the phase III JUNIPER study (A Study of Abemaciclib [LY2835219] in Participants With Previously Treated KRAS Mutated Lung Cancer), examining abemaciclib in previously treated mutant KRAS NSCLC, presented its findings, expressing that the trial did not reach its primary endpoint of OS.[3] Four hundred fifty-three previously treated KRAS mutant NSCLC patients were randomized 3:2 to receive abemaciclib (n = 270) or erlotinib (n = 183). Although abemaciclib did not improved median OS compared to erlotinib (7.4 versus 7.9 months, respectively), it did demonstrate an improvement in median PFS (3.6 versus 1.9 months; HR [95% CI]: 0.58 [0.47–0.72], $p < 0.001$) as well as a significantly higher ORR (8.9% versus 2.7%, $p = 0.01$). Palbociclib (CDK4/6 inhibitor) is under investigation in the National Lung Matrix Trial (NCT02664935) for patients diagnosed with NSCLC with a CDK4/6 genomic alteration. The RAS signaling pathway shows some level of redundancy or cross-activation, and single synthetic lethal targets may not be sufficient to block transformation by mutant KRAS *in vivo*. These studies highlight the difficulty in achieving effective target inhibition of synthetic lethal partners of KRAS in patients. Recent preclinical data have demonstrated that inhibitors against KRAS-dependent pathway targets, including mTOR and insulin-like growth factor 1, combined with KRAS G12C inhibitors instead of MEK inhibitors, significantly improved the efficacy and selectivity of blocking downstream MEK/ERK signaling in KRAS G12C mutant tumor cells *in vitro* and in mouse models.[70] Effective treatment of KRAS tumors could involve a combination approach of direct KRAS targeting and indirect targeting through synthetic lethality of downstream effectors.

Targeting KRAS-regulated pathways

KRAS is involved in various downstream signaling pathways that could allow for the indirect targeting of KRAS. Mutations in KRAS result in dysregulated signaling of different cascades, including MET overexpression, the RAF/MEK/ERK, the PI3K/AKT/mTOR, the RHO/FAK, and the Ral/GEF pathways.[71] Targeting patients with KRAS mutant cancers via downstream pathways as a strategy to affect KRAS has been examined in clinical trials (Table 1) with average response rates of <20%,[72] as compared to EGFR or ALK response rates of 60% to 80% when treated with TKIs.[73,74] These differential outcomes can be explained considering that EGFR and ALK can be targeted with specific inhibitors, while direct inhibition of KRAS has been made possible only recently.

Fatty acid synthase (FASN)

It was previously shown that KRAS induces fatty acid synthesis by activating lipogenesis and ERK2 kinase activity. Also, inhibition of one of the FASN subunits with cerulenin in KRAS mutant lung adenocarcinoma cell lines showed a significant decrease in cell growth.[77] The small-molecule drug TVB-2640, an orally bioavailable and first-in-class inhibitor of FASN, was studied as monotherapy or with paclitaxel in a phase I clinical trial of NSCLC patients.[78] Preliminary results from the phase I trial showed promising clinical activity in KRAS mutant NSCLC.[75,79,80] Of the 31 NSCLC patients included in the study, 18 were KRAS mutant. Six patients on monotherapy TVB-2640 and 5 patients on the combination regimen achieved stable disease for at least 19 and 23 weeks, respectively. KRAS mutant NSCLC patients achieved a longer PFS on TVB-2640 monotherapy, with 60% of KRAS mutant patients still on trial at 12 weeks versus 0% of KRAS wild-type patients. A phase II clinical trial investigating TVB-2640 is under way in KRAS mutant NSCLC (NCT03808558).

