Review

Direct GDP-KRAS^{G12C} inhibitors and mechanisms of resistance: the tip of the iceberg

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***Abstract*:** Kirsten rat sarcoma viral oncogene homolog mutations are observed in 25% of lung adenocarcinoma and 40% of these are G12C mutations. Historically, no approved targeted agents were available for patients with any *KRAS* mutation, and response rates to standard-of-care therapies were suboptimal. Newly developed inhibitors directed toward KRAS^{G12C} have been successful in clinical trials with overall response rates ranging between 32% and 46%, and two FDA approvals were granted in May 2021 and December 2022 as second-line or later monotherapies. However, rapid tumor resistance complicates their use as a monotherapy. With the rapid development of this novel class of inhibitors, it is important to discern the different types of tumor resistance that may arise and how each can differently contribute to tumor growth and survival. G12C inhibitor resistance is under investigation and combinations of therapies with G12C inhibitors have been proposed. Much of this insight is gleaned from preclinical investigations, as our knowledge of clinical resistance is in its infancy. In this review, we summarize the preclinical development of KRAS^{G12C} inhibitors, their clinical evaluations, different types of resistance mechanisms to these compounds, and ways of overcoming them. Finally, we underscore the importance of basic and translational investigations of these molecules in a landscape where their clinical evaluations garner the most attention, and we set the stage for what is to come.

Keywords: clinical trial, drug resistance, G12C, KRAS, preclinical, targeted therapy

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Introduction

Over the last several decades, many oncogenes have been identified in non-small-cell lung cancer (NSCLC).[1] Research has led to the development and approval of small-molecule inhibitors which specifically target mutant oncogenic proteins.[2] However, until recently,[3] the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) was deemed undruggable by small-molecule inhibitors. With the identification of a previously under-appreciated pocket in the GDP-bound form of KRAS^{G12C}, compounds capable of trapping KRAS^{G12C} in its inactive, GDP-bound state have been generated.[4] Many inhibitors of this class are currently under clinical investigation for NSCLC and colorectal cancer (CRC) (Table 1), and objective response rate (ORR) or disease control rates (DCRs) of leading compounds, sotorasib and adagrasib, ranging between 32% and 46% and 88% and 96% for NSCLC, respectively.[5,6] To date, both sotorasib and adagrasib are approved as a second-line or later monotherapy in locally advanced or metastatic *KRAS*^{G12C} NSCLC.[7,8]

Preclinical studies have identified secondary resistance mechanisms to these G12C inhibitors,[9–17] and investigators have recently begun a search for potential combination strategies to overcome these resistance mechanisms using cell lines and patient-derived xenograft (PDX) models.[9–17] While clinical data for these resistance mechanisms are scarce thus far[18–20] likely due to the novelty of these inhibitors, it is expected that more data are forthcoming. Here, we summarize

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the rapidly progressing study of KRAS^{G12C} inhibitors (G12Ci) and the advances in our understanding of intrinsic, adaptive, and acquired resistance to this novel class of inhibitor.

KRAS

KRAS is a 21 kDa GTPase which cycles between its inactive GDP-bound and active GTP-bound forms with the help of guanine exchange factors (GEFs) and GTPase activating proteins (GAPs).[21] Three mammalian *RAS* genes (*KRAS*, *NRAS*, and *HRAS*) encode for four proteins (KRAS4A/4B, NRAS, and HRAS), and all RAS proteins possess highly conserved G-domains but dissimilar hypervariable regions[22,23] (Figure 1(a)). The effector lobe (residues 1–86) of the G-domain contains the p-loop (facilitates guanine nucleotide binding), and the switch-I and switch-II regions[24,25] (binding interfaces for effector proteins and RAS regulators) (Figure 1(b)). The allosteric lobe (residues 87–166) contains membrane-interacting regions and motifs that allow for guanine nucleotide specificity and binding.[26]

