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Review

On target: Rational approaches to KRAS inhibition for treatment of non-small cell lung carcinoma

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ABSTRACT

Non-small cell lung carcinoma (NSCLC) is a leading cause of cancer death. Approximately one-third of patients with NSCLC have a *KRAS* mutation. *KRAS*^{G12C}, the most common mutation, is found in ~13% of patients. While KRAS was long considered 'undruggable', several novel direct KRAS^{G12C} inhibitors have shown encouraging signs of efficacy in phase I/II trials and one of these (sotorasib) has recently been approved by the US Food and Drug Administration. This review examines the role of *KRAS* mutations in NSCLC and the challenges in targeting KRAS. Based on specific KRAS biology, it reports exciting progress, exploring the use of novel direct KRAS inhibitors as monotherapy or in combination with other targeted therapies, chemotherapy, and immunotherapy.

1. Introduction

Lung cancer is a leading cause of cancer deaths worldwide [1]. Approximately 85% of lung cancers are non-small cell lung carcinomas (NSCLCs) [2], with the most common subtypes being lung adenocarcinoma followed by squamous cell carcinoma [3]. Over the past 20 years, the treatment of advanced NSCLC has evolved from cytotoxic therapies that result in low response rates, rapid recurrence of disease, and short survival, to therapies targeting specific genomic alterations of NSCLC identified through molecular profiling, or immunotherapies such as immune checkpoint inhibitors [3]. These new therapies have led to survival benefits in many patients; however, the long-term survival rate remains very low for patients with metastatic disease [3]. A rapidly growing list of actionable or emerging oncogenic drivers in NSCLC includes alterations such as point mutations, insertions/deletions, amplifications (e.g., *EGFR*, *BRAF*, *KRAS*, *MET*, *ERBB2/HER2*), or rearrangements (e.g., *ALK*, *ROS1*, *RET*, *NTRK*, *FGFR 1/2/3*, *NRG1*) [4–6]. Efficacious targeted treatments have recently emerged for patients with NSCLC driven by many of these genomic alterations.

In contrast to most of the oncogenic drivers named above, KRAS was for many years considered 'undruggable' because of its extremely high affinity for GTP, a lack of well-defined hydrophobic pockets, and the complexity of its downstream pathways [7,8]. Accordingly, promising preclinical results with KRAS inhibitors in early studies failed to translate into success in clinical trials [8]. The treatment landscape is now looking much more promising, following the recent US Food and Drug Administration (FDA) approval of the direct KRAS^{G12C} inhibitor sotorasib for adult patients with *KRAS*^{G12C}-mutated locally advanced or metastatic NSCLC, who have received at least one prior systemic therapy [9,10]. The accelerated approval of sotorasib was based on the favorable efficacy and safety results from phase I/II trials and represents the first approved targeted treatment for patients with a *KRAS* mutation [10–13]. Adagrasib is an investigational KRAS^{G12C} inhibitor that is also showing promise in phase I/II trials [14,15], with other similar drugs currently entering early-phase clinical trials (GDC-6036 [NCT04449874], JDQ443 [NCT04699188], and D-1553 [NCT04585035]) or preclinical (e.g. LY3537982, JAB-21822, BAY-293, and RMC-6291) testing.

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This review examines the role of *KRAS* mutations in NSCLC and the challenges faced in targeting KRAS to date, while also exploring the benefits and disadvantages of novel agents that directly inhibit KRAS as monotherapy or that are used in combination with other targeted therapies, chemotherapy, and immunotherapy.

2. *KRAS* mutations in NSCLC

2.1. *KRAS* and oncogenesis

KRAS is one of three isoforms of the *RAS* family of oncogenes, the most frequently mutated oncogene family in human cancers [16]. RAS proteins activate signaling cascades essential for cell survival, proliferation, and differentiation [17]. Approximately 86% of *RAS*-mutant cancers involve mutations in *KRAS* [16]. KRAS is a small GTPase that functions as a molecular switch, changing between an active GTP-bound state ('ON') and an inactive GDP-bound state ('OFF'). GTP-bound KRAS is located on the inner face of the cell membrane, where it interacts with and directly activates downstream signaling pathways, in particular the MAPK/ERK pathway and the PI3K pathway [18,19]. Oncogenic mutations in KRAS reduce its conversion from its GTP-bound to GDP-bound state, locking KRAS into a constitutively active form that can initiate intracellular cascades independent of extracellular signals. This leads to uncontrolled cell proliferation and survival, and contributes to tumor growth [20,21].

KRAS can have a variety of effects in cancer cells and controls complex signaling pathways [21]. Nevertheless, the TRACERx study demonstrated that most *KRAS* mutations in patients with early-stage NSCLC were clonal events [22], and an analysis of a Guardant360® database found that the $KRAS^{G12C}$ mutation was clonal in the majority of patients with NSCLC [23] (see *KRAS* mutation heterogeneity section); thus, its activation is likely an important and early event in oncogenesis. Furthermore, the prolonged response rate obtained with specific inhibitors of KRAS (see direct inhibition of KRAS section) confirm *KRAS*-dependent oncogenic addiction in this group of patients with smoking-related lung cancer.

2.2. *KRAS* mutation prevalence and prognosis

KRAS mutations are the most frequent molecular alteration in lung cancer and among the hardest to target. *KRAS* mutations are present in up to one-third of patients with NSCLC adenocarcinoma [24], although the prevalence may be lower in non-white populations (e.g., ~15% in Asians) [25]. In comparison, the frequencies of other oncogenic drivers in the white population are lower; e.g., ~15% for *EGFR* mutations (~35% in Asians; see Fig. 1 for the approximate prevalence of common oncogenic driver alterations in white patients with NSCLC adenocarcinoma) [4,5,24]. The vast majority of *KRAS* mutations are in codons 12 or 13, with a minority of mutations in codon 61 [26,27]. The most common *KRAS* mutation in NSCLC is a transversion in which the guanine at codon 12 is replaced by a cysteine (G12C). The $KRAS^{G12C}$ transversion accounts for ~41% of *KRAS* mutations in lung adenocarcinoma or NSCLC, equating to a presence in ~13% of patients with NSCLC [24]. Other mutations in *KRAS* include the substitutions G12A, G12D, G12R, G12S, G12V, G13A, C13C, G12D, G13R, G13S, and G13, and substitutions in G61 [27]; of these, G12V is the most common, occurring in ~18–20% of all *KRAS*-mutant NSCLC [24,28,29]. Although the *KRAS* mutational landscape seems complex, it may be more straightforward than for other oncogenes, as there are a finite number of alterations that allow opportunities for drug discovery. Additionally, the high frequency of *KRAS* mutations in patients with NSCLC justifies considerable molecular focus to facilitate development of new effective therapies.