RAF

RAF inhibition was examined in preclinical and clinical models to determine potential efficacy against KRAS. BRAF inhibitors vemurafenib and dabrafenib are contraindicated for use in KRAS tumors since they stimulate tumorigenesis through binding to BRAF, also known as the MAPK paradox.[81] Thus, targeting other

Table 1. Select completed trials investigating inhibitors against KRAS-regulated pathway targets in KRAS-mutant NSCLC

Therapeutic drug	Target	Trial	Activity/results in KRAS subgroup	Toxicity
TVB-2640	FASN	NCT02223247: A Phase 1, First-In-Human Study of Escalating Doses of Oral TVB-2640 in Patients With Solid Tumors[75]	● 11/18 patients demonstrated stable disease for a minimum of 19 weeks	common related AEs include alopecia (41%), palmar-plantar erythrodysesthesia (47%), and decreased appetite (13%)
Sorafenib	RAF	NCT00863746: A Phase III, Multicenter, Placebo-Controlled Trial of Sorafenib (BAY43-9006) in Patients With Relapsed or Refractory Advanced Predominantly Non-Squamous Non-Small Cell Lung Cancer (NSCLC) After 2 or 3 Previous Treatment Regimens for Advanced Disease[76]	● median OS sorafenib 6.4 mo versus placebo 5.1 mo ● median PFS sorafenib 2.6 mo versus placebo 1.7 mo ● ORR sorafenib 2.9% versus placebo 0%	common related AEs include skin toxicities (40.5%), fatigue (36.1%), and diarrhea (35.8%)
Selumetinib + docetaxel	MEK	NCT01933932: A Phase III, Double-Blind, Randomized, Placebo-Controlled Study to Assess the Efficacy and Safety of Selumetinib (AZD6244; ARRY-142886) (Hyd-Sulfate) in Combination With Docetaxel, in Patients Receiving Second Line Treatment for KRAS Mutation-Positive Locally Advanced or Metastatic Non Small Cell Lung Cancer (Stage IIIB–IV) (SELECT 1)[6]	● median OS selumetinibc+cdocetaxel 8.7 months versus placeboc+cdocetaxel 7.9 mo ● median PFS selumetinibc+cdocetaxel 3.9 months versus placeboc+cdocetaxel 2.8 mo ● ORR selumetinibc+cdocetaxel 20.1% versus placeboc+cdocetaxel 13.7%	common related AEs in the selumetinibc+cdocetaxel group were diarrhea (61%), nausea (38%), rash (34%), and peripheral edema (30%)
Ridaforolimus	mTOR	NCT00818675: A Randomized Discontinuation Phase II Trial of Ridaforolimus in Non-Small Cell Lung Cancer (NSCLC) Patients With KRAS Mutations[5]	● median OS ridaforolimus 18 mo versus placebo 5 mo ● median PFS ridaforolimus 4 mo versus placebo 2 mo ● ORR at 8 weeks ridaforolimus 1%	common related AEs include fatigue (10%), mucositis/stomatitis (10%), pneumonia (10%), dyspnea (9%), diarrhea (6%), and hyperglycemia (6%)

See also https://clinicaltrials.gov/. AEs, adverse events; FASN, fatty acid synthase; KRAS, Kirsten rat sarcoma viral oncogene homolog; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RAF, rapidly accelerated fibrosarcoma kinase.

members of the RAF family was explored in KRAS-mutated NSCLC. Preclinical data demonstrated the inhibition of cell growth in KRAS mutant NSCLC cells when treated with sorafenib, an oral multikinase inhibitor directed at RAF and similar transmembrane receptors.[4] Although promising, clinical models did not demonstrate great efficacy when using sorafenib. A phase II clinical trial investigated sorafenib in KRAS-mutated stage IIIB/IV NSCLC patients that progressed on a previous line of platinum chemotherapy with a primary endpoint of the DCR.[82] Fifty-seven patients treated with sorafenib demonstrated an observed DCR of 52.6%, a median PFS of 2.3 months, and a median OS of 5.3 months. Grade III/IV adverse events (AEs) secondary to sorafenib, including skin and gastrointestinal toxicities, were reported in 10 patients (17.5%). This modest clinical activity warranted further investigation and resulted in a phase III multicenter, placebo-controlled clinical trial in relapsed or refractory non-squamous NSCLC patients treated with monotherapy sorafenib after progression on at least two treatment lines.[76] Although OS was comparable, PFS was significantly longer in the KRAS mutant subpopulation as compared to the KRAS wild-type group when treated with sorafenib. Common AEs included skin toxicities (40.5%), fatigue (36.1%), and diarrhea (35.8%). Finally, in the BATTLE-1 trial, although sorafenib demonstrated better DCR in KRAS mutant patients compared with all of the other TKIs (61% versus 32%), this was not significant ($p = 0.11$).[83]

MEK

Clinical trials targeting the KRAS effector MEK with CI-1040 or PD-0325901 in various solid tumor cancers demonstrated insufficient antitumor activity.[84] Selumetinib, a potent, selective MEK inhibitor, was shown to inhibit tumor growth and proliferation in KRAS mutant xenograft models.[85] This was significantly enhanced when combined with cytotoxic chemotherapy docetaxel or irinotecan. Several phase II clinical trials were initiated to examine selumetinib versus single-agent chemotherapy agents. Selumetinib versus pemetrexed was first evaluated in advanced NSCLC patients who progressed on first-line treatment and no clinical benefit over the standard of care was found.[86] Based on the previous preclinical study, the second trial investigated selumetinib with docetaxel in previously treated KRAS mutant NSCLC. Eighty-seven patients were enrolled in the trial and were randomly assigned to receive selumetinib and docetaxel ($n = 44$) or placebo and docetaxel ($n = 43$).[87] Adding selumetinib to docetaxel demonstrated promising clinical activity in terms of ORR and PFS. However, the phase III SELECT-1 trial of selumetinib and docetaxel in KRAS mutant NSCLC failed to demonstrate improved PFS versus docetaxel alone.[6] Of the 510 patients who were randomized to receive treatment, 505 actually received treatment with either selumetinib and docetaxel ($n = 251$) or placebo and docetaxel ($n = 254$). Median OS (8.7 versus 7.9 months, $p = 0.64$) and median PFS (3.9 versus 2.8 months, $p = 0.44$) were not significant in the selumetinib and docetaxel group compared to the placebo and docetaxel group. The trial reported an ORR of 20.1% in the combination regimen and 13.7% in the placebo and docetaxel cohort ($p = 0.05$); however, the addition of selumetinib to docetaxel ultimately did not improve OS or PFS. A phase II clinical trial examined monotherapy selumetinib or in combination with erlotinib in previously treated advanced NSCLC patients.[88] Although the ORR improved in the mutant KRAS combination cohort (0% for selumetinib alone versus 10% for combination), combination therapy led to increased AEs. Trametinib, a selective allosteric MEK inhibitor, has also been examined in clinical trials and demonstrated results comparable to those of selumetinib.[89–91] Several trials investigating MEK inhibitors combined with other therapies are ongoing (NCT02964689, NCT01859026, NCT03170206, NCT03299088, and NCT02185690).

PI3K/AKT/mTOR pathway

Although KRAS alterations can occur with PI3K alterations, research has shown that PI3K inhibitors as monotherapy are ineffective. Recently, a preclinical study reported that combination treatment with KRAS G12C inhibitor ARS1620 and a PI3K inhibitor overcame resistance in patient-derived xenograft models resistant to the KRAS inhibitor.[92] mTOR inhibitor ridaforolimus was investigated in a phase II clinical trial in KRAS mutant advanced NSCLC.[5] Seventy-nine patients were enrolled and randomized 1:1 to receive ridaforolimus or placebo. Although the trial investigators reported a low ORR (1%) and no significant benefit in OS, they reported prolonged median PFS in patients treated with ridaforolimus (4 versus 2 months, $p = 0.013$, HR 0.36). Common grade 3/4 AEs were fatigue (10%), mucositis/stomatitis (10%), pneumonia (10%), dyspnea (9%), diarrhea (6%), and hyperglycemia (6%). Overall, preclinical and clinical data on targeting this pathway in KRAS mutant tumors suggest that combination treatment with other inhibitors could enhance therapeutic efficacy.

KRAS inhibitors

Since RAS proteins must localize to the plasma membrane to be functionally active, it was long thought that blocking KRAS farnesylation would be beneficial. The majority of the effort was focused on developing farnesyltransferase inhibitors (FTIs) due to lipid post-translational modifications catalyzed by these enzymes that are required for the oncogenic activity of KRAS.[93] Unfortunately, clinical trial testing of this class of inhibitors yielded poor results.[94–97] The development of novel inhibitors that directly target KRAS is expected to transform the therapeutic landscape of cancers affected by this oncogenic driver. Although none of these inhibitors are FDA approved, several promising drugs have entered clinical trials (Table 2). Ideally, efforts began in developing a small-molecule inhibitor targeted at the KRAS-binding site to prevent GTP-KRAS interactions, but they have failed primarily due to the high affinity of GTP for KRAS and the lack of suitable binding pockets for small-molecule drugs that could interfere with RAS function. Thus, the focus has shifted away from approaching KRAS inhibition as "one-size-fits-all" and instead to approaching it from many sides. Drugs including the pan-KRAS inhibitor BBP-454 (BridgeBio Pharma) may change the paradigm of KRAS as an undruggable target as it is designed to bind to previously unrecognized binding pockets.[98,99] It is in the preclinical phase and data have not yet been formally reported. Also, interest in targeting KRAS G12C arose when it was discovered that KRAS had an allosteric regulatory site that could lead to inhibition.[100] Preliminary research on targeting the allosteric site revealed that it was mutation specific to KRAS G12C.[101–103] Thus, various pharmaceutical companies have begun to develop KRAS G12C-specific

Table 2. Select clinical trials in KRAS mutant cancers

Therapeutic drug	Target	Trial	Primary objective(s)
AMG 510	KRAS G12C	NCT03600883: A Phase I/II, Open-label Study Evaluating the Safety, Tolerability, Pharmacokinetics, and Efficacy of AMG 510 in Subjects With Solid Tumors With a KRAS p.G12C Mutation and AMG 510 Combination Therapy in Subjects with Advanced NSCLC with KRAS p.G12C Mutation (CodeBreak 100)	evaluate the safety and tolerability of AMG 510, estimate the MTD and/or a recommended phase II dose, ORR assessed by RECIST 1.1 criteria of AMG 510 as monotherapy
MRTX849	KRAS G12C	NCT03785249: Phase 1/2 Multiple Expansion Cohort Trial of MRTX849 in Patients with Advanced Solid Tumors with KRAS G12C mutation KRYSTAL-1	characterize the safety of MRTX849 and evaluate the pharmacokinetics of the drug, ORR assessed by RECIST 1.1 criteria of MRTX849 as monotherapy
JNJ-74699157/ ARS-3248	KRAS G12C	NCT04006301: A First-in-Human Study of the Safety, Pharmacokinetics, Pharmacodynamics, and Preliminary Antitumor Activity of JNJ-74699157 in Participants with Advanced Solid Tumors Harboring the KRAS G12C Mutation	determine the MTD and RP2D of JNJ-74699157, determine the safety and preliminary antitumor activity of JNJ-74699157
BI 1701963	Pan-KRAS	NCT04111458: A Phase I Open-label Dose Escalation Trial of BI 1701963 as Monotherapy and in Combination With Trametinib in Patients With KRAS Mutated Advanced or Metastatic Solid Tumors	determine the MTD and RP2D of BI 1701963 as monotherapy and in combination with trametinib
mRNA-5671 (cancer vaccine)	KRAS G12C/ G12D/ G13D/ G12V	NCT03948763: A Phase 1, Open-Label, Multicenter Study to Assess the Safety and Tolerability of mRNA-5671/V941 as a Monotherapy and in Combination With Pembrolizumab in Participants With KRAS Mutant Advanced or Metastatic Non-Small Cell Lung Cancer, Colorectal Cancer or Pancreatic Adenocarcinoma	determine the safety and tolerability and establish a preliminary RP2D of mRNA-5671/V941 as a monotherapy and in combination with pembrolizumab infusion
KRAS-targeted long peptide vaccine	KRAS	NCT04117087: Pooled Mutant KRAS-Targeted Long Peptide Vaccine Combined With Nivolumab and Ipilimumab for Patients With Resected MMR-p Colorectal and Pancreatic Cancer	evaluate the safety and tolerability of the KRAS-based peptide vaccine, determine the drug-related toxicities, determine the fold change in interferon-producing mutant-KRAS-specific CD8 and CD4 T cells at 16 weeks
mDC3/8-KRAS vaccine	KRAS G12C/ G12D/ G12R/ G12V	NCT03592888: Pilot Study of Mature Dendritic Cell Vaccination Against Mutated KRAS in Patients With Resectable Pancreatic Cancer	evaluate the safety and tolerability of the KRAS mature dendritic cell vaccine

(*Continued on next page*)

Table 2. *Continued*

Therapeutic drug	Target	Trial	Primary objective(s)
Mutant KRAS G12V-specific TCR transduced autologous T cells	KRAS G12V	NCT04146298: Clinical Trial Evaluating the Safety and Activity of Mutant KRAS G12V-specific TCR Transduced T Cell Therapy for Advanced Pancreatic Cancer	evaluate the safety and tolerability of the KRAS G12V-specific TCR transduced T cell therapy; ORR assessed by RECIST 1.1 criteria
Anti-KRAS G12D murine TCR peripheral blood lymphocytes	KRAS G12D	NCT03745326: A Phase I/II Study Administering Peripheral Blood Lymphocytes Transduced With a Murine T-Cell Receptor Recognizing the G12D Variant of Mutated RAS in HLA-A*11:01 Patients	evaluate the safety and tolerability of anti-KRAS G12D murine TCR peripheral blood lymphocytes, ORR assessed by RECIST 1.1 criteria
Anti-KRAS G12V murine TCR peripheral blood lymphocytes	KRAS G12V	NCT03190941: A Phase I/II Study Administering Peripheral Blood Lymphocytes Transduced With a Murine T-Cell Receptor Recognizing the G12V Variant of Mutated RAS in HLA-A*11:01 Patients	evaluate the safety and tolerability of anti-KRAS G12V murine TCR peripheral blood lymphocytes, ORR assessed by RECIST 1.1 criteria

See also https://clinicaltrials.gov/. MMR-p, mismatch repair protein; MTD, maximum tolerated dose; RP2D, the highest dose with acceptable toxicity, usually defined as the dose level producing ~20% of dose-limiting toxicity; TCR, T cell receptor.

inhibitors, many of which are being explored in ongoing clinical trials, as noted in the following four subsections.

BI 1701963 (Boehringer Ingelheim)

BI 1701963 is a pan-KRAS inhibitor that interferes with KRAS binding to SOS1, a guanine nucleotide exchange factor essential in activating KRAS. By targeting and binding to SOS1, RAS activation is disrupted through the inhibition of the RAS-SOS1 interaction. Thus, due to its design, it can target mutated KRAS beyond the G12C mutation. The drug demonstrated promising antitumor activity against KRAS, and it was advanced into an ongoing phase I clinical trial as monotherapy or in combination with trametinib (NCT04111458).

AMG 510/sotorasib (Amgen)

The KRAS G12C inhibitor AMG 510/sotorasib was reported in preclinical studies to inhibit KRAS signaling and tumor growth in cell lines and patient-derived xenograft models.[104] Furthermore, the drug enhanced *in vitro* antitumor activity and tumor immune infiltration in immunocompetent mouse models when combined with targeted therapy/chemotherapy and immunotherapy, respectively. The G12C inhibitor is being investigated in an ongoing phase I/II clinical trial in advanced KRAS mutant solid tumor patients (NCT03600883).[105] The trial also contains an arm examining AMG 510 in combination with anti-PD-1/PD-L1 antibodies. The primary endpoints include the safety and tolerability of the drug as well as ORR. Preliminary data from the first 29 patients enrolled in the trial were presented at ESMO 2019.[106] Ten of the 29 patients were diagnosed with NSCLC, 5 patients of whom exhibited a partial response, 4 exhibited stable disease, and 1 patient progressed. Based on the preliminary data, no disease-limiting toxicities were noted, and the majority of patients demonstrated good clinical activity. Recently, the final results from the phase I trial showed a median PFS of 6.3 months and a median duration of response of 10.9 months in their NSCLC subgroup (n = 59).[10] Nineteen NSCLC patients (32.2%) had a confirmed ORR and 52 patients (88.1%) demonstrated disease control. At the first restaging scan at 6 weeks, 42 patients (71%) showed a decrease in tumor burden, with a median time to response of 1.4 months. The trial confirmed that AMG 510 exhibits antitumor efficacy in pretreated KRAS G12C mutant NSCLC, consistent with previous preliminary results.

JNJ-74699157/ARS-3248 (Janssen Biotech and Wellspring Biosciences)

Preclinical data allowed Nagasaka et al.[11] to move the KRAS G12C inhibitor into a phase I clinical trial in advanced solid tumors harboring KRAS G12C mutations (NCT04006301).

MRTX849/adagrasib (Mirati Therapeutics)

Similar to its competitor drugs, MRTX849/adagrasib, a KRAS G12C inhibitor, exhibited significant inhibition of KRAS signaling and tumor growth *in vivo*, as well as tumor regression in mutant KRAS G12C cell lines and patient-derived xenograft models.[107] KRAS G12C mutant patients are enrolling in the expansion cohort of the KRYSTAL (A Phase I/II Multiple Expansion Cohort Trial of MRTX849 in Patients With Advanced Solid Tumors With KRAS G12C Mutation) phase I/II clinical trial (NCT03785249). Preliminary data from the trial demonstrated great antitumor activity in 6 pretreated metastatic NSCLC patients with a >30% tumor decrease in half of those patients after 6 weeks.[12]

As the KRAS G12C trials continue, future directions will focus on strategies to prolong PFS, overcome treatment resistance by developing combination therapies, and develop more KRAS inhibitors to target other mutations. Recent preclinical work has attempted to elucidate how to maximize KRAS G12C mutant cell response to targeted therapy.[108] It has been previously reported that while KRAS G12C cells fluctuate between active and inactive forms, KRAS G12C inhibitors only bind to its inactive state, or the GDP-bound state, and leave the cell in its inactivated form.[109] The same study showed that in KRAS G12C mutant cell lines, suppressing the nucleotide exchange activity that allows cells to switch from active to inactive enhanced the inhibition of the drug. Researchers have discovered that this limitation allows tumor cells to undergo adaptive changes after the inhibitor is introduced to the tumor environment to become insusceptible to the drug.[108] Instead of relying on the drug-inhibited MEK pathway to proliferate, these adapted cells now use EGFR and aurora kinase downstream signaling cascades as an escape mechanism to remain in their active form. Another preclinical study demonstrated similar results of feedback reactivation of the RAS pathway via receptor tyrosine kinase (RTK)-mediated activation of non-mutant RAS.[110] Mainardi et al.[111] found that Src homology region 2 (SHP2), a cytoplasmic Src homology 2 domain containing protein tyrosine phosphatase that regulates several cellular processes, is necessary for KRAS mutant tumor cell growth in *in vivo* models of NSCLC. The authors also discovered that the inhibition of SHP2 promoted a senescence response in KRAS mutant NSCLC cells, suggesting a preclinical rationale for targeting SHP2 in KRAS mutant tumors.[111] Interestingly, a recent study reported that combined inhibition of KRAS and SHP2 effectively suppressed feedback reactivation of the RAS pathway and produced significant tumor regression in xenograft models.[110] These data help elucidate why the majority of KRAS G12C patients in these clinical trials tend to exhibit partial responses to treatment versus complete radiographic response. Escape mechanisms such as these need to be addressed through the development of combination targeted therapies to create a more durable response to KRAS G12C inhibitors.

ICIs and mutant KRAS

Prior research has shown that PD-L1 expression is associated with KRAS alterations in KRAS NSCLC cell lines and patient tumor tissue.[112] Also, mutated KRAS contributes to an immunosuppressive environment through phenotypic conversion of T cells[113] and is able to upregulate PD-L1 expression in preclinical lung adenocarcinoma models through MEK/ERK signaling,[112,114] suggesting a role for active KRAS in immunoediting of NSCLC. In lung adenocarcinoma tumor cells, PD-L1 expression variation correlates with KRAS, TP53, and STK11 alterations.[115] Furthermore, KRAS alterations measured in the blood by liquid biopsy next-generation sequencing was associated with favorable clinical responses to ICIs.[116] Consistent with previous reports,[8,117] a recent preclinical study found a strong connection between KRAS mutation and immune markers associated with responses to ICI treatment, such as inflammatory tumor microenvironment, increased tumor-infiltrating lymphocytes, and high tumor mutation burden.[118] These results indicate that mutant KRAS NSCLC patients in particular would gain clinical benefit from ICI treatment.

Immunotherapy with ICIs is already a lead standard-of-care treatment in NSCLC.[63,119–123] A subgroup analysis of the KRAS NSCLC population in the KEYNOTE-042 clinical trial was performed.[124] The results showed clinical benefits in patients treated with pembrolizumab monotherapy versus chemotherapy, with an ORR of 56.7% versus 18.0% in patients with any KRAS mutation (n = 69) and an ORR of 66.7% versus 23.5% in patients with KRAS G12C mutations (n = 29), respectively. The median PFS was comparable between any KRAS mutation and KRAS G12C. KRAS wild-type patients (n = 232) had a significantly smaller ORR (29.1% versus 21.0%) and observed no differences in median PFS between each arm (6 months for each treatment). A subgroup analysis of NSCLC patients treated with PD-1 inhibitors identified KRAS and STK11 co-mutations as negative predictors of response to ICIs.[62] The median PFS was significantly shorter in the KRAS-STK11 co-mutation group when compared to the KRAS-TP53 co-mutation group or the KRAS-only group (1.8 versus 3.0 and 2.7 months, respectively, $p = 0.0018$).

Overall, these clinical studies report clinical activity in KRAS mutant NSCLC patients treated with ICIs; however, further analysis is necessary to confirm a distinct benefit of KRAS mutations and immunotherapy treatment. Preclinical data have shown that KRAS G12C inhibitor AMG 510 promotes a pro-inflammatory tumor microenvironment, suggesting potential synergy between KRAS G12C inhibitors and ICIs.[104] This study also combined AMG 510 and anti-PD-1 therapy in mouse models, which produced long-term T cell responses against KRAS mutant tumor cells, providing preclinical rationale for the combination treatment of KRAS inhibitors and PD-1 blockade. There is an ongoing phase I/II study of AMG 510 treatment in KRAS G12C mutant tumors with an experimental arm to evaluate the combination therapy of AMG 510 and anti-PD-1/PD-L1 monoclonal antibodies (NCT03600883).

Targeting KRAS neoantigens

KRAS mutations create new epitopes that can be recognized by the immune system as neoantigens. The goal is to either elicit a T cell response against these neoantigens through vaccination or through adoptive T cell therapy, which are under investigation in several clinical trials (Table 2). mRNA-5671, a cancer vaccine against KRAS G12C, G12D, G13D, and G12V (from Moderna Therapeutics and Merck), is the only mRNA vaccine currently being considered. This vaccine is encoded as a neoantigen concatemer in a single RNA molecule, and once administered, is expected to be optimally presented on HLA-A11:01 and/or HLA-C*08:02 receptors. Preclinical data reported an enhanced $CD8^+$ T cell response against KRAS antigens after KRAS mRNA vaccination.[125] A phase I clinical trial examining mRNA-5671 with or without pembrolizumab is recruiting advanced or metastatic KRAS mutant NSCLC patients (NCT03948763). In resected pancreatic ductal adenocarcinoma, an ongoing pilot study is recruiting eligible patients to undergo treatment with a vaccine composed of autologous dendritic cells pulsed with mutant KRAS peptides corresponding to the KRAS mutational variant of each patient, including G12C, G12D, G12R, and

G12V (NCT03592888). A different strategy uses pooled KRAS neoantigen peptide-based vaccines in a clinical trial in combination with nivolumab and ipilimumab in patients with resected mismatch repair-proficient colorectal cancer and resected pancreatic cancer (NCT04117087). Preclinical reports in humanized mouse models demonstrated a strong induction of cytotoxic and T cell helper response against mutations through KRAS-targeted long peptide vaccinations.[126] Another strategy is to infuse in patients autologous T cells transduced with mutant KRAS-specific T cell receptors (TCRs). Preclinical data showed that KRAS mutant-specific TCRs inhibited tumor growth in immunodeficient mouse models.[127] A single case report demonstrated specific tumor response when using four different T cell clonotypes from a patient who specifically targeted KRAS G12D.[128] In another case of successful adoptive T cell therapy, four different TCRs were identified within the tumor-infiltrating T cell population, recognizing a nonamer and a decamer neoantigen specific to KRAS G12D.[129] There is a phase I/II clinical trial evaluating the safety and tolerability of KRAS G12V-specific TCR transduced T cell therapy in advanced pancreatic cancer (NCT04146298). There are also several ongoing phase I/II clinical trials examining gene transfer with murine TCRs that are equipped to recognize KRAS mutational variants such as G12D (NCT03745326) and G12V (NCT03190941) in KRAS mutant cancer patients that are HLA-A11:01, based on preclinical studies of murine T cells against epitopes in KRAS G12D/G12V mutant cells produced from HLA-A11:01 transgenic mice.[127] Even though some of the therapeutics are initially not tested in lung cancers, these approaches are expected to have more general applicability. It is also important to note that these therapeutic approaches require meaningful MHC1-peptide display of the KRAS neoantigens, which has not been demonstrated by physical detection methods, and immunoediting may select against this.

Conclusions

NSCLC is a heterogenous disease, initially subclassified histologically and currently classified by molecular markers as well. We have made strides against EGFR mutations, ALK translocations, and other genomic alterations in lung cancer. The ICIs have also led to enhanced OS in certain tumors. KRAS mutations in lung cancer have been very difficult to treat. Recent developments in therapeutics have led to the resurgence of KRAS as one of the most promising oncogenic drivers with therapeutic potential in NSCLC, and efforts to directly target KRAS oncoproteins are becoming clinically relevant. For many years, KRAS had built a reputation as being "undruggable," but several ongoing clinical trials are redefining the efficacy of different direct and indirect KRAS inhibitors through appropriate patient stratification and identification of subgroup mutations. Other treatment strategies including the inhibition of specific pathway genes such as MEK, RAF, and others are also under clinical investigation. Encouraging preliminary response and safety results for the direct allosteric irreversible inhibition of KRAS G12C has sparked a therapeutic race for FDA approval. This also triggered the development of a number of novel pan-KRAS inhibitors that interact with active KRAS independent of mutational status. KRAS inhibitors targeting specific mutations such as AMG 510 and MRTX849 are the most promising therapeutic approaches, and the biggest challenge that could prevent them from having a lasting impact in the clinic is therapeutic resistance caused by resistance mutations, phenotypic switching, and tumor plasticity, which has been noted in other targeted therapies such as EGFR and ALK. The role of ICIs in the treatment of mutant KRAS cancers is also under consideration and may enhance or may be enhanced by targeted therapy approaches. KRAS is an early mutational event in the development of NSCLC,[46,130–132] and it would therefore be interesting to see whether immunological approaches that target the mutant KRAS neoantigen have the capacity to efficiently eradicate cancer stem cells to cause a durable response. Specific KRAS inhibition has been difficult to accomplish due to the small size of the protein and a surface area with few deep pockets for drug interaction, but numerous agents under investigation in preclinical and clinical models have overcome this challenge by using the distinct alterations of KRAS mutant tumors to provide targeted therapeutics that may finally provide significantly improved survival for this patient group.

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AUTHOR CONTRIBUTIONS

All of the authors contributed to the manuscript.

DECLARATION OF INTERESTS

R.S. reports speakers' bureau participation with AstraZeneca and Merck and is an advisory board member for Janssen and Novartis. R.P., I.M., A.N., and M.S. declare no competing interests.

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