Several KRAS downstream pathways exist and the best defined in the NSCLC repertoire are MAPK, PI3K/AKT, and RalGEF, with each playing sometimes overlapping roles in cell proliferation (MAPK), differentiation (MAPK), apoptosis (PI3K/AKT), survival (PI3K/AKT), and migration (RalGEF), among other cellular processes.[23]

KRAS gain-of-function mutations are observed in ~25% of lung adenocarcinomas (LUAD),[27,28] are more prevalent in Caucasians *versus* Asians, and in current or former smokers *versus* never smokers.[29] KRAS glycine (G)-12 mutations lead to accumulation of active, GTP-bound KRAS[30] and account for roughly 90% of all *KRAS* mutations, where *KRAS*^{G12C} is the most prevalent G12 mutation in LUAD (~40%).[31,32] G12 mutations were previously believed to lock KRAS in its active, GTP-bound state.[21] However, active intrinsic GTP hydrolysis does occur in mutant-KRAS,[30,33] with KRAS^{G12C} having the highest rate of the most common KRAS mutants, comparable to wild-type KRAS.[30] GTP-bound KRAS^{G12C} also uniquely engaged the rasGEF, sons-of-sevenless (SOS), in a similar fashion to wild-type KRAS.[34] These data highlight the structural variation between KRAS-mutants and the uniqueness of KRAS^{G12C}.

Changes in spatial molecular dynamics also lead to functional differences. Immortalized human bronchial epithelial cells (HBEC) stably expressing *KRAS*^{G12C} and shRNA knockdown of *TP53* (HBECsi-*TP53*) expressed less p-AKT than the *KRAS*^{G12D} HBECs, and this was validated in *KRAS*^{G12C} NSCLC lines, which expressed less p-AKT than other mutants and *KRAS*^{WT}.[35] As well, *KRAS*^{G12C}-expressing HBECsi-*TP53* expressed the RalA and RalB effectors to a greater extent than other mutants.[35] These data suggest the need for unique therapeutic regimens depending on the specific *KRAS* mutation identified. Extensive efforts have been made to target mutant-*KRAS*, and the first clinical success has come with inhibitors directed toward KRAS^{G12C}.

KRAS^{G12C} direct inhibitors

In 1997, Ganguly *et al.* first targeted the switch-II pocket of GDP-KRAS^{G12C} with the non-covalent inhibitors SCH 54292 and SCH5434.[36] Ostrem *et al.*[4] expanded on this work and in 2013 led an exploration of cysteine reactive small molecules that bound to this underappreciated pocket, and the lead compound 12 was identified as the first GDP-KRAS^{G12C} direct-targeting irreversible inhibitor specific for KRAS^{G12C}. Upon binding to KRAS, two mechanisms impaired KRAS signaling: (1) nucleotide affinity was shifted to prefer GDP *versus* GTP, leading to the accumulation of inactive KRAS and (2) interactions with effectors and regulatory proteins were hindered, further reducing KRAS signaling. Unfortunately, this compound could not be studied *in vivo* due to poor pharmacologic properties.

Patricelli *et al.* subsequently utilized structure-based drug design to identify ARS-853, which engaged KRAS^{G12C} to a 600-fold greater extent than compound 12 in the NCI-H358 cell line.[4,37] The group later optimized the scaffold of ARS-853 using a quinazoline core[38] to make ARS-1620, which had a 10-fold improvement in covalently modifying KRAS^{G12C} over ARS-853 and possessed pharmacokinetic and pharmacodynamic properties suitable for *in vivo* use.[39] ARS-1620 reduced the pool of active KRAS, and a resultant inhibition in MAPK and PI3K/AKT signaling was observed in all NSCLC cell lines tested.[11,13,37,40] Cell-derived xenografts also had varying responses to ARS-1620 monotherapy, between complete regression to no impact whatsoever.[10,11,39,41] Overall, while these data were promising, it was

Table 1. List of current clinical trials evaluating GDP-KRAS^{G12C} inhibitors for all indications.

Drug	Trial name	Participants (Phase #)	Indication	Combinations	Trial identifier
Leading clinical candidates					
Sotorasib	CodeBreak 100	793 (PI/II)	Adv. solid tumors	PD-1i, PD-L1i	NCT03600883
	CodeBreak 101	1054 (PI/II)	Adv. solid tumors	Chemotherapy (NSCLC, CRC), CDK4/6i, EGFR TKI, MEKi, mTORi, PD-1i, PD-L1i, SHP2i, anti-EGFR Ab, anti-VEGF Ab	NCT04185883
	CodeBreak 105	12 (PI)	Adv. solid tumors	–	NCT04380753
	CodeBreak 200	345 (PIII)	Adv. NSCLC	Versus docetaxel	NCT04303780
	CodeBreak 201	170 (PII)	Adv. NSCLC, as a first line	–	NCT04933695
	CodeBreak 300	153 (PIII)	Adv. CRC	Versus chemotherapy (CRC), combination with anti-EGFR Ab	NCT05198934
	–	Expanded access	Adv. NSCLC	–	NCT04667234
	–	43 (PI/II)	Adv. NSCLC with untreated brain mets	Anti-VEGF Ab	NCT05180422
	–	59 (PI/II)	Adv. pancreatic cancer	Chemotherapy (pancreatic cancer)	NCT05251038
	LungKG12Ci	300 (Retrospective)	Adv. NSCLC	–	NCT05273047
	–	27 (PII)	Neoadjuvant, Stage IIA-IIIB non-squamous NSCLC	Chemotherapy (NSCLC)	NCT05118854
	–	46 (PII)	Adv. NSCLC	SHP2i	NCT05054725
	Lung-MAP	116 (PII)	Stage IV/recurrent non-squamous NSCLC	–	NCT04625647
	RAMP203	53 (PI/II)	Adv. NSCLC	Raf/MEKi	NCT05074810
	–	140 (PI)	Adv. NSCLC	AURKAi	NCT05374538
	MERIT-lung	43 (PII)	Stage III unresectable NSCLC	–	NCT05398094
	–	25 (PII)	Stage Ib-IIIA NSCLC	–	NCT05400577
	Argonaut	85 (PI)	Adv. solid tumors	SHP2i	NCT05480865
	–	30 (PI/II)	All NSCLC	Pan-HER TKI	NCT05313009
		37 (PII)	Adv. NSCLC	–	NCT05451056
	SOLUCOM	100 (PII)	Adv. NSCLC	–	NCT05311709
	HERKULES-2	200 (PI/II)	Adv. NSCLC	ERK1/2i, SHP2i, EGFR TKI	NCT04959981
Adagrasib	KRYSTAL-1	740 (PI/II)	Adv. solid tumors	EGFR TKI, PD-1i, anti-EGFR Ab	NCT03785249
	KRYSTAL-2	86 (PI/II)	Adv. solid tumors	SHP2i	NCT04330664
	KRYSTAL-7	250 (PII)	Adv. NSCLC	PD-1i	NCT04613596

(Continued)

Table 1. (Continued)

Drug	Trial name	Participants (Phase #)	Indication	Combinations	Trial identifier
	KRYSTAL-10	420 (PIII)	Adv. CRC	Versus chemotherapy (CRC), combination with anti-EGFR Ab	NCT04793958
	KRYSTAL-12	340 (PIII)	Adv. NSCLC	Versus docetaxel	NCT04685135
	KRYSTAL-14	100 (PI)	Adv. NSCLC	SOS1i	NCT04975256
	KRYSTAL-16	50 (PI)	Adv. NSCLC	CDK4/6i	NCT05178888
	RAMP204	85 (PI/II)	Adv. NSCLC	Raf/MEKi	NCT05375994
	Neo-Kan	42 (PII)	Stage IB-IIIA NSCLC	PD-1i	NCT05472623
	–	24 (PI)	Adv. solid tumors	–	NCT05263986
	–	Expanded access	Adv. solid tumors	–	NCT05162443
	–	133 (PI/II)	Adv. solid cancers	SHP2i, PD-1i	NCT04418661
JDQ443	kONTrasT-01	425 (PI/II)	Adv. solid tumors	PD-1i, SHP2i	NCT04699188
	kONTrasT-02	360 (PIII)	Adv. NSCLC	Versus docetaxel	NCT05132075
	kONTrasT-03	346 (PI/II)	Adv. solid tumors	MEKi, anti-EGFR-Ab, CDK4/6i	NCT05358249
	–	120 (PII)	Adv. NSCLC with $<1\%$ PD-L1 or $\geqslant 1\%$ and *STK11* co-mutation	–	NCT05445843
GDC-6036	–	498 (PI)	Adv. solid tumors	EGFR TKI, PD-L1i, SHP2i, anti-EGFR Ab, anti-VEGF Ab, PI3Kαi	NCT04449874
	B-FAST	1000 (PII/III)	Adv. NSCLC	Versus docetaxel	NCT03178552
Additional promising clinical compounds					
BI 1823911	–	72 (PI)	Adv. lung, CRC, pancreatic, bile duct cancers	SOS1i	NCT04973163
D-1553	–	200 (PI/II)	Adv. solid tumors	NSCLC standard-of-care	NCT04585035
	–	144 (PI/II)	Adv. NSCLC	NSCLC standard-of-care	NCT05492045
	–	203 (PI/II)	Adv. NSCLC	–	NCT05383898
LY3537982	–	360 (PI)	Adv. solid tumors	AURKAi, CDK4/6i, EGFR TKI, ERK1/2i, PD-1i, Anti-EGFR Ab, SHP2i	NCT04956640
Other KRAS^{G12C} inhibitors in development					
BPI-421286	–	80 (PI)	Adv. solid tumors	–	NCT05315180
D3S-001		98 (PI)	Adv. solid tumors	–	NCT05410145
GFH925/IBI351	–	128 (PI/II)	Adv. NSCLC	–	NCT05005234
		102 (PI)	Metastatic CRC	Anti-EGFR Ab	NCT05497336
		144 (PI)	Adv. NSCLC	Chemotherapy, PD-1i	NCT05504278
HBI-2438	–	44 (PI)	Adv. solid tumors	–	NCT05485974

(Continued)

Table 1. (Continued)

Drug	Trial name	Participants (Phase #)	Indication	Combinations	Trial identifier
HS-10370	–	176 (PI/II)	Adv. solid tumors		NCT05367778
JAB-21822	–	144 (PI/II)	Adv. solid tumors	–	NCT05009329
	–	100 (PI/II)	Adv. solid tumors, NSCLC, CRC	Anti-EGFR Ab	NCT05002270
	–	62 (PI/II)	Adv. CRC, intestinal, appendiceal	Anti-EGFR Ab	NCT05194995
	–	104 (PI/II)	Adv. NSCLC with *STK11* mutation and $KEAP1^{WT}$	–	NCT05276726
	–	124 (PI/II)	Adv. solid tumors	SHP2i	NCT05288205
MK-1084	–	185 (PI)	Adv. solid tumors	PD-1i	NCT05067283
YL-15293	–	55 (PI/II)	Adv. solid tumors	–	NCT05119933
	–	90 (PI/II)	Adv. solid tumors	–	NCT05173805

CRC, colorectal cancer; EGFR, epidermal growth factor receptor; NSCLC; non-small-cell lung cancer; PD-1i, programmed death-1 inhibitor; PD-L1i, programmed death-ligand 1 inhibitor; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

understood that improvements to ARS-1620 were necessary before this class of compound could enter clinical trials. For this review, we will summarize G12Ci that are being investigated clinically for $KRAS^{G12C}$-mutant tumors but not mRNA vaccines[42,43] (NCT03948763 and NCT04853017) or pan-KRAS inhibitors.[44–46]

Leading clinical candidates

Among several G12Ci under clinical evaluation, sotorasib (AMG 510), adagrasib (MRTX849), JDQ443, and GDC-6036 are the furthest along in their scrutinization (Table 1).

Sotorasib. Sotorasib/LUMAKRAS®/AMG510 (Amgen, Thousand Oaks, CA, USA) was conceived by a KRAS H95 groove-binding molecule optimization.[47] The isopropyl-methylpyridine component of sotorasib that occupies the KRAS H95 groove engages in 25 ligand–protein van der Waals interactions, leading to improved binding over ARS-1620, despite sharing significant molecular structure. This improved binding translated to a 10-fold improved potency over ARS-1620 in a nucleotide exchange assay, and a 40-fold increase in reducing NCI-H358 LUAD and MIA PaCa-2 pancreatic ductal adenocarcinoma (PDAC) cell line viabilities *versus* ARS-1620.[47] *In vivo*, sotorasib led to maximal inhibition of p-ERK 2–4h after treatment which was sustained for 48h, and cell-derived xenografts regressed over 3weeks of treatment.

Sotorasib was the first $KRAS^{G12C}$ inhibitor to enter phase I/II clinical trials targeting CRC[48] and LUAD[49] (CodeBreak 100; NCT03600883) (Table 1). At the highest dose (960mg daily) of sotorasib, this rate reached 37.1% in 126 NSCLC phase II patients.[50] Of these, 125 patients had adverse events, the most common being diarrhea (50.8%), nausea (31%), and increases in aspartate/alanine aminotransferase levels (21.4%; 20.6%).[50] An updated ORR from combining phases I and II NSCLC patients ($n=174$) was 40.7% [95% confidence interval (CI): 33.2–48.4] and median progression-free survival (PFS) and overall survival were 6.3months (95% CI: 5.3–8.2) and 12.5months (95% CI: 10.5–17.8), respectively.[51]

After the success of CodeBreak 100 for NSCLC, the phase I/II CodeBreak 101 was initiated to study sotorasib combinations in all advanced-stage tumors with chemotherapies, targeted agents, or various antibodies (NCT04185883). These combinations are based on preclinical studies by Amgen,[47] and they align with combinations of targeted agents in *KRAS*-mutant tumors proposed by studies predating G12Ci.[52–56] With positive data from CodeBreak 100 and CodeBreak 101, the FDA granted approval as a second-line

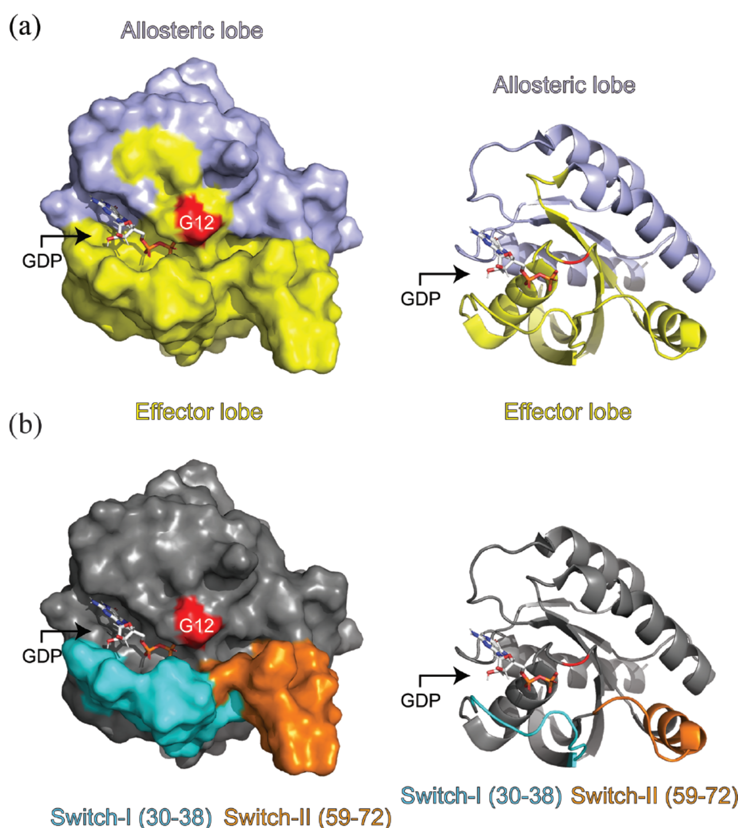

Figure 1. KRAS^{WT} complexed with GDP (PDB: 4OBE). (a) Allosteric and effector domains denoted by either purple or yellow colors, respectively. (b) Switch-I and Switch-II regions are colored cyan and orange, respectively. The red highlighted residue is G12. Switch-I is residues 30–38 inclusive while switch-II was considered residues 59–72 inclusive. Images were generated using PyMol version 2.5.2.

or later therapy in locally advanced or metastatic *KRAS*^{G12C} NSCLC in May 2021.[57] The cell-free DNA (cfDNA) Guardant360® CDx liquid biopsy test was also approved by the FDA as a companion diagnostic tool for sotorasib at the same time.[58]

CodeBreak 200 is a phase III trial to compare sotorasib to the standard-of-care chemotherapy, docetaxel, in advanced-stage NSCLC as a second-line therapy (NCT04303780). An ORR of 28.1% was observed for sotorasib *versus* 13.2% for docetaxel ($p<0.001$), while PFS was 24.8% for sotorasib *versus* 10.1% with docetaxel (hazard ratio: 0.66, 95% CI: 0.51–0.86; $p=0.002$).[59] Also, a phase II trial was initiated to assess sotorasib as a first-line therapy in Stage IV NSCLC patients (NCT04933695) (Table 1). Other trials are ongoing to study sotorasib in combination with an anti-vascular endothelial growth factor (VEGF) antibody in Stage IV NSCLC with untreated brain metastases (NCT05180422), and in combination with a dual RAF/MEK inhibitor (NCT05074810) or with a SHP2 inhibitor (NCT05054725)[60] in advanced-stage NSCLC.

Adagrasib. Mirati Therapeutics (San Diego, CA, USA) developed their own KRAS^{G12C} inhibitor called compound 4.[61] This molecule shared similarities with ARS-1620 and sotorasib, despite being identified from an independent screen[62] (Figure 2). Improvements to compound 4 led to compounds 18 and 19, which reduced cellular IC_{50} to 1nM.[63] Slight electrophilic substitution led to compound 20 (adagrasib/KRAZATI™/MRTX849).[63] Adagrasib has a 3-h cellular IC_{50} of 14nM (NCI-H358) and 5nM (MIA PaCa-2). These IC_{50} values were comparable to those observed with sotorasib, where NCI-H358 and MIA PaCa-2 cells had IC_{50} values of 6nM and 9nM, respectively.[12,47]

Adagrasib was the second GDP-KRAS^{G12C} inhibitor to enter clinical trials. In the KRYSTAL-1 trial, 42.9% (48/112) of NSCLC patients had an objective response (NCT03785249).[64] Adagrasib is also being evaluated in combination with the programmed death-1 (PD-1) inhibitor, pembrolizumab, the anti-epidermal growth factor receptor (EGFR) antibody, cetuximab, or the EGFR/Her2 inhibitor, afatinib, in the phase Ib portion of the trial. On 12 December 2022, based on data from the KRYSTAL-1 trial, adagrasib was granted accelerated approval as a second-line therapy for *KRAS*^{G12C} locally advanced or metastatic NSCLC.[8] In addition, the QIAGEN therascreen KRAS RGQ PCR kit (tissue) and the Agilent Resolution ctDx FIRST Assay (plasma) were both approved by the FDA as companion diagnostic tools for adagrasib.[8] Finally, adagrasib received breakthrough designation in combination with cetuximab in *KRAS*^{G12C} advanced CRC after chemotherapy and anti-VEGF therapy.[65] This designation came the same day as a KRYSTAL-1 report highlighting a 23% (10/43) ORR in advanced CRC patients with adagrasib alone, whereas the ORR with an adagrasib and cetuximab combination jumped to 46% (13/28).[66]

With a modest ORR of 42.9% in NSCLC, several phase I–III trials in advanced-stage NSCLC have been initiated to investigate adagrasib in combination with SHP2 inhibitors (NCT04330664), PD-1 antibodies (NCT04613596), SOS1 inhibitors (NCT04975256), or CDK4/6 inhibitors (NCT05178888) as second-line therapies

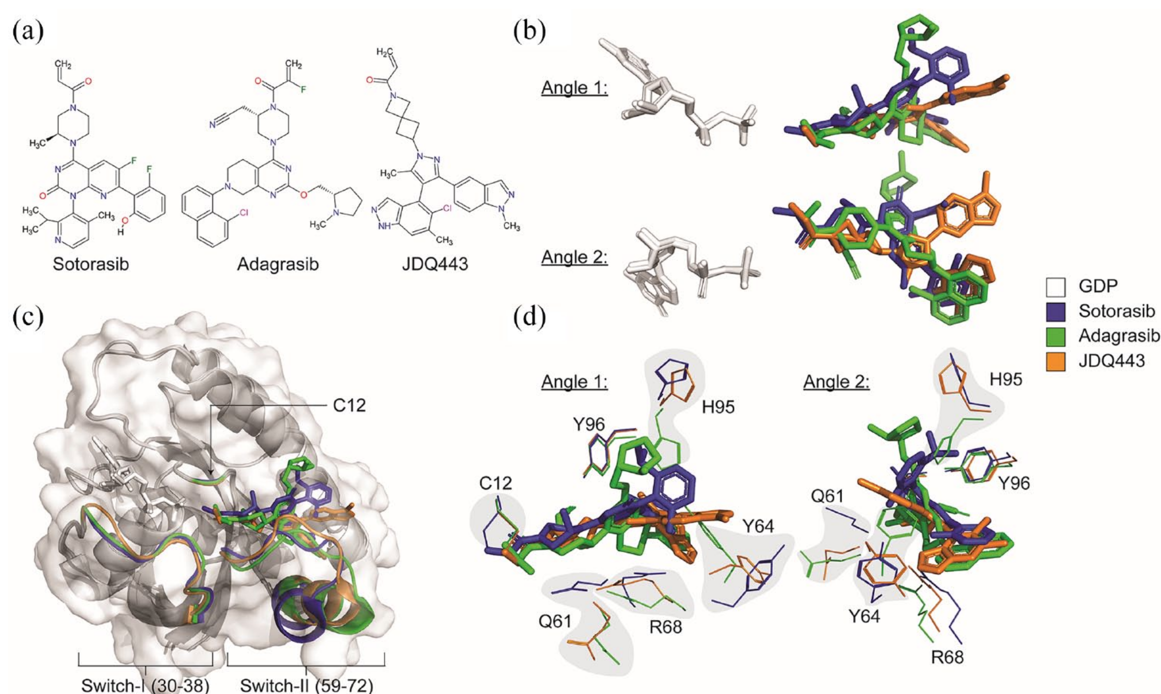

Figure 2. Binding of different inhibitors on KRAS^{G12C}. (a) Molecular structure of sotorasib, adagrasib, and JDQ443. (b) Two different angles of aligned GDP with aligned sotorasib, adagrasib, and JDQ443 on KRAS^{G12C}. (c) Illustration of how different KRAS-G12Ci binding impacts KRAS protein conformation. (d) Two different angles of sotorasib, adagrasib, and JDQ443 binding on GDP-KRAS^{G12C} with several key KRAS residues highlighted. AMG 510 (PDB: 6OIM); MRTX849 (PDB: 6UT0); JDQ443 (PDB: