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Targeting KRAS G12C with Covalent Inhibitors

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Abstract

KRAS is the most frequently mutated oncogene in cancer. Following numerous attempts to inhibit KRAS spanning multiple decades, recent efforts aimed at covalently targeting the mutant cysteine of KRAS G12C have yielded very encouraging results. Indeed, one such molecule, sotorasib, has already received accelerated US Food and Drug Administration approval with phase III clinical trials currently underway. A second molecule, adagrasib, has also progressed to phase III, and several others have entered early-phase clinical trials. The success of these efforts has inspired an array of novel approaches targeting KRAS, with some reporting extension to the two most common oncogenic KRAS mutations, G12V and G12D.

1. BACKGROUND: THE RAS PATHWAY

RAS proteins are small guanine nucleotide–binding proteins that cycle between an inactive GDP-bound state and an active GTP-bound state. RAS is localized to the inner leaflet of the plasma membrane, and it is activated by upstream signaling through receptor tyrosine kinases (RTKs), such as the epidermal growth factor receptor (EGFR), in response to extracellular stimulation by growth factors (**Figure 1*a***). RTK activation by growth factors induces autophosphorylation of tyrosine (Tyr) residues on their C-terminal tail. These phosphotyrosine residues serve as the binding sites for two SH2-containing adapter proteins, SHC and GRB2, which in turn recruit the guanine nucleotide exchange factor SOS to the membrane. Colocalization of SOS with RAS results in exchange of GDP for GTP on RAS, and activation of downstream signaling (Aronheim et al. 1994). Signaling through RAS is then terminated by the activity of GTPase-activating proteins (GAPs), which stimulate hydrolysis of GTP to GDP, with release of phosphate (Trahey & McCormick 1987, Xu et al. 1990). In the active state, RAS signals through multiple downstream pathways, including RAF/MEK/ERK and PI3K/AKT, among others, to regulate transcription, translation, proliferation, and survival (reviewed in Downward 2003).

2. RAS STRUCTURE AND MUTATIONAL ACTIVATION

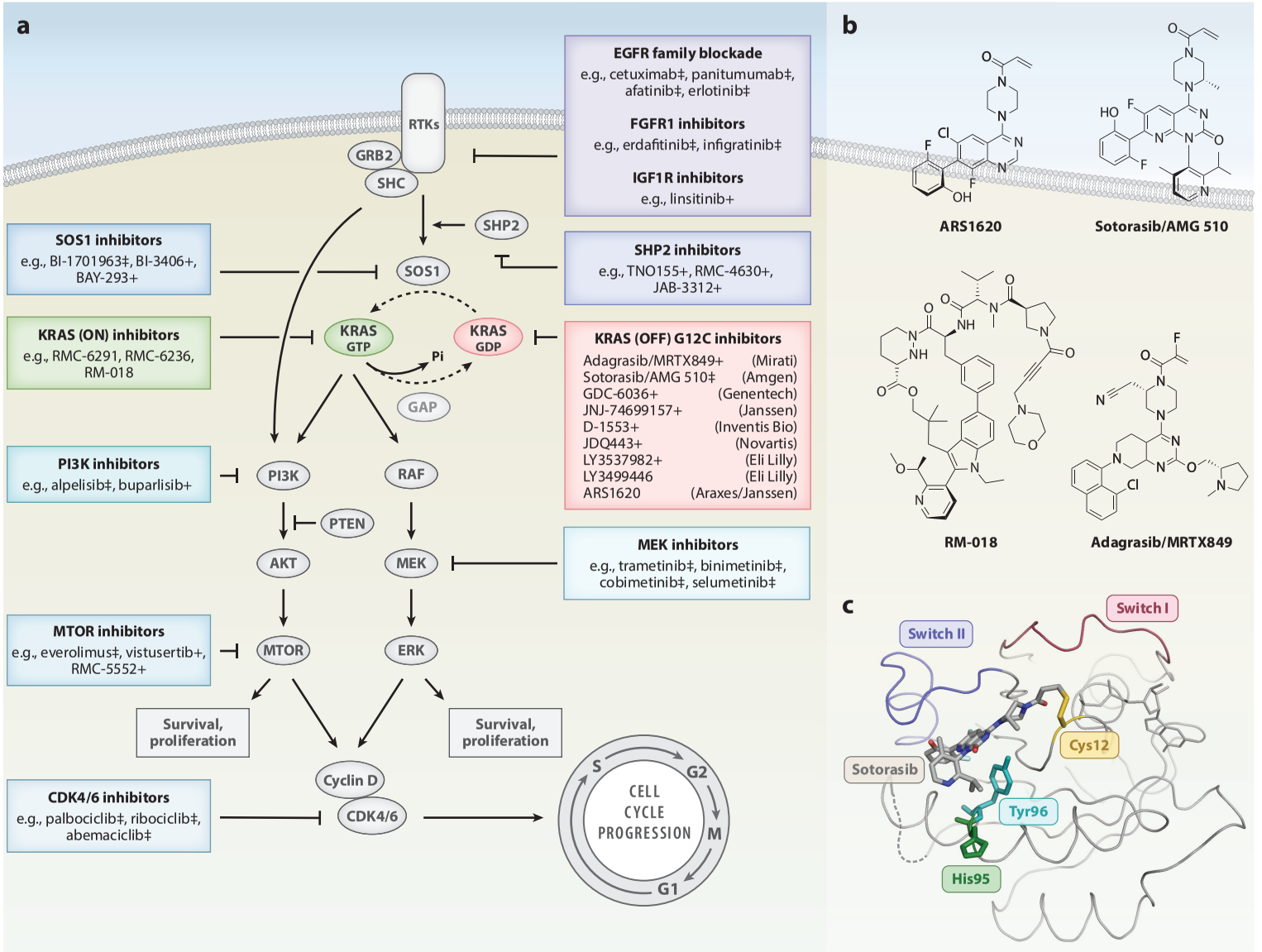
RAS proteins are expressed by three genes (*HRAS*, *NRAS*, and *KRAS*), encoding a total of four proteins with high sequence homology, particularly in the amino-terminal 165 residues. The C-terminal 23–24 residues make up the hypervariable region of RAS, differing significantly between isoforms. The hypervariable region of each RAS protein includes sequences and posttranslational modifications involved in localization to the inner leaflet of the cell membrane. All RAS proteins undergo lipidation with a farnesyl isoprenoid at the cysteine (Cys) of the C-terminal C-A-A-X moiety (Casey et al. 1989) consisting of Cys (C), two aliphatic amino acids (A-A), and a terminal amino acid (X). This is followed by cleavage of the terminal A-A-X and methylation of the new C terminus. Each RAS hypervariable region contains at least one additional membrane-localizing feature, including a second lipidation or a polybasic sequence of amino acids.

The most common sites of oncogenic RAS mutations, glycine-12 (Gly12), Gly13, and glutamine-61 (Gln61), all border the nucleotide-binding pocket, adjacent to the terminal phosphate of the nucleotide. Mutations at positions 12 and 13 sterically clash with the critical arginine (Arg) finger of the GAP involved in stabilizing the transition state, and thus generally impair GAP-catalyzed GTP hydrolysis (Scheffzek et al. 1997, Trahey & McCormick 1987). The side chain of Gln61 plays a role in activating the water molecule involved in GTP hydrolysis, and mutations at this position can affect both intrinsic and GAP-catalyzed hydrolysis (Frech et al. 1994).

KRAS mutations differ in their biochemical effects. While most mutations at Gly12 make RAS insensitive to GAP-stimulated hydrolysis, they vary in their effects on intrinsic GTP hydrolysis and nucleotide exchange. Some mutations at Gly13 and Gln61 only partially block GAP-stimulated hydrolysis, while also increasing intrinsic nucleotide exchange (Hunter et al. 2015, Smith et al. 2013). These differences between KRAS mutations can be clinically meaningful. While mutations at Gly12 confer insensitivity to EGFR antibody therapy in colorectal cancer, tumors expressing KRAS G13D do show some sensitivity (De Roock et al. 2010, Tejpar et al. 2012). Conversely, in the context of ERK inhibition, tumors expressing mutations at Gly12 become dependent upon the phosphatase SHP2, whereas those expressing G13D do not (Ahmed et al. 2019).

3. RAS MUTATIONS IN CANCER

While mutations in each of the RAS proteins are found in cancers, KRAS mutations account for ~85% of RAS mutations overall. KRAS mutations are especially common in pancreatic



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

KRAS signaling pathway and potential combination therapies. (*a*) Schematic representation of the KRAS signaling pathway listing GDP state–targeting KRAS (OFF) G12C inhibitors (*red box*) and GTP state–targeting KRAS (ON) inhibitors (*green box*). Many studies point toward benefit of combination therapies with inhibitors of additional nodes in the pathway, and these are highlighted as well, including molecules that have advanced to clinical trials (+) or have achieved US Food and Drug Administration approval (‡). (*b*) Chemical structures of KRAS G12C inhibitors ARS1620, adagrasib, and sotorasib are shown along with the KRAS (ON) inhibitor RM-018. (*c*) X-ray crystal structure of sotorasib bound to KRAS G12C (Protein Data Bank ID: 6OIM). Abbreviations: CDK4/6, cyclin-dependent kinases 4 and 6; EGFR, epidermal growth factor receptor; GAP, GTPase-activating protein; RTKs, receptor tyrosine kinases; Pi, phosphate.

(>90%), colorectal (25–40%), and lung (15–25%) adenocarcinomas (Biankin et al. 2012, Boch et al. 2013, Buttitta et al. 2006, Neumann et al. 2009, Reinersman et al. 2011, Staudacher et al. 2017). NRAS mutations occur at highest frequency in cutaneous melanoma (12–20%) (Akslen et al. 2008, Curtin et al. 2005, Lee et al. 2011), hepatocellular carcinoma (15%) (Challen et al. 1992), and acute myeloid leukemia (~10%) (Auewarakul et al. 2006, Bowen et al. 2005), and less frequent HRAS mutations have been identified in cancers of the bladder and head and neck, among others (Kodaz 2017).

Specific mutations in RAS proteins vary across cancer types. KRAS G12C is the most common KRAS mutation in lung adenocarcinoma, accounting for 40–50% of KRAS mutations and present in more than 13% of advanced lung adenocarcinomas (Nassar et al. 2021, Riely et al. 2008, Rodenhuis et al. 1987). In resected stage I–III lung cancers, this mutation was found in 17% of adenocarcinomas with a prevalence of 10.5% across all histologies (Finn et al. 2021). KRAS G12C also occurs at lower frequency in colorectal, small bowel, and appendiceal cancers (~3% each), and at ~1% each in gynecologic and pancreatic cancers. KRAS mutations in lung cancer occur at a higher rate among those with a history of smoking tobacco than those with limited to no exposure to tobacco (so-called never-smokers), and among these patients G12C is most common. When KRAS mutations do occur in never-smokers, KRAS G12D appears to predominate (Riely et al. 2008). In contrast with lung adenocarcinoma, in colorectal and pancreatic adenocarcinomas, KRAS G12D is most common, followed by KRAS G12V.

Although KRAS G12C is a less common mutation in colorectal cancer, it occurs at a high rate in adenocarcinomas and benign adenomas of patients with MYH-associated polyposis (Aime et al. 2015, Jones et al. 2004). In addition, KRAS G12C has been reported as a resistance mechanism in a patient with BRAF-mutant colorectal cancer treated with combined BRAF/MEK inhibition as well as in a patient with EGFR-mutant lung cancer treated with an EGFR inhibitor (Belchis et al. 2016, Oddo et al. 2016).

4. KRAS MUTATIONS AND CURRENT CANCER TREATMENT

In addition to their role as driver mutations in a variety of cancers, KRAS mutations have been shown to play a predictive or prognostic role in some cancers. As discussed above, KRAS mutations other than G13D predict lack of response to EGFR-directed therapy in colorectal cancers (Karapetis et al. 2008, Lièvre et al. 2006). In non-small-cell lung cancer (NSCLC), KRAS mutations are not independent predictive markers for response to chemotherapy, targeted therapy, or immunotherapy. KRAS mutations are associated with a worse prognosis in both NSCLC and colorectal cancer, with some differences between mutations (Andreyev et al. 1998, Guan et al. 2013). KRAS G12C in particular may be associated with worse prognosis compared with other KRAS mutations in resected stage I–III NSCLC (Finn et al. 2021). However, the same association was not seen in advanced lung cancer (Sebastian et al. 2021). In advanced and recurrent colorectal

cancer, *KRAS* codon 12 mutations are associated with worse prognosis, and this effect is driven primarily by KRAS G12C and G12V (Jones et al. 2017).

Comutations also play an important role. In particular, comutation of KRAS with KEAP1 or STK11 appears to be associated with shorter response to therapy and worse prognosis overall (Arbour et al. 2018). STK11 mutations result in decreased immune surveillance (Schabath et al. 2016). In combination with KRAS mutations, STK11 mutations predict primary resistance to immune checkpoint inhibitors, which are now part of first-line therapy for most KRAS-mutant lung cancers (Skoulidis et al. 2018). KRAS mutations themselves have immunomodulatory effects. Activation of KRAS has been associated with downregulation of MHC class I (MHC-I), which plays a critical role in immune response by cytotoxic T lymphocytes (He et al. 2013). In models of KRAS-mutant pancreatic ductal adenocarcinoma and NSCLC, MHC-I was shown to undergo lysosomal degradation (Yamamoto et al. 2020). Conversely, genomic or antibody-based inactivation of KRAS increased MHC-I surface expression in colorectal cancer cell lines (El-Jawhari et al. 2014). Despite this, multiple studies have shown that patients with KRAS-mutant NSCLC benefit from immune checkpoint therapy at least as well as unselected cohorts and may even obtain greater benefit (Jeanson et al. 2019, Kim et al. 2017).

While the addition of immunotherapy has benefited many patients with advanced KRAS-mutant NSCLC, the duration of response to first-line therapy still tends to be relatively short for most (median 9–10 months), and second-line therapy generally yields even shorter responses (median 3–4 months) (Gandhi et al. 2018, Garon et al. 2014, Reck et al. 2016). In colorectal cancer, apart from those with microsatellite instability, most patients do not benefit from current immunotherapies regardless of KRAS mutational status (André et al. 2020). First-line therapy generally consists of combination chemotherapy, and it is also associated with a fairly short duration of response (median ~7 months), with subsequent therapies yielding even lower likelihood and duration of response (Bokemeyer et al. 2011). In both diseases the need to prolong treatment response is clear.

5. MUTANT-SELECTIVE INHIBITORS OF KRAS G12C

Efforts to inhibit RAS in cancer span the past 40 years, with limited success until recently. In 2013, we reported the development of covalent, mutant-selective inhibitors of KRAS G12C (Ostrem et al. 2013). This class of inhibitors critically depends on the presence of the mutant Cys, and therefore affects only the mutant protein while sparing wild-type KRAS. These molecules bind beneath switch II, in a pocket that is only accessible in the inactive, GDP-bound state of KRAS (**Figure 1**). In doing so, they prevent KRAS from adopting the active, GTP-bound conformation in which KRAS interacts with downstream effector proteins such as RAF and PI3K. An extensive medical chemistry effort yielded compounds with potent activity in G12C-mutant cancer cell lines (Lito et al. 2016, Patricelli et al. 2016). Ultimately these efforts led to the discovery of ARS1620, the first KRAS G12C inhibitor with efficacy in vivo in patient-derived xenografts (Janes et al. 2018) (**Figure 1*b***).

One key residue adjacent to the switch II pocket, histidine-95 (His95), plays a critical role in enhancing the potency of switch II–binding molecules (**Figure 1*c***). Efforts to identify molecules capable of targeting the GTP-bound, active state of KRAS led to the discovery of a cryptic groove adjacent to the switch II pocket that is created by rotation of His95 (Gentile et al. 2017), facilitating a π–π stacking interaction with this residue. A similar interaction with His95 was used to enhance the potency of switch II binders based on earlier analogs of ARS1620 (Zeng et al. 2017). In this case a functional group is extended around His95 without inducing rotation of the residue. Two subsequent compounds based on modified scaffolds, adagrasib (MRTX849) and

Table 1 Clinical trials of KRAS G12C inhibitors initiated to date

KRAS G12C inhibitor	Combination therapies under investigation	Trial phase	Study sponsor	Monotherapy in NSCLC	Monotherapy in CRC
Sotorasib	PD-1, PD-L1, SHP2, MEK, pan-ErbB, EGFR, mTOR, CDK4/6, chemotherapy	I/II/III	Amgen	$N = 124$, ORR 31%, DCR 80%	$N = 42$, ORR 7%, DCR 74%
Adagrasib	PD-L1, EGFR, pan-ErbB, SHP2	I/II/III	Mirati Therapeutics	$N = 51$, ORR 45%, DCR 96%	$N = 24$, ORR 17%, DCR 94%
JDQ443	PD-1, SHP2	I/II	Novartis	ND	ND
D-1553	Chemotherapy	I/II	InventisBio	ND	ND
GDC-6036	PD-1, VEGF, EGFR	I	Genentech	ND	ND
JNJ-74699157	NA	I	Janssen Pharmaceuticals	ND	ND
LY3499446	EGFR, CDK4/6	I/II	Eli Lilly and Company	Study terminated due to toxicity	

Abbreviations: CRC, colorectal cancer; DCR, disease control rate; NA, not any; ND, no data; NSCLC, non-small-cell lung cancer; ORR, objective response rate.

sotorasib (AMG 510), each employ one of these modes of interaction with His95 to maximize potency (Canon et al. 2019, Hallin et al. 2020) (**Figure 1*b***).

The availability of compounds capable of potent inhibition of KRAS in cells has facilitated additional insights into the biology of KRAS and oncogenic mutants. Despite early biochemical data suggesting ongoing flux through the RAS cycle, the prevailing view for multiple decades was that RAS mutants existed locked in the active GTP-bound conformation (John et al. 1988). However, this view is directly contradicted by the effectiveness of KRAS G12C inhibitors. These inhibitors exclusively target the GDP-bound conformation of RAS, yet they potently inhibit KRAS G12C in cells. Indeed, perturbations directed at increasing KRAS GTP levels, such as EGF stimulation, have been shown to decrease target engagement and decrease sensitivity to G12C inhibitors in cells (Patricelli et al. 2016). Conversely, cotreatment with EGFR inhibitors such as erlotinib and afatinib decreases the proportion of KRAS-GTP in G12C-mutant cell lines, facilitating increased target engagement and increased sensitivity to G12C inhibitors. Similarly, the introduction of the second-site mutations A59G or Q61L, which further impair GTPase activity and increase KRAS-GTP, decreases sensitivity to G12C inhibitors (Lito et al. 2016).

6. KRAS G12C INHIBITORS IN CLINICAL TRIALS AND BEYOND

To date, a total of seven irreversible inhibitors of KRAS G12C have advanced to clinical trials, including sotorasib (AMG 510, Amgen), adagrasib (MRTX849, Mirati Therapeutics), JNJ-74699157 (Janssen), LY3499446 (Eli Lilly), GDC-6036 (Genentech), D-1553 (InventisBio), and JDQ443 (Novartis), with a trial planned to open soon for an eighth, LY3537982 (Eli Lilly) (**Table 1**). Early efficacy data from both sotorasib and adagrasib have been very encouraging, and both appear to be well tolerated, with predominantly gastrointestinal side effects (Hong et al. 2020, Jänne et al. 2020). Evaluation of 124 NSCLC patients from the phase II portion of the sotorasib trial revealed an objective response in 37% of patients and disease control in 81% (Skoulidis et al. 2021) (**Table 1**). Median progression-free survival was 6.8 months, and median overall survival was 12.5 months. Evaluation of the phase I colorectal cancer cohort showed a lower rate of response (7%), but disease control was confirmed in 74% of patients (Hong et al. 2020). Based on the results of this trial, sotorasib was granted accelerated FDA (US Food and Drug Administration) approval for the treatment of locally advanced or metastatic G12C-mutant NSCLC in May 2021. Results have been equally encouraging for adagrasib. Although results

from the phase I/II trial have not yet been published, a recent presentation reported interim analysis of 51 NSCLC patients with a disease control rate of 96% and objective response rate of 45% (Jänne et al. 2020). In addition, of 18 evaluable patients with colorectal cancer, they reported disease control in 94% and an objective response in 17%.

Metastases to the central nervous system (CNS) occur frequently in NSCLC, and only a fraction of available therapies cross the blood–brain barrier sufficiently to provide benefit. Encouragingly, preclinical evaluation of adagrasib revealed significant CNS penetration in mice, and at least one patient experienced response of a small CNS metastasis after initiating adagrasib (Jänne et al. 2020).

All of the abovementioned clinical G12C inhibitors contain a chemically reactive acrylamide group. Selectivity is provided by interaction with the switch II pocket of KRAS G12C, which facilitates reaction with the mutant Cys. Despite this, some inhibitors based on chemically related scaffolds have had different toxicity profiles. Indeed, at least one clinical candidate has failed in trials due to toxicity (LY3499446). The covalent adduct formed upon reaction with the acrylamide group lends itself to mass spectrometric analyses to identify off-targets. However, such proteome-wide analyses in G12C-mutant cell lines did not identify significant engagement of non-KRAS, off-target Cys residues either for the preclinical compound ARS1620 or for sotorasib (Canon et al. 2019, Patricelli et al. 2016).

One challenge facing ATP-competitive kinase inhibitors is that the ATP-binding pocket is highly conserved across kinases. This leads to difficulty in achieving specificity for one kinase over others. Toxicity related to kinase inhibitors frequently stems from either off-target inhibition of other kinases or on-target/off-tissue toxicity from inhibition of the kinase in normal tissues (Klaeger et al. 2017). As described above, KRAS G12C inhibitors do not suffer from on-target/off-tissue toxicity by virtue of the fact that they are incapable of inhibiting wild-type KRAS. In addition, while there are approximately 150 small GTPases expressed in the cell, with over 30 in the RAS family alone, these are unlikely sources of toxicity due to the nature of the pocket targeted by G12C inhibitors. Unlike most kinase inhibitors, KRAS G12C inhibitors bind in an allosteric pocket that is only formed upon compound binding. In addition, only two GTPases contain a natural Cys at the position analogous to Gly12 in KRAS. These factors together make off-target inhibition of a small GTPase an unlikely source of toxicity.

7. RESISTANCE TO G12C INHIBITORS

While KRAS G12C inhibitors have shown encouraging results as single agents in early-phase clinical trials, it is already clear that resistance can develop rapidly. To maximize benefit from this approach, it will be critical to elucidate the mechanisms by which tumors adapt and evade these inhibitors. A wide range of both mutational and nonmutational mechanisms of resistance to G12C inhibitors have already been described, with all clinical resistance data so far coming from patients treated with adagrasib. Several putative resistance mechanisms have been identified through biopsy or circulating tumor DNA from patients with NSCLC, colorectal cancer, and appendiceal cancer following progression on adagrasib (Awad et al. 2021, Tanaka et al. 2021). No single mechanism appears to dominate in terms of frequency, and a surprising number of patients show evidence of multiple mechanisms of resistance arising simultaneously. In fact, among those with identified resistance mechanisms, no two patients' tumors harbored the same pattern of new genomic alterations.

As one would expect, second-site mutations within the drug-binding pocket do arise in patients treated with KRAS G12C inhibitors. In particular, mutations at His95, Tyr96, or Arg68 were identified in multiple patients, and such mutations appear to interfere with drug binding by eliminating

critical interactions (Awad et al. 2021). In vitro analyses suggest that additional second-site mutations can also confer resistance, either by directly interfering with drug binding or by changing the balance between the GDP and GTP states of KRAS. As discussed above, current clinical G12C inhibitors are only capable of binding to the GDP-bound state of KRAS. Second-site mutations that enhance accumulation of GTP-bound KRAS by either decreasing nucleotide hydrolysis or increasing the intrinsic exchange rate also confer resistance in cellular assays.

Mutational heterogeneity, that is, tumors harboring cells driven by distinct KRAS mutations, may also play a role in resistance to G12C inhibitors. Such mutational heterogeneity may be common in KRAS-mutant lung cancer. KRAS G12C mutations in NSCLC occur more frequently in patients with a significant history of tobacco smoke exposure, and more extensive smoking history also correlates with increased tumor mutational burden (Wang et al. 2021). Prior to the development of G12C inhibitors, separate biopsies from at least one patient had been reported to harbor two different KRAS mutations, with G12C identified in the primary lung tumor and G12R identified in a lymph node (Schmid et al. 2009). While they were not detected at diagnosis, codon changes corresponding to non-Cys driver mutations at Gly12, Gly13, and Gln61 were identified by circulating tumor DNA in patients with adagrasib-resistant NSCLC and colorectal cancer (Awad et al. 2021, Tanaka et al. 2021). Mutational heterogeneity may play an even bigger role in resistance in colorectal cancer. Whereas in lung cancer, KRAS mutations are thought to be initiating events, in colorectal cancer KRAS mutations tend to occur later in tumorigenesis. Consistent with this, alternative driver mutations in KRAS appeared more common in patients with colorectal cancer at time of progression (4 of 6 patients) than in NSCLC (2 of 10 patients), although this difference was not statistically significant (Awad et al. 2021).

In addition to mutations within KRAS itself, many additional RAS pathway alterations have been identified in patients progressing on adagrasib. These changes include mutational activation of EGFR, RET, NRAS, BRAF, MEK, and PI3K and inactivating mutations in NF1 and PTEN, as well as gene fusions involving ALK, RET, FGFR3, and RAF proteins (Awad et al. 2021, Tanaka et al. 2021). Indeed, only three patients showed isolated changes at the KRAS locus without an additional resistance mechanism identified: one with an isolated KRAS second-site mutation and two with amplification of KRAS G12C. All other patients with on-target KRAS resistance mutations had evidence of additional RAS pathway alterations, raising the possibility that next-generation KRAS inhibitors may not overcome resistance in these cases.

Another potential source of resistance is the rewiring of signaling pathways to decrease dependence on KRAS. Such adaptation may explain resistance in some cases where no new genomic alteration is identified. Pathway rewiring is well documented in the case of kinase inhibitors. In HER2-driven breast cancer, for example, RTK inhibitors targeting EGFR and HER2 achieve only transient inhibition of downstream PI3K/AKT signaling due to a compensatory increase in signaling through HER3 (Sergina et al. 2007). Similarly, in BRAF-mutant melanoma and colorectal cancer, inhibition of BRAF results in loss of negative feedback from activated ERK, which in turn leads to accumulation of RAS-GTP and reactivation of ERK through CRAF (Corcoran et al. 2012, Lito et al. 2012). While dramatic responses were seen in studies evaluating single-agent BRAF inhibitors in melanoma and a majority of patients benefitted, rapid reactivation of ERK in colorectal cancer led to primary resistance for most patients, likely due to higher levels of EGFR activation in colorectal cancer cells compared with melanoma cells (Corcoran et al. 2012, Kopetz et al. 2015, Sosman et al. 2012).

The GDP state dependence of KRAS G12C inhibitors predicts another potential source of resistance. In vitro analyses suggest that sensitivity to G12C inhibitors is affected by perturbations that alter the proportions of GDP- and GTP-bound KRAS (Lito et al. 2016, Lou et al. 2019, Patricelli et al. 2016). In many lung cancer cell lines, KRAS inhibition leads to only transient

suppression of downstream signaling, with rapid reaccumulation of both KRAS-GTP and phosphorylated ERK (Xue et al. 2020). While this reaccumulation correlates with insensitivity in 2D growth, cells remain sensitive in 3D growth (Patricelli et al. 2016). Moreover, unlike for BRAF inhibitors in colorectal cancer, this finding has not corresponded to a high rate of intrinsic resistance to G12C inhibitors in lung cancer. In cell culture, there appears to be a heterogenous response to G12C inhibition even among genetically identical cells, driven in part by adaptive activation of KRAS through EGFR and aurora kinase (Xue et al. 2020). However, the disconnect between sensitivities in 2D culture and those in 3D culture and in patients does raise the question of how well signaling responses seen in 2D culture reflect those occurring in patient tumors.

Finally, persistent signaling through PI3K/AKT may represent an important mechanism of resistance. Signaling through the PI3K/AKT pathway can be activated by KRAS but can also occur through KRAS-independent mechanisms. While G12C inhibitors effectively block signaling through RAF/MEK/ERK, activation of PI3K/AKT signaling frequently persists (Misale et al. 2019). Signaling through PI3K/AKT has been shown to play an important role in epithelial-to-mesenchymal transition (EMT), which is also a potential mechanism of resistance to G12C inhibitors (Adachi et al. 2020, Xu et al. 2015). Shifts in cell state such as EMT broadly confer therapy resistance by making cells resistant to apoptosis (Viswanathan et al. 2017). While this shift generally renders cells susceptible to inhibition of the lipid peroxidase GPX4, to date no clinical inhibitors of GPX4 have been reported.

8. OVERCOMING RESISTANCE WITH COMBINATION TREATMENTS

The GDP state specificity of KRAS G12C inhibitors predicts mechanisms of resistance, but also points toward potential combination therapies. Due to the ongoing nucleotide cycling of KRAS G12C, this mutant remains partially dependent upon upstream signaling for activation, and the balance of GDP- and GTP-bound KRAS is easily shifted. While cells are likely to adapt to treatment by increasing KRAS-GTP and thus decreasing engagement by G12C inhibitors, combination treatments aimed at shifting the balance toward the GDP state may help to prevent this adaptation (**Figure 1*a***).

The state dependence of G12C inhibitors has led to the evaluation of an array of combination treatments targeting upstream signaling. As discussed above, EGFR inhibitors were shown to enhance sensitivity to G12C inhibitors in lung cancer cell lines (Lito et al. 2016, Lou et al. 2019). A similar, though less pronounced, effect was seen for inhibitors of other RTKs, including MET, FGFR, and SRC. In colorectal cancer cells, while multiple RTKs appear to feed into KRAS activation, EGFR was identified as a central driver of intrinsic resistance (Amodio et al. 2020). The addition of EGFR blockade to KRAS G12C inhibitors halted proliferation and increased cytotoxicity in cancer cell lines, and rapidly induced tumor regression in patient-derived xenografts. In pancreatic cancer cells, signaling through FGFR appears to drive compensatory KRAS activation, and FGFR inhibitors strongly sensitize cells to G12C inhibitors (Lou et al. 2019). Knockdown of the exchange factor SOS1 was also shown to increase sensitivity to G12C inhibitors (Lito et al. 2016). In addition, early work with the KRAS-SOS1 interaction inhibitor BAY-293 suggests synergy with G12C inhibition in proliferation assays, and another SOS1 inhibitor, BI 1701963, is already under investigation in phase I trials, alone and in combination with trametinib (Gerlach et al. 2020, Hillig et al. 2019, Hofmann et al. 2021).

As discussed above, SHP2 appears to play an important role in RTK-mediated KRAS activation. Multiple studies have identified SHP2 as a candidate for combinatorial treatment with G12C inhibitors. SHP2 inhibitors enhance the antiproliferative effect of G12C inhibitors in cell culture (Lou et al. 2019, Santana-Codina et al. 2020). In addition, SHP2 inhibitors have been shown to

overcome adaptive resistance to G12C inhibitors in vitro and in vivo in models of lung, colorectal, and pancreatic cancer (Fedele et al. 2021, Liu et al. 2021, Ryan et al. 2020). Trials combining G12C inhibitors with SHP2 inhibition are already underway.

Another rational strategy for which there is strong scientific basis is the combination of G12C inhibitors with inhibitors of cyclin-dependent kinases 4 and 6 (CDK4/6). Cell cycle progression through G1-S phase depends on phosphorylation of retinoblastoma protein (RB) by CDK4 or its close homolog, CDK6. Cdk4 was identified as having a synthetic lethal interaction with oncogenic Kras in a mouse model of NSCLC (Puyol et al. 2010). Despite this promising finding, monotherapy with CDK4/6 inhibitors in KRAS-mutant NSCLC showed no survival benefit in a phase III clinical trial (Goldman et al. 2018). Temporal proteomics studies showed upregulation of cell cycle–related proteins following prolonged exposure to G12C inhibitors, and both this analysis and genome-wide CRISPR interference implicated CDK4/6 inhibitors as having potential benefit in combination (Lou et al. 2019, Santana-Codina et al. 2020). Consistent with this, the addition of a CDK4/6 inhibitor enhanced the antiproliferative effect of the G12C inhibitor ARS1620, and the effect was even more pronounced in 3D culture and in mouse xenografts. Clinical trials evaluating combined G12C and CDK4/6 inhibition are also underway.

Activated KRAS can signal through a wide array of downstream pathways. However, RAF/MEK/ERK and PI3K/AKT/mTOR pathways appear to be the most critical for survival of KRAS-mutant cancers, and successful blockade of both pathways is critical to overcome adaptation to inhibition of either pathway individually (Engelman et al. 2008, Sos et al. 2009, Wee et al. 2009). Preclinical studies of KRAS G12C–mutant lung, colon, and pancreatic cancers, including patient-derived xenografts, suggest broad activity of PI3K inhibitors in combination with direct inhibitors of KRAS G12C (Lou et al. 2019, Misale et al. 2019, Santana-Codina et al. 2020). Highlighting the potential for KRAS-independent activation of PI3K signaling, IGF1R (insulin-like growth factor 1 receptor) inhibition was also found to enhance sensitivity to G12C inhibitors, particularly in combination with mTOR inhibitors (Molina-Arcas et al. 2019).

Beyond pathway-related approaches to overcoming resistance, the combination of G12C inhibitors with immunotherapy holds particular promise. As discussed above, patients with KRAS-mutant NSCLC are among those who respond well to immunotherapy. In addition, mouse studies suggest that treatment with sotorasib induces a proinflammatory tumor microenvironment that might enhance response to immune checkpoint blockade (Canon et al. 2019). Such combination regimens are already under investigation in the clinic, including a first-line trial combining adagrasib with the immune checkpoint inhibitor pembrolizumab.

9. APPROACHES TO EXTEND TARGETING OF KRAS G12C

Since the initial description of covalent KRAS G12C inhibitors, several efforts have developed with the hopes of maximizing the effect of target engagement. These have primarily focused on the induction of new protein-protein interactions. PROTACs (proteolysis-targeting chimeras) engaging Cys12 have led to successful degradation of KRAS when based on a VHL ligand but were incapable of inducing ubiquitination of KRAS when based on a thalidomide analog (Bond et al. 2020, Zeng et al. 2020). Bifunctional molecules coupling the G12C inhibitor ARS1620 with immunophilin ligands are capable of inducing association of KRAS with FKBP12 and cyclophilin A (Zhang & Shokat 2019). Similar bifunctional molecules based on KRAS binders targeting the non-natural M72C mutant successfully induce association with cyclophilin A and block binding to BRAF. Warp Drive Bio, now a part of Revolution Medicines, has also developed molecules that induce association between KRAS and immunophilins. In contrast with other G12C inhibitors, these molecules are capable of binding the GTP-bound conformation and have therefore been

dubbed RAS(ON) inhibitors. One such molecule, RM-018, was shown to overcome resistance conferred by the Y96D second-site mutation (Tanaka et al. 2021) (**Figure 1*b***).

10. PROSPECTS TO DIRECTLY TARGET KRAS MUTANTS BEYOND KRAS G12C

The clinical successes of sotorasib and adagrasib in patients with KRAS G12C–driven tumors importantly demonstrate dependence on this oncogenic lesion and show that therapeutic benefit can be achieved by targeting KRAS, much like Gleevec in 1999 represented a breakthrough for targeting a new class of signaling molecules essential for cancer therapy. The identification of a druggable allosteric binding site on KRAS G12C has opened a new avenue for directly targeting other even more common oncogenic alleles of KRAS, including G12D and G12V. In lacking the covalent handle provided by the mutant Cys, these mutations present an even greater challenge in achieving potent binding. Now the race is on to find small-molecule drug approaches that target tumors driven by these so-called non-G12C KRAS mutants, which represent more than 150,000 new patients each year. Indeed, several press releases from separate companies suggest that such molecules may be coming soon.

11. CONCLUSIONS

Since the initial report of small molecules targeting KRAS G12C, we have seen truly remarkable progress leading to a growing number of clinical candidates, with sotorasib the first to receive accelerated FDA approval. While KRAS G12C inhibitors have not immediately led to durable responses for patients, clinical trials in pretreated lung and colorectal cancers have demonstrated disease control in a majority of patients for both sotorasib and adagrasib, with prolonged responses in a subset. Despite this, it is clear that the potential of inhibitors targeting KRAS G12C is far from maximized.

So far, all G12C-targeted agents to reach clinical trials target the same switch II pocket, and all exclusively engage the GDP state of KRAS. While the state dependence of G12C inhibitors has helped uncover a surprising degree of flux through the GTPase cycle, this feature is also a liability in the treatment of KRAS-driven cancers. KRAS is a central signaling node, and cells have a multitude of ways to push the balance toward the active GTP-bound state in response to KRAS inhibition. The mechanism by which cells achieve this depends to some extent on cell type, but many such mechanisms overlap. Indeed, despite differing collateral dependencies on RTKs, reactivation of KRAS appears to be broadly dependent on SHP2 across different tissues of origin.

The expanding base of knowledge on mechanisms of resistance to G12C inhibitors has inspired an array of combination therapies now under investigation in the clinic. While single-agent trials have already shown the potential for G12C inhibitors to benefit a vast number of patients, as for BRAF inhibitors in melanoma, it is highly likely that combination therapies will help to enhance and prolong responses. Novel methods that leverage the G12C and the switch II pocket also hold promise, with the induction of unnatural protein-protein interactions having the potential to overcome some, but not all, mechanisms of resistance. Looking forward, overcoming the state dependence of current G12C inhibitors and expanding to additional KRAS mutants will be two of the most important advances to come, and they appear to be just on the horizon.

DISCLOSURE STATEMENT

J.M.L.O. and K.M.S. are joint inventors on a University of California Board of Regents–owned patent application covering inhibitors of KRAS, which has been licensed to Araxes Pharma LLC.

They also hold stock in and are consultants to Araxes Pharma LLC. In addition, K.M.S. has consulting agreements, which involve monetary and/or stock compensation, for the following companies: Black Diamond Therapeutics, BridGene Biosciences, Denali Therapeutics, Dice Molecules, eFFECTOR Therapeutics, Erasca, Genentech/Roche, Janssen Pharmaceuticals, Kumquat Biosciences, Kura Oncology, Mitokinin, Revolution Medicines, Type6 Therapeutics, Venthera, Turning Point, Ikena, Initial Therapeutics, and BioTheryX.

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