Whether *KRAS* mutations affect prognosis in NSCLC is unclear, with

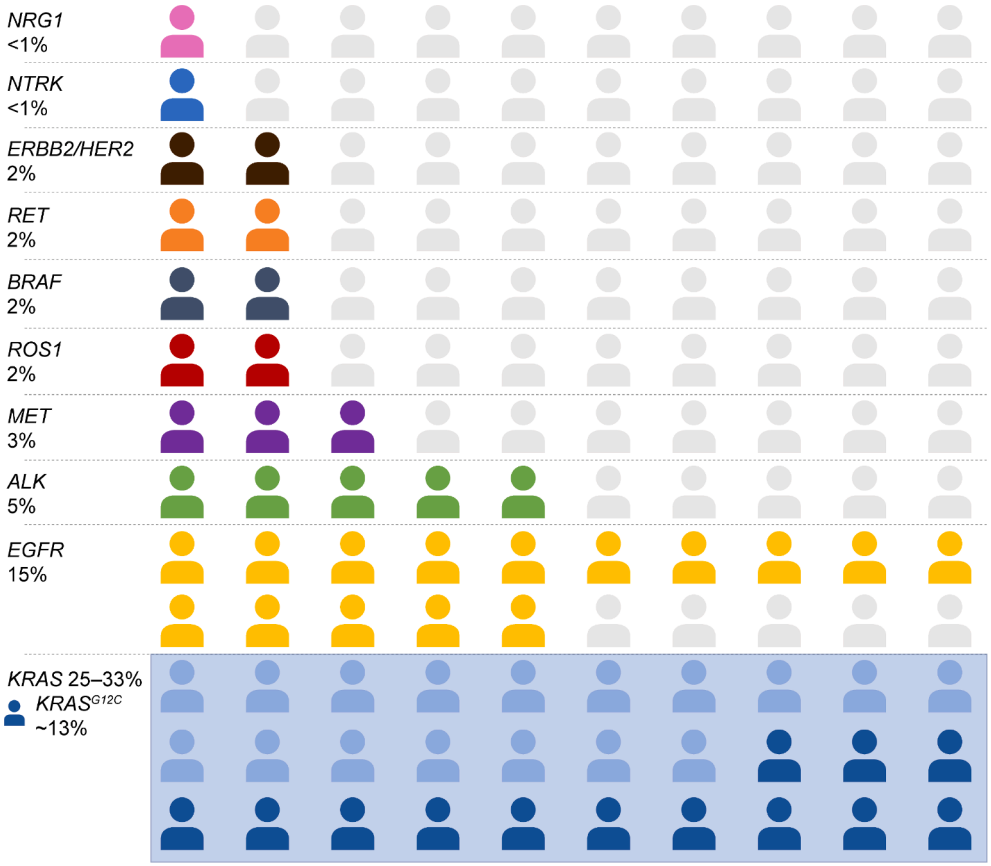

Fig. 1. Prevalence of common oncogenic driver alterations in NSCLC adenocarcinoma [4,5,24].

some studies reporting poorer survival outcomes in patients with *KRAS* mutations than in those with wild-type *KRAS* [28,30,31], and other studies finding no effect of *KRAS* mutational status on overall survival (OS) [32–36]. It has been reported that different *KRAS* variants have distinct patterns of tumor dissemination, with $KRAS^{G12C}$ associated with more bone and brain metastases and $KRAS^{G12V}$ associated with more pleural/pericardial metastases [37,38]. *KRAS* variants also differ histologically, with $KRAS^{G12C}$ less frequently associated with mucinous adenocarcinoma than $KRAS^{G12D}$ or $KRAS^{G12V}$ [39].

Most patients with *KRAS*-mutant NSCLC are current/former smokers [21] (Table S1); however, *KRAS*-mutant tumors can be present in patients without a history of smoking. One study found that 11.7% of patients who had never smoked harbored a *KRAS* mutation, and almost two-thirds of these were female [40]. This subgroup had marked genetic and clinical differences to the well-characterized smoker cohort; for example, $KRAS^{G12D}$ was the most common mutation found in 31% of patients, whereas $KRAS^{G12C}$ was found in 18.1%. Co-occurring mutations were seen primarily with *TP53* (35.2% of patients), with *KEAP1* and *PIK3CA* mutations each occurring in 4.3% of patients. Further characterization of the *KRAS*-mutant never-smoking population is warranted in light of new targeted therapies on the horizon.

2.3. KRAS co-occurring mutations

KRAS mutations are often mutually exclusive of those in other oncogenic drivers, which makes identification of other driver mutations unlikely in patients with observed *KRAS* mutations [41,42]. However, important *KRAS* co-occurring mutations in non-oncogenic drivers are relatively common [32]. One analysis found that slightly over half of patients with *KRAS* mutations had at least one additional functionally significant co-occurring mutation [32]. Co-occurring mutations observed with *KRAS* in NSCLC with potentially functional significant effects include *TP53*, *STK11*, *KEAP1 MET, RBM10*, *EPHA5*, and *ERBB2/HER2* (with the $KRAS^{G12C}$ subtype only) [32,43–45]. Co-occurring mutations in genes such as *STK11*, *KEAP1*, and *TP53* may cooperate with *KRAS* mutations to drive oncogenesis and influence therapeutic responses, as well as the ability of tumor cells to acquire resistance [46–48]. Co-occurring mutations have the potential to alter prognosis, with *STK11* and *KEAP1* mutations having a proposed negative impact on survival [45,49].

2.4. KRAS mutation heterogeneity

KRAS mutational status may be heterogeneous within a primary tumor, or between a primary tumor and metastases, although these occurrences are rare. In an analysis of *KRAS*-mutant tumors in NSCLC, some samples from individual tumors had wild-type *KRAS*, while other tumors had two different *KRAS* mutations [50]. Other studies in NSCLC have reported limited heterogeneity in *KRAS* mutational status within and between tumors [51,52], illustrating the complexity of KRAS genetics. The heterogeneity in *KRAS* mutational status observed in NSCLC is also seen in other solid tumors: in metastatic colorectal cancer (CRC), in which ~40% of patients have a *KRAS* mutation [18], ~10% of patients had discordant *KRAS* mutational status within primary tumors [53] or between a primary tumor and metastases [54].

Another important aspect of *KRAS* mutation heterogeneity is clonality. While somatic clonal mutations can occur in *KRAS,* mutations (including G12C) can arise as subclones [32], which act as a resistance mechanism to therapy [55]. In the TRACERx study, multi-region whole-exome sequencing was performed on 100 early-stage NSCLC tumors that had been resected before systemic therapy, to classify somatic mutations as clonal (present in all tumor cells) or subclonal (present in only a subset of cells) [22]. In lung adenocarcinoma, most *KRAS* mutations were found to be clonal, suggesting their emergence early in carcinogenesis, whereas only 3/26 *KRAS* mutations were subclonal somatic events. In squamous cell carcinoma, by contrast, 2/2 mutations identified in *KRAS* were subclonal late-driver mutations [22]. However, as the TRACERx study focused on early-stage NSCLC (stages IA–IIIA), the results may not fully extrapolate to stage IV disease. Nevertheless, an analysis of a Guardant360® database, which included 1783 patients with non-squamous NSCLC, also found that the $KRAS^{G12C}$ mutation was clonal (clonality >0.9) in approximately 60% of this cohort [23].

A recent study identified that $KRAS^{G12C}$-mutant tumor cells are heterogeneous in how they respond to KRAS inhibition at the single-cell level [56]. Some tumor cells, in response to suppressed MAPK output, produce new $KRAS^{G12C}$, which is maintained in its active, drug-insensitive state by EGFR and aurora kinase signaling [56]. Single-cell RNA-seq may facilitate the understanding of *KRAS* mutation heterogeneity with respect to clonality and the effects on drug resistance.

2.5. KRAS and brain metastases

About 20–40% of patients with NSCLC, especially adenocarcinoma, develop brain metastases during disease progression [26,57]. The limited ability of conventional treatments such as chemotherapy to penetrate the central nervous system (CNS) has hampered survival outcomes for these patients. Brain metastases are increasingly likely to be identified, while treatments for extracranial NSCLC continue to improve. Testing the CNS penetrance of the new KRAS inhibitors will be of importance. As third-generation EGFR and ALK inhibitors have high CNS penetrance [58,59], this might also be possible with KRAS inhibitors.

2.6. KRAS molecular analyses

KRAS can be detected through numerous procedures including PCR, Sanger sequencing or next-generation sequencing from tissue or liquid biopsy [60,61]. However, testing is currently often confined to clinical trials or academic institutions, and biomarker testing algorithms vary according to country and regional guidelines (reviewed in Kerr et al. [62]). In terms of liquid biopsy testing, non-tumor derived mutations that expand during clonal hematopoiesis are an important consideration as they can cause false positives if the results are misinterpreted [63,64]. However, as *KRAS* mutations arising from clonal hematopoiesis are rare, the impact on liquid biopsy testing may be minimal [63]. Laboratories should consider appropriate methodology and use relevant controls to mitigate the risk of false positives from liquid biopsy [65].

While the high prevalence of *KRAS* point mutations in NSCLC justifies significant molecular and clinical focus, it is possible that analysis of other *KRAS*-related features could be considered in the future to help guide clinical decision making. For example, recent data from Awad and colleagues demonstrated that mutant allele specific imbalances caused by amplification of $KRAS^{G12C}$ may be involved in resistance to $KRAS^{G12C}$ inhibitors in some patients [66]. Additionally, Nagy and colleagues indicated that the expression changes of genes affected by mutant KRAS signaling may have higher prognostic relevance than the primary genomic alteration itself [67].

3. A brief history of KRAS targeting

KRAS-mutant NSCLC has historically proven challenging to treat, given the lack of advanced targeted treatments. KRAS-inhibition strategies have included reducing the proportion of active RAS-GTP, disrupting protein–protein interactions, decreasing RAS at the plasma membrane, inhibiting downstream effector signaling, and synthetic lethality [7,68].

Despite promising preclinical/early-phase studies, KRAS drug development has been marked by repeated failures to translate into clinical success as standard of care (Table S2). Initial development focused on preventing RAS from associating with the cell membrane, e.g. by inhibiting the post-translational farnesylation of its C-terminal domain. However, the farnesyltransferase inhibitor tipifarnib

Table 1
KRAS inhibitors, including mode of action, development stage, and clinical results to date.

Drug	ClinicalTrials.gov	Mode of delivery	KRAS isoform targeted	Mode of action	Development stage	Clinical results in NSCLCto date
Clinical studies						
Sotorasib [11–13,77,81]	NCT03600883 NCT04303780	Oral	G12C (KRAS (OFF))	Covalent allosteric inhibitor	Phase I/II ongoing (CodeBreaK 100) Phase III ongoing (CodeBreaK 200)	DCR: 80.6%[a] Confirmed ORR: 37.1%[a] Grade 3 TRAEs: 19.8%[a] No DLTs
Adagrasib [14,15,78,82]	NCT03785249 NCT04685135	Oral	G12C (KRAS (OFF))	Covalent allosteric inhibitor	Phase I/II ongoing Phase III ongoing (KRYSTAL-12)	DCR: 96%[b,c]; ORR: 45%[b,c] Grade 3–4 TRAEs: 30%[b,c,d]
GDC-6036	NCT04449874	Oral	G12C	Covalent allosteric inhibitor (TBC)	Phase I recruiting	N/A
D-1553	NCT04585035	Oral	G12C	TBC	Phase I/II recruiting	N/A
JDQ443	NCT04699188	Oral	G12C	Covalent allosteric inhibitor	Phase I/II recruiting	N/A
BI-1701963 (BI-3406)	NCT04111458	Oral	Pan-KRAS	SOS1: KRAS inhibitor that binds to the catalytic domain of the GEF SOS1, preventing interaction with KRAS-GDP	Phase I ongoing	N/A
mRNA-5671 (V941)	NCT03948763	IM	G12C, G12D, G12V, G13D	Lipid nanoparticle-formulated mRNA-based cancer vaccine	Phase I ongoing, including NSCLC	N/A
Anti-KRAS engineered T-cell receptor therapy	NCT01174121	IV	–	Gene transfer therapy	Phase I/II suspended (note that trial enrolled patients with several types of solid tumors, but not NSCLC)	N/A
Preclinical studies						
LY3537982	N/A	TBC	G12C	Covalent inhibitor	Preclinical	N/A
JAB-21822	N/A	TBC	G12C	TBC	Preclinical	N/A
BAY-293	N/A	TBC	Pan-KRAS	SOS1: KRAS inhibitor	Preclinical	N/A
RMC-6291	N/A	Oral	G12C (KRAS (ON))	Promotes a ternary complex between KRAS(ON) and immunophilin cyclophilin A, causing steric occlusion of the effector face and inhibiting oncogenic signaling	Preclinical	N/A
Tri-complex inhibitors	N/A	TBC	G12C, G13C, G12D (KRAS(ON))	Promotes a ternary complex between KRAS(ON) and immunophilin cyclophilin A, causing steric occlusion of the effector face and inhibiting oncogenic signaling	Preclinical	N/A
Tetrahydroquinazoline derivatives	N/A	TBC			Patent registered	N/A
Tetracyclic compound [79]	N/A	TBC			Patent registered	N/A
KRAS-G13C inhibitor [80]	N/A	TBC	G13C		Preclinical inhibition	N/A

[a] Data from patients with NSCLC in the phase II cohort of CodeBreaK 100 (NCT03600883) [13].
[b] Data from phase I/II study (NCT03785249).
[c] Includes patients with unconfirmed partial responses.
[d] All patients (includes NSCLC, CRC, and other tumor cohorts). DCR, disease control rate; DLT, disease-limiting toxicity; IM, intramuscular; IV, intravenous; N/A, not applicable; NSCLC, non-small cell lung cancer; ORR, objective response rate; TBC, to be confirmed; TRAE, treatment-related adverse event.

(R115777), which primarily dislodges the HRAS isoform from its membrane-anchoring site, showed only minimal activity in a phase II trial [69–71], possibly due to the predominance of *KRAS* mutations in patients with NSCLC. Salirasib, an inhibitor of all RAS isoforms, also showed insufficient activity in the treatment of *KRAS*-mutant lung adenocarcinoma [69]. KRAS acquired a reputation for being undruggable due to its picomolar affinity for GTP, relatively high cellular levels of GTP compared with GDP (10-fold that of GDP), and because its structure appears to lack a suitable hydrophobic 'pocket' that could accept a small-molecule inhibitor [16].

Attention turned to strategies for targeting KRAS indirectly, by inhibiting upstream, downstream, or parallel pathways (Table S2). Experimental approaches included inhibitors of signaling proteins such as Raf, FAK, MEK, MET, and mTOR, but were associated with relatively limited success [68].

Synthetic lethality screens have been used to identify targets that would theoretically result in selective death of *KRAS*-mutant, but not *KRAS*-wild-type, cells. One identified target has been cyclin-dependent kinase (CDK) 4 [72]. In the phase III JUNIPER study (patients with *KRAS*-mutant NSCLC), the CDK4 inhibitor abemaciclib did not improve OS compared with erlotinib, a drug recognized to perform poorly in this setting [73]. Additionally, a recent UK study, the National Lung Matrix Trial, reported that only 1/30 patients with *KRAS*-mutant NSCLC showed a response to the CDK4/6 inhibitor palbociclib [74]. Overall, synthetic lethality approaches may not be optimal in *KRAS*-mutant NSCLC, where pre-existing variation could result in tumors being unresponsive to therapies targeting non-oncogene addicted pathways [75].

4. Direct inhibition of KRAS

A breakthrough in the direct targeting of KRAS occurred when advances in nuclear magnetic resonance spectroscopy and X-ray crystallography revealed the complete structure of the $KRAS^{G12C}$ mutant protein. Ostrem and colleagues discovered a switch-II pocket allosteric binding site near the effector region of mutant $KRAS^{G12C}$, and identified a small molecule, ARS-1620, that could bind covalently to this pocket [76]. This raises the possibility of developing an allosteric inhibitor of mutant $KRAS^{G12C}$ that could inhibit KRAS-dependent oncogenic

signaling without affecting signaling by wild-type KRAS. Such drugs will likely have lower toxicity than those targeting both wild-type and mutant protein. Many pharmaceutical companies are now developing more potent inhibitors of $KRAS^{G12C}$; some of these are entering clinical trials, while others remain in early development (Table 1) [11–15,77–82].

4.1. Sotorasib

The first direct $KRAS^{G12C}$ inhibitor to enter clinical testing is the small-molecule inhibitor sotorasib (formerly AMG 510). Analysis of the X-ray crystal structure of the $KRAS^{G12C}$–ARS-1620 covalent complex revealed a hidden groove in $KRAS^{G12C}$ that becomes accessible upon changing the orientation of the histidine at position 95 [11,83]. A systematic effort to optimize binding to this groove led to the development of sotorasib, with enhanced potency over the compound ARS-1620 [83].

Sotorasib is an oral drug that selectively inhibits mutant $KRAS^{G12C}$ by covalently and irreversibly binding to the cysteine in the $KRAS^{G12C}$ transversion, locking the protein in its inactive GDP-bound state ('OFF') (Fig. 2) [78,84,85]. In preclinical testing, sotorasib inhibited ERK phosphorylation and tumor cell growth in multiple *KRAS*^{G12C}-mutant cell lines *in vitro*, and in mice with xenografts of human tumor cells [11,86]. Sotorasib did not affect PI3K signaling [8]. Complete regression of tumors was observed at higher doses, with durable cures achieved in 8/10 mice [11].

Based on preclinical data, a phase I/II, first-in-human, open-label, multicenter trial evaluated the safety, tolerability, pharmacokinetics, and efficacy of sotorasib as monotherapy in heavily pretreated patients with locally advanced/metastatic $KRAS^{G12C}$ solid tumors (CodeBreaK 100; NCT03600883) [12,13,81]. The sotorasib doses for the four escalation cohorts were 180 mg, 360 mg, 720 mg and 960 mg; the exposure to sotorasib at the 960 mg dose markedly exceeded the 90% maximal inhibitory concentration identified in preclinical studies indicating near total inhibition of $KRAS^{G12C}$ during the dosing interval [12].

The phase II cohort comprised 126 patients with locally advanced/metastatic $KRAS^{G12C}$ NSCLC, who had progressed on prior standard therapies (81.0% had progressed on platinum-based chemotherapy plus PD-1/PD-L1 inhibitors); patients received oral sotorasib 960 mg once daily. Objective response rate (ORR) was 37.1% (including 4 [3.2%] patients who had a complete response), 80.6% of patients had disease control, and the median duration of response was 11.1 months. Median progression-free survival and overall survival were 6.8 and 12.5 months, respectively. Treatment-related adverse events (TRAEs) were reported in 69.8% of patients, the most common being diarrhea (31.7%), nausea (19.0%), alanine aminotransferase increase and aspartate aminotransferase increase (each 15.1%) [13,81]. Discontinuations due to TRAEs were low (7.1%); grade 3 TRAEs were reported in 19.8% of patients. One patient (0.8%) reported grade 4 TRAEs of pneumonitis and dyspnea; there were no fatal TRAEs [13,81]. Exploratory analyses demonstrated clinical activity of sotorasib across a range of co-occurring mutation profiles [13] (including patients with *KEAP1* and/or *STK11* co-occurring mutations, which may be associated with poor outcomes to therapy [87]). However, subgroup sample sizes were small and future prospective studies are warranted. Clinical activity was also demonstrated across a range of mutational allele frequencies, PD-L1 expression levels, and tumor mutational burden (TMB) levels [13,77]. Finally, an analysis of patient-reported outcome measures from CodeBreaK 100 suggested that sotorasib maintained or improved quality of life, physical functioning, and the severity of key lung cancer-related symptoms [88]. The efficacy and safety data from CodeBreaK 100 supported the recent FDA approval of sotorasib for the treatment of patients with *KRAS*-mutant NSCLC [10]. Sotorasib has recently been added to the National Comprehensive Cancer Network NSCLC guidelines as a subsequent therapy option for patients who progressed on or after platinum-based chemotherapy with/without immunotherapy [89].

A global randomized phase III trial in NSCLC, comparing sotorasib daily versus standard-of-care docetaxel in second-line therapy, is currently ongoing (CodeBreaK 200, NCT04303780) [90]. Sotorasib is also currently being tested in combination with other anticancer therapies (including EGFR, MEK, SHP2, pan-ErbB, mTOR, and CDK inhibitors, as well as immunotherapy [PD-1/PD-L1 inhibitors] and chemotherapy) in the CodeBreaK 101 trial in patients with advanced solid tumors (NCT04185883) [91].

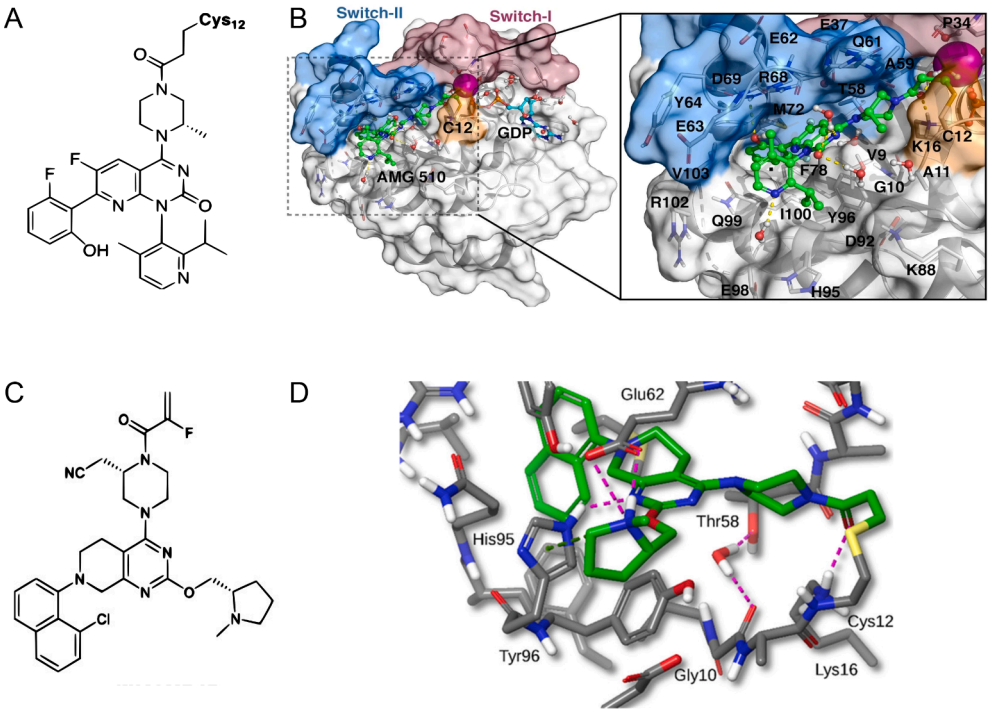

Fig. 2. Targeting $KRAS^{G12C}$ with sotorasib and adagrasib. **A–B,** The cryptic groove in $KRAS^{G12C}$ targeted by sotorasib [84]. **A,** Chemical structure of sotorasib. **B,** crystal structure of $KRAS^{G12C}$–sotorasib complex: sotorasib (green) binds to the SII-P and exploits a cryptic groove formed by H95/Y96/Q99. **C–D,** Adagrasib binds to $KRAS^{G12C}$ [78]. **C,** Chemical structure of adagrasib. **D,** X-ray crystal structure of adagrasib bound to $KRAS^{G12C}$. Panels A–B adapted from Pantsar T [84], with permission from Springer Nature in accordance with the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). Panels C–D adapted from Fell JB, et al. [78], with permission from the American Chemical Society (ACS) in accordance with the ACS Author Choice license (https://pubs.acs.org/page/policy/authorchoice_termsofuse.html).

4.2. Adagrasib

The second small-molecule inhibitor of KRAS^{G12C} to enter clinical trials is adagrasib (formerly MRTX849) [14,78]. In common with sotorasib, adagrasib locks KRAS^{G12C} into its inactive GDP-bound form ('OFF') (Fig. 2). In preclinical studies, adagrasib inhibited cell growth in multiple KRAS^{G12C} cell lines, and inhibited tumor growth *in vivo* in mice bearing patient-derived xenografts, with complete tumor regression observed in a subset of the models [92]. Beyond *KRAS^{G12C}*, no single genomic co-alteration predicted the antitumor activity of adagrasib (also the case for sotorasib), but baseline gene/protein expression of selected members of the HER family of receptor tyrosine kinases showed trend associations with the magnitude of the antitumor response [14]. As for sotorasib, adagrasib did not affect PI3K signaling [8].

Adagrasib is now being tested in a phase I/II trial (KRYSTAL-1) involving pretreated patients with advanced solid tumors (NCT03785249) [15]. The doses for the escalation cohorts were 150 mg, 300 mg, 600 mg and 1200 mg once daily, and 600 mg twice daily. The 600 mg twice daily dose maintained adagrasib above the target plasma thresholds (identified in preclinical studies) throughout the 24 dosing interval [15]. A recent update confirmed that 79 patients had received adagrasib 600 mg twice daily; of the 51 patients evaluable for clinical activity, 45% had an objective response (includes unconfirmed partial responses) and 96% had disease control (median follow-up 3.6 months) [15]. TRAEs were identified in 85% of all patients (including the NSCLC, CRC, and other tumor cohorts); the most commonly reported were nausea (54%), diarrhea (51%), and vomiting (35%). Grade 3/4 TRAEs were reported in 30% of all patients, most commonly fatigue (6%), increased alanine aminotransferase (5%), and increase aspartate aminotransferase (5%). Additionally, a phase III trial (KRYSTAL-12) of adagrasib versus docetaxel recently began enrollment of pretreated patients with *KRAS^{G12C}*-mutated NSCLC (NCT04685135). Several combination clinical trials are enrolling or planned in NSCLC with afatinib (NCT03785249), pembrolizumab (NCT03785249), TNO155 (SHP2 inhibitor; NCT04330664), and palbociclib [15].

4.3. Other KRAS-targeted molecules

Several other direct KRAS^{G12C} allosteric inhibitors are in the early stages of clinical testing as monotherapy and in combination with other therapies. These KRAS^{G12C} inhibitors include GDC-6036 (phase I; NCT04449874), D-1553 (phase I/II; NCT04585035) and JDQ443 (phase I/II; NCT04699188).

Notably, all drugs that target the GDP-bound 'OFF' KRAS state may be vulnerable to resistance mutations that induce the exchange of GDP for GTP, or that block the GTPase activity required for the formation of the GDP-bound state. Researchers are, therefore, developing inhibitors of active GTP-bound forms (RAS(ON)) of KRAS^{G12C}, KRAS^{G13C}, and KRAS^{G12D}. The inhibitors use steric occlusion to prevent the target protein from interacting with effector proteins such as Raf, required for oncogenic signaling [80], and the KRAS^{G12C} ON inhibitor induced tumor regression in a mouse NSCLC xenograft model [80].

V941, a lipid nanoparticle-formulated mRNA-based cancer vaccine that targets four of the most commonly occurring *KRAS* mutations (G12D, G12V, G13D, and G12C), is also being developed. An ongoing phase I study of patients with advanced or metastatic NSCLC, CRC, or pancreatic adenocarcinoma is evaluating V941 alone or in combination with pembrolizumab (NCT03948763).

5. Indirect inhibition of KRAS

While the *KRAS^{G12C}* mutation is the most common in NSCLC, a variety of other common driver mutations in *KRAS* can be found and may be more prevalent in other solid tumors. As a result, additional drugs are being developed that could be effective against multiple *KRAS* mutations. These include BI-1701963, a potent and selective orally bioavailable SOS1:KRAS inhibitor that binds to the catalytic domain of the guanine nucleotide exchange factor SOS1, preventing it from interacting with KRAS-GDP [93]. A phase I trial of BI-1701963 in solid tumors is ongoing (NCT04111458).

Encouraging results have also been obtained with inhibition of SHP2. SHP2 is required for complete RAS-MAPK activation, and is essential for *KRAS*-mutant carcinogenesis (Fig. 3) [94]. A SHP2 inhibitor, RMC-4630, has shown a reasonable safety profile and signs of clinical activity with a disease control rate of 67% for all *KRAS* mutations, and 75% for *KRAS^{G12C}* mutations, in a phase I trial in NSCLC (NCT03989115) [95]. In this study, 1/18 patients with *KRAS* mutations had a confirmed partial response. Preliminary data from this study also suggested that tolerability was improved with an intermittent dosing schedule versus daily dosing. Another SHP2 inhibitor, TNO155, is being studied in a phase I trial in advanced solid tumors, for use after disease progression following standard-of-care therapy (NCT03114319). This study will also include patients with *KRAS^{G12C}*-mutated NSCLC. Initial results with TNO155 indicated favorable pharmacokinetic properties with rapid absorption and a half-life of 34 h [96]. AEs were mostly grade 1/2; the most common grade $\geq$3 AEs were decreased platelets (4%), increased aspartate aminotransferase, diarrhea and decreased neutrophils (3% each) [96]. The optimal dosing schedule of TNO155 is under investigation.

6. Better together: the need for combination therapy

Even if direct inhibitors of mutant KRAS show promising efficacy in clinical trials, they are unlikely to provide enduring benefit if used as monotherapies. The activation of bypass signaling pathways, and the emergence of resistant subclones within previously responsive tumors as experienced with virtually all targeted approaches (e.g., EGFR, ALK, ROS1), means that combination therapies are likely to be essential. Targeting vertical and/or parallel pathways in addition to direct KRAS inhibition may be necessary to overcome both *ab initio* and acquired resistance mechanisms, and may result in more durable responses.

To determine how cancer cells bypass inhibition to prevent complete tumor responses, Xue and colleagues examined the effects of direct KRAS^{G12C} inhibition at single-cell resolution [56]. They found that after treatment with a KRAS^{G12C} inhibitor, only some of the tumor cells remained in the inactive 'OFF' state, whereas others responded to the suppression of MAPK signaling by producing new KRAS^{G12C} proteins that resumed KRAS-oncogenic signaling. The new KRAS^{G12C} proteins were maintained in their active 'ON' configuration by EGFR and aurora kinase signaling. This flexible adaptation thus enabled a subset of cells within the tumor to bypass the effects of KRAS^{G12C} inhibition—indicating that combination-therapy approaches, aimed at multiple targets and their associated signaling pathways (Fig. 3), will likely be required to achieve a longer and higher rate of response. An important area for further consideration and research is to define patient selection criteria for the different combinations.

Toxicity is another key research area when considering combination therapy, and should be assessed in relation to potential additive or synergistic effects. Notably, recent clinical trials reported that 20.6% and 30% of patients had grade 3/4 toxicity when receiving monotherapy with sotorasib or adagrasib, respectively (toxicities were reported for the NSCLC and all tumor cohort in the sotorasib and adagrasib trials, respectively, making any comparisons challenging). Adding chemotherapy or combining with other targeting agents could increase toxicity. Overlapping toxicity profiles between treatment combinations need careful consideration; e.g., chemotherapy often causes gastrointestinal issues in patients with NSCLC [97], which may exacerbate gastrointestinal side effects seen in some patients treated with direct KRAS inhibitors.

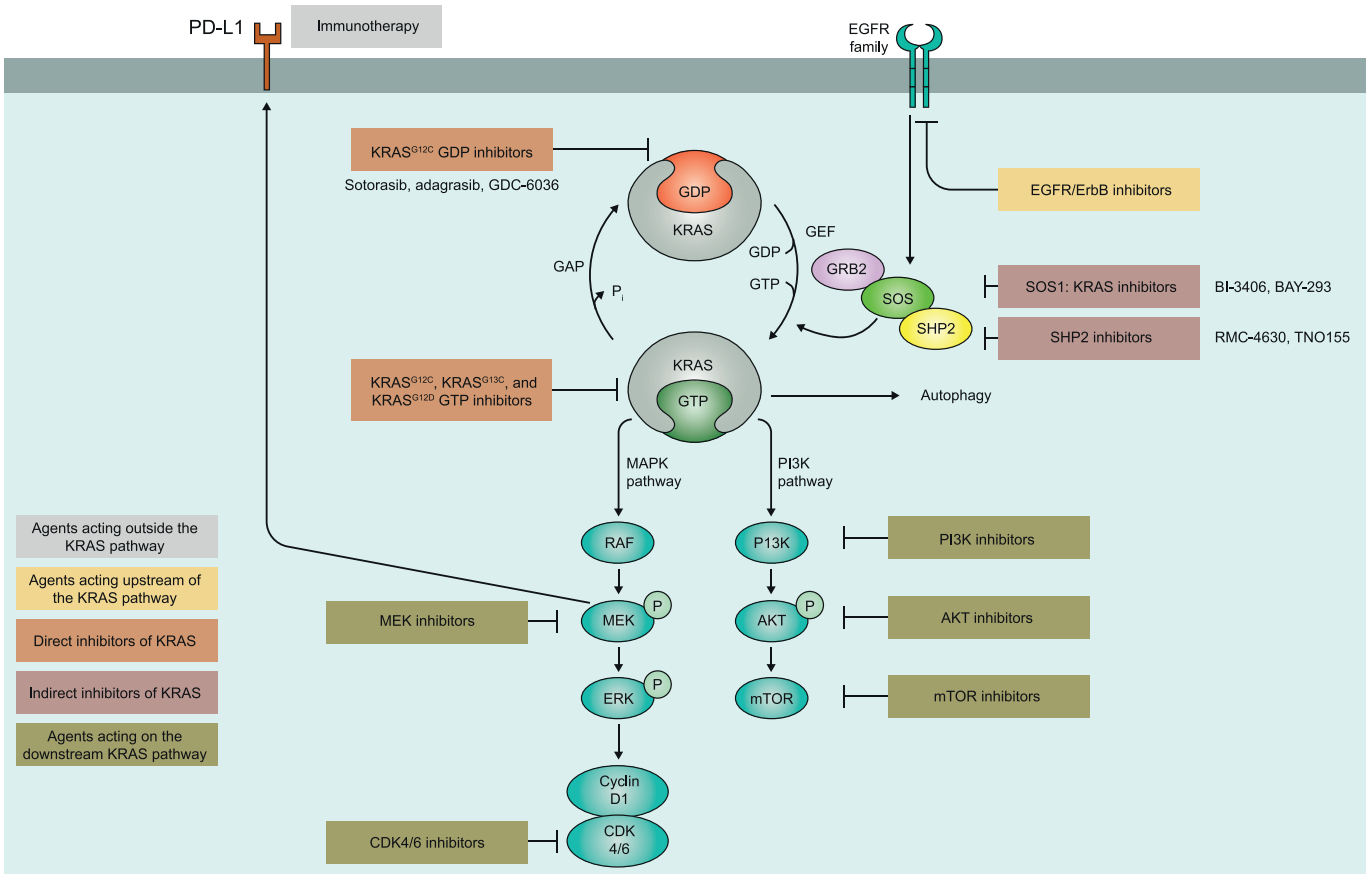

Fig. 3. Targeting *KRAS*-mutant tumors for the treatment of NSCLC. GAP, GTPase-activating proteins; GRB2, growth factor receptor-bound protein 2.

6.1. Anti-KRAS agents and chemotherapy

Chemotherapy is traditional standard-of-care for patients with *KRAS*-mutant NSCLC, but response rates have often been low and outcomes poor [98]. The LACE *meta*-analysis of four randomized trials of adjuvant chemotherapy found that *KRAS* mutations did not predict OS in patients with resected early-stage NSCLC [34]. However, elsewhere in NSCLC, combining chemotherapy with direct KRAS inhibition might boost therapeutic responses compared with either agent alone. For example, combining EGFR inhibitors with chemotherapy has shown great promise in early clinical trials [99], and the phase III FLAURA2 study (osimertinib with or without chemotherapy) is currently recruiting (NCT04035486). Trametinib, a MEK inhibitor, has shown promising results in combination with chemotherapy [100]. The MEK inhibitor selumetinib also increased chemotherapeutic efficacy and progression-free survival (PFS) in a mouse model of $KRAS^{G12C}$ NSCLC [101]. By contrast, the SELECT-1 randomized controlled trial of 510 patients with *KRAS*-mutant NSCLC found that adding selumetinib to docetaxel did not improve PFS versus docetaxel alone [102]. Preclinical evidence with direct KRAS inhibition plus chemotherapy has been promising, with the direct $KRAS^{G12C}$ inhibitor sotorasib showing significantly increased tumor cell killing when combined with carboplatin in *in vivo* mouse xenograft models [11]. Sotorasib is currently being tested in combination with chemotherapy in the CodeBreaK 101 trial (NCT04185883).

Choosing the most appropriate chemotherapy backbone could be critical, as subtypes of *KRAS*-mutant NSCLC may respond differently to chemotherapy regimens [103]. Subgroups could also exist even with the $KRAS^{G12C}$ population, perhaps requiring further treatment modifications. Commonly used chemotherapy and chemotherapy-immunotherapy backbones in NSCLC include carboplatin-pemetrexed-pembrolizumab and platinum-pemetrexed. Chemotherapy-only regimens have been almost completely replaced by combinations with immune checkpoint inhibitors for first-line treatment. An ongoing randomized phase III trial, conducted by the Netherlands Society of Pulmonologists, is comparing bevacizumab plus chemotherapy (carboplatin plus paclitaxel) with cisplatin plus pemetrexed in patients with *KRAS*-mutant advanced NSCLC (NVALT 22, NCT02743923) [104].

6.2. Direct and indirect anti-KRAS agents

KRAS mutations are negatively associated with response to other targeted therapies, potentially due to altered associations with downstream signaling transducers [105]. To identify the most promising targets for combination therapies, Misale and colleagues used high-throughput screening to examine the responses of a panel of $KRAS^{G12C}$ NSCLC models to 112 drugs in combination with the $KRAS^{G12C}$ inhibitor ARS-1620 [106]. Reactivation of the MAPK pathway and a failure to inactivate the PI3K-AKT pathway were identified as the mechanisms most likely to underlie the emergence of resistance [106]. The combination of a PI3K inhibitor plus ARS-1620 reduced tumor growth in patient-derived xenograft models that had shown resistance to ARS-1620 monotherapy, suggesting that activation of PI3K may have the potential to overcome resistance to $KRAS^{G12C}$ inhibitors [106]. Consistent with this, the combination of an mTOR inhibitor, an insulin-like growth factor 1 receptor (IGF1R) inhibitor, and ARS-1620 improved the effectiveness of KRAS inhibition in mutant lung cancer cells *in vitro* and in KRAS-driven mouse models [107].

Direct inhibitors of $KRAS^{G12C}$ have been tested in preclinical trials in combination with upstream, downstream, and parallel inhibitors of KRAS signaling (Table 2) [11,14,80,106–110], with most studies finding that the response to any given combination varies between cell lines and models. Sotorasib, for example, was tested *in vitro* in combination with

Table 2
Ongoing clinical and preclinical studies examining combinations with direct and indirect targeting of KRAS and immunotherapy.

Reference	KRAS inhibitor	Drugs tested	Trial status/key preclinical findings	Most promising combination(s)
Clinical studies				
NCT04185883 CodeBreaK 101	Sotorasib	SHP2, EGFR, pan-ErbB, mTOR, CDK, and MEK inhibitors; PD-1 and PD-L1 inhibitors	Ongoing	–
NCT04330664	Adagrasib	TNO155 (SHP2 inhibitor)	Ongoing	–
NCT03785249	Adagrasib	Afatinib, pembrolizumab (PD-1 inhibitor)	Ongoing	–
NCT04111458	BI1701963	Trametinib (MEK inhibitor)	Ongoing	–
NCT04449874	GDC-6036	Atezolizumab (PD-L1 inhibitor), cetuximab (EGFR inhibitor), bevacizumab (VEGF inhibitor), erlotinib (TKI)	Ongoing	–
NCT04699188	JDQ443	TNO155 and spartalizumab (PD-1 inhibitor)	Ongoing	–
Preclinical studies				
Misale 2019 [106]	ARS-1620 ($KRAS^{G12C}$)	Tested 112 drugs *in vitro*, and then examined top candidates *in vitro* and *in vivo*	Reactivation of MAPK pathway and failure to inhibit PI3K pathway most likely to underlie ARS-1620 resistance	PI3K inhibitor
Molina-Arcas 2019 [107]	ARS-1620 ($KRAS^{G12C}$)	mTOR and IGF1R inhibitor	Combination of mTOR inhibitor and IGF1R inhibitor increased the effectiveness of ARS-1620 in $KRAS^{G12C}$ mutant cells *in vitro* and in mouse models	mTOR inhibitor and IGF1R inhibitor used together
Canon 2019 [11]	Sotorasib ($KRAS^{G12C}$)	Inhibitors of HER kinases, EFGR, SHP2, MEK, PI3K, AKT, and anti-PD-1	Synergy was observed with multiple agents and varied between cell lines, but the combination with a MEK inhibitor was synergistic in multiple settings. Combination of sotorasib and anti-PD-1 therapy enhanced survival over sotorasib alone	MEK inhibitor Anti-PD-1
Briere 2019 [108]	Adagrasib ($KRAS^{G12C}$)	Anti-PD-1	Combination of adagrasib and anti-PD-1 therapy enhanced survival over adagrasib alone	Anti-PD-1
Hallin 2020 [14]	Adagrasib ($KRAS^{G12C}$)	Afatinib (ErbB inhibitor), mTOR inhibitors (e.g., vistusertib), and CDK4/6 inhibitor (palbociclib)	Afatinib was synergistic in most of the cell lines evaluated, and was the top hit in a combination screen *in vitro*. mTOR inhibitors and a CDK4/6 inhibitor showed synergy with adagrasib in a subset of models	ErbB inhibitor
Ryan 2020 [109]	ARS-1620 and sotorasib ($KRAS^{G12C}$)	Inhibitors of RTKs, SHP2, and MEK/ERK	Combined $KRAS^{G12C}$ inhibition and SHP2 inhibition showed the strongest synergy, with sustained RAS pathway suppression and improved efficacy *in vitro* and *in vivo*	SHP2 inhibitor
Hoffmann 2019 [110]	BI-3406 (pan-KRAS)	MEK inhibitor	Combination led to MAPK pathway blockade of regression of KRAS tumors *in vitro*	MEK inhibitor
Schulze 2019 [80]	Tri-complex inhibitor of $KRAS^{G12C}$	MEK inhibitor	Submaximal concentrations of a MEK inhibitor drove pronounced tumor cell death in combination with the $KRAS^{G12C}$ tri-complex inhibitor	MEK inhibitor

inhibitors of HER kinases, EFGR, SHP2, MEK, PI3K, and AKT [11]. Synergy was observed with a variety of agents, to variable degrees in different cell lines, but combination with a MEK inhibitor was synergistic in multiple settings. The antitumor activity of a minimally efficacious dose of sotorasib was also enhanced when combined with a MEK inhibitor *in vivo*, with efficacy greater than that seen with either agent alone. Maximal inhibition of MAPK signaling may be required for maximum efficacy of sotorasib [8,11]. Adagrasib was tested in combination with 70 different agents in an initial *in vitro* screen, before a subset of agents were selected for further examination [14]. In common with sotorasib, synergy was observed with multiple agents but varied between cell lines and xenograft models. Adagrasib showed maximum synergy with the ErbB inhibitor afatinib, followed by mTOR inhibitors such as vistusertib, the CDK4/6 inhibitor palbociclib, and the SHP2 inhibitor RMC-4550 [14]. The combination of adagrasib and TNO155 is being studied in advanced solid tumors in a phase I/II trial (NCT04330664). The combinations of GDC-6036 with erlotinib (phase I; NCT04449874) and JDQ443 with TNO155 (phase I/II; NCT04699188) are in the early stages of clinical testing. Clinical studies will need to evaluate whether synergistic efficacy can be achieved by combining KRAS inhibitors with other targeted therapies, whether toxicity profiles are acceptable (Table 2), and whether patient selection beyond $KRAS^{G12C}$ mutational status is necessary.

It is important to fully characterize direct $KRAS^{G12C}$ inhibitors and investigate mechanisms of acquired resistance in order to inform combination strategies in the clinic. Recent data indicate that both sotorasib and adagrasib bind to the histidine (H)95 surface groove in the P2 pocket of $KRAS^{G12C}$, and models of acquired resistance may involve several mechanisms [111]. However, a direct comparison of resistance mechanisms between the two inhibitors is not yet possible [66]. One recent study focusing on adagrasib found that both RAS-dependent (*KRAS* alterations and amplification of $KRAS^{G12C}$) and non-RAS-dependent (acquired bypass mechanisms involving amplifications, activating mutations, fusions, and loss-of-function mutations in other genes) resistance mechanisms may be involved [66].

Another approach under preclinical investigation is the exploitation of KRAS-driven metabolic vulnerabilities in combination with other therapies [112]. One study found that the co-occurrence of *KRAS/STK11* mutations defined a specific subgroup of tumors with high sensitivity to the combination of metformin, which induced metabolic stress, and cisplatin [113]. Further studies are warranted to identify other sensitive subgroups and the optimal drug combinations for therapy.

Finally, autophagy, a compensatory survival mechanism in tumors, can be activated by inhibition of the KRAS pathway [114]. Selectively blocking autophagy via inhibition of ULK in combination with MAPK pathway inhibition has shown promise *in vitro* [114]. Autophagy inhibitors could theoretically be combined with direct anti-KRAS strategies; however, data from such combinations have yet to be reported.

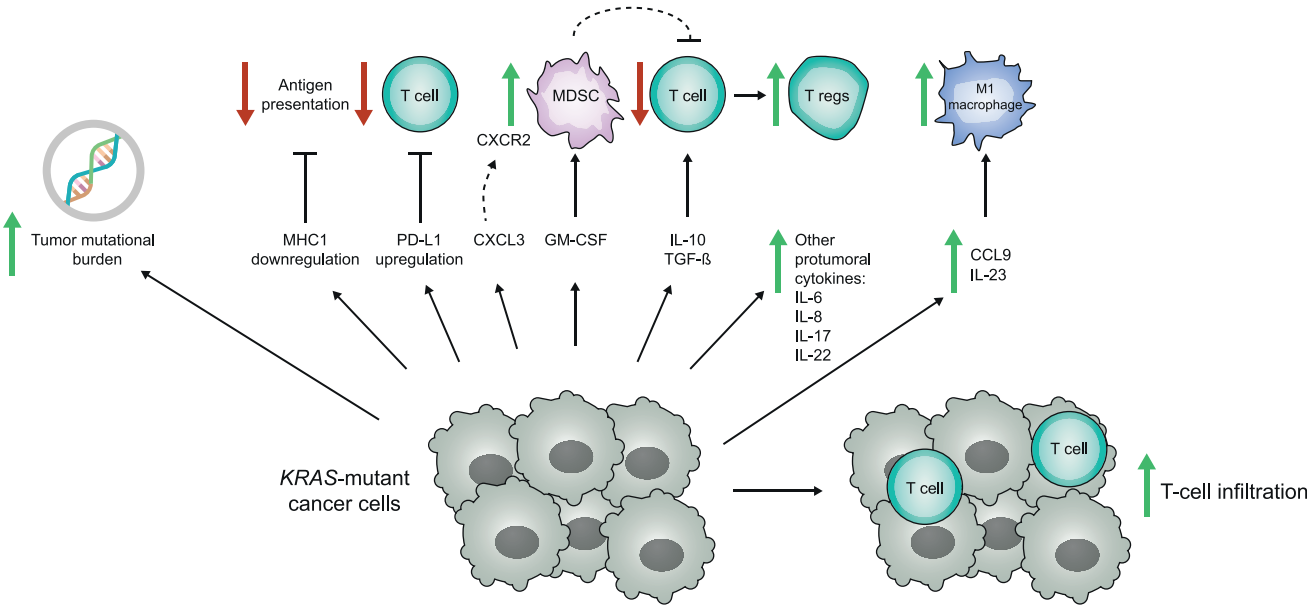

Fig. 4. Impact of *KRAS*-mutant tumors on the immune environment. CCL9, chemokine (C–C motif) ligand 9; CXCL3, chemokine (C-X-C motif) ligand 3; CXCR2, chemokine (C-X-C motif) receptor 2; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; MDSC, myeloid-derived suppressor cell.

6.3. Anti-KRAS agents and immunotherapy

Generally, response to cancer immunotherapy tends to be improved when the tumor has a proinflammatory microenvironment, high infiltration of immune cells, a high mutational burden, and/or high expression of PD-L1 [115]. *KRAS* mutations in lung adenocarcinoma are associated with tobacco smoking, a high TMB, and an inflammatory tumor microenvironment with high T-cell infiltration (Fig. 4) [116]. Mutant *KRAS* has also been shown to induce the formation of immunosuppressive regulatory T cells through the secretion of IL-10 and TGF-β1 [117]. Importantly, expression of PD-L1 is higher in *KRAS*-mutant tumors than in their wild-type counterparts [116,118–121], with median PD-L1 tumor proportion scores between 30 and 60% and 5–35% in patients with and without *KRAS* mutations, respectively [118,121]. Furthermore, oncogenic RAS signaling has been shown to increase PD-L1 expression through activation of various KRAS downstream pathways [122], including MAPK and STAT3 signaling [123,124], and by increasing phosphorylation of ERK [125]. Oncogenic RAS signaling also increases the stability of PD–L1 mRNA, via modulation of the AU-rich element-binding protein tristetraprolin [126]. Notably, one study reported that patients with $KRAS^{G12C}$-mutant tumors have a high ORR to pembrolizumab monotherapy in PD-L1-positive advanced non-squamous NSCLC [121]. However, another study reported that ORRs to anti-PD-(L)1 treatment were similar in patients with $KRAS^{G12C}$- and non-$KRAS^{G12C}$-mutated disease [33].

Co-occurring mutations can also impact response to immunotherapy. For example, PD-L1 expression is associated with *TP53* mutation in $KRAS^{G12C}$-mutant NSCLC, which correlates to a positive response to anti-PD-1 therapy [127]. In contrast, *STK11* co-occurring mutations may drive resistance to PD-1/PD-L1 inhibitors in the context of *KRAS*-mutant disease [128], highlighting the complexity when developing optimal treatment regimens.

Clinical trials have reported better responses to immunotherapy in patients with heavily pretreated NSCLC caused by *KRAS* mutations than in patients with wild-type *KRAS*. A *meta*-analysis of three studies found that the anti-PD-1 antibody nivolumab or anti-PD-L1 antibody atezolizumab extended OS in pretreated patients with *KRAS* mutations but not in patients with wild-type *KRAS* [129]. In a retrospective study of patients receiving anti-PD-1/PD-L1 immunotherapy for advanced NSCLC with at least one oncogenic driver alteration, patients with *KRAS* mutations showed a better ORR (26%) than those with other driver mutations (e.g. EGFR mutations, 12%; ALK mutations, 0%) [130]. The same investigators reported that patients with *KRAS* mutations, particularly $KRAS^{G12C}$, seem to derive a greater benefit from immune checkpoint inhibitor monotherapy than patients with other *KRAS* alterations [131]. However, there are also immunosuppressive aspects of *KRAS*-mutant tumors, which mean that immunotherapy alone has not been a comprehensive answer for *KRAS*-mutant NSCLC. For example, mutant *KRAS* is associated with the downregulation of MHC1, and production of immunosuppressive and protumoral cytokines (Fig. 4) [132,133]. Combination therapies, particularly ones that counter some of the immunosuppressive effects of *KRAS* mutations, are a rational next step.

Accordingly, immunotherapy is being investigated for use in combination with direct targeted inhibition of mutant *KRAS*. The long-term cures induced by sotorasib in mice with patient-derived xenografts depended on immune system engagement [11], suggesting that immune-checkpoint inhibition may synergize with sotorasib. Similarly, combined treatment with sotorasib and an anti-PD-1 inhibitor led to complete and durable tumor regression in 9/10 mice bearing $KRAS^{G12C}$-mutated tumors, whereas responses occurred in only 1/10 mice with either treatment alone. Further analysis showed that in mice with an intact immune system, sotorasib increased tumor infiltration by $CD8^+$ and $CD3^+$ T cells, as well as by macrophages and dendritic cells. Overall, sotorasib induced a proinflammatory tumor microenvironment, with increased markers of innate immune system activation as well as increased interferon activation, chemokine production, cytotoxic and natural killer cell activity, and MHC1 expression [11]. A phase I study of sotorasib in combination with PD-1 and PD-L1 inhibitors is ongoing (CodeBreaK 101, NCT04185883).

The direct $KRAS^{G12C}$ inhibitor adagrasib has also been shown to activate the immune system [108]. In mouse models of NSCLC, adagrasib reduced intratumoral immunosuppressive myeloid-derived suppressor cell populations, and increased populations of macrophages, dendritic cells, and $CD4^+$ and $CD8^+$ T cells. In xenograft models, adagrasib increased MHC1 protein expression. Adagrasib in combination with anti-PD-1 treatment showed durable antitumor activity [108]. A phase Ib study of adagrasib plus pembrolizumab in patients with advanced solid tumors is underway (NCT03785249). Early-stage clinical trials are recruiting with GDC-6036 plus atezolizumab, cetuximab, or bevacizumab (phase I; NCT04449874), and with JDQ443 plus

spartalizumab (phase I/II; NCT04699188).

One challenge related to the use of immunotherapy is the potential for rare but serious toxicities [134]. The timing of these may be unpredictable, sometimes emerging months after completion of immunotherapy (possibly due to the long half-life of antibodies [134]), which can impact later-line therapies [135]. If immunotherapy is to form part of combination approaches, appropriate sequencing of KRAS inhibitors and immunotherapy will be crucial. Increasing evidence suggests that concurrent use of immunotherapy and targeted therapies in NSCLC may increase the risk of adverse events [136–142]. Concurrent use of an EGFR tyrosine kinase inhibitor (TKI) plus immunotherapy, for example, was associated with relatively high rates of toxicity (including hepatotoxicity, pyrexia, diarrhea, and interstitial lung disease) [136,141,142]. Additionally, an increased incidence of grade 3/4 hepatoxicity has been reported in patients who received immunotherapy before the ALK/ROS1 inhibitor crizotinib (45.5%) compared with those who received crizotinib alone (8%) [140].

It remains unclear whether the toxicity concerns for immunotherapy combined with EGFR TKIs and ALK inhibitors will apply to KRAS-targeted therapy. As EGFR TKIs and ALK inhibitors work upstream of the KRAS pathway, they have different toxicity profiles from the direct anti-KRAS agents. Currently, most patients with *KRAS*-mutant NSCLC who receive immunotherapy do so in the first-line setting, either as monotherapy or in combination with chemotherapy. Further investigation into optimal combinations of immunotherapy with anti-KRAS agents is required, especially to determine whether use of an anti-KRAS agent before immunotherapy could improve safety and efficacy.

Data from the IMMUNOTARGET registry study in NSCLC have suggested that, in general, patients with actionable tumor alterations should receive targeted therapies and chemotherapy before considering immunotherapy as a single agent [130]. Indeed, in practice, immunotherapy is often discouraged in never-smokers with driver mutations such as *EGFR*, and targeted therapy is preferred. However, this approach cannot be translated to patients with NSCLC and *KRAS* mutations, many of whom are smokers (with a potentially increased TMB) and who may also respond well to immunotherapy; further investigation is warranted. One potential approach under investigation in a phase I clinical trial is the combination of a KRAS inhibitor (GDC-6036) with anti-VEGF antibody (bevacizumab; NCT04449874).

7. Conclusions

KRAS is the most commonly mutated oncogenic driver in NSCLC, found in approximately one-third of patients with lung adenocarcinoma; ~13% of these harbor $KRAS^{G12C}$. Thus, the development of effective KRAS targeted therapies in NSCLC could have a positive impact on the largest proportion of patients compared with targeted therapies for other less common oncogenic drivers. Until recently, attempts to develop targeted therapies for *KRAS*-mutant NSCLC repeatedly failed. This changed when advances in spectroscopy and crystallography revealed the complete structure of the $KRAS^{G12C}$ mutant protein, enabling development of direct inhibitors. One of these inhibitors (sotorasib) was recently approved by the FDA in patients with NSCLC, demonstrating that KRAS is no longer undruggable; early data on investigational $KRAS^{G12C}$ inhibitors also appear promising. While notable advancements have been made with the development $KRAS^{G12C}$ inhibitors, further studies are required to identify effective inhibitors to other KRAS mutants. Overall, there is a strong rationale to investigate combining or sequencing anti-KRAS agents with other treatment approaches, including chemotherapeutic regimens, inhibitors of signaling pathways upstream, downstream, or parallel to KRAS, as well as immune checkpoint inhibitors. Upcoming data from clinical trials with $KRAS^{G12C}$ inhibitors will inform the most promising strategies for achieving robust responses and better survival, limiting toxicity, and overcoming acquired resistance.

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Author contributions

All authors meet the International Committee of Medical Journal Editors criteria for authorship of this article, take responsibility for the integrity of the work as a whole, and have given their approval for publication of the final version.

CRediT authorship contribution statement

Colin R. Lindsay: Conceptualization, Writing - review & editing. **Marina C. Garassino:** Conceptualization, Writing - review & editing. **Ernest Nadal:** Conceptualization, Writing - review & editing. **Katarina Öhrling:** Conceptualization, Writing - review & editing. **Matthias Scheffler:** Conceptualization, Writing - review & editing. **Julien Mazières:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

C. R. Lindsay has received personal fees from Amgen for lectures/workshops and is a consultant/advisor for Amgen and CBPartners. He has received institutional research funding from Amgen, Apollomics, Boehringer Ingelheim, Mirati Therapeutics, Revolution Medicines, and Roche for his work as a Chief/Principal Investigator on clinical trials.

M. C. Garassino has, over the past 5 years, received grants, personal fees, and/or participated in advisory boards for AstraZeneca, Bayer, Blueprint Medicine, Bristol Myers Squibb, Celgene, Eli Lilly, GlaxoSmithKline, Incyte, Janssen, Novartis, Otsuka Pharma, MSD, Pfizer, Regeneron, Roche, Sanofi-Aventis, and Spectrum Pharmaceuticals; grants from Clovis, Exelixis, Ipsen, MedImmune, Merck, Merck Serono, Tiziana Sciences, and United Therapeutics Corporation; personal fees and/or participated in advisory boards for Boehringer Ingelheim, Daiichi Sankyo, Inivata, Mirati Therapeutics, Seattle Genetics, and Takeda; and non-financial support from Eli Lilly and MSD. M. C. Garassino also participated as a Principal Investigator in clinical studies for AstraZeneca, Blueprint Medicine, Bristol Myers Squibb, Celgene, Clovis, Eli Lilly, Exelisis, Ipsen, MedImmune, Merck Serono, MSD, Novartis, Otsuka Pharma, Pfizer, Roche, Sanofi-Aventis, Spectrum Pharmaceuticals, and Tiziana Sciences.

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K. Öhrling is an employee of Amgen (Europe) GmbH, and holds stocks in Amgen.

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Data sharing statement

This manuscript does not include any unpublished patient data; all reported data are publicly available in the scientific literature.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lungcan.2021.07.005.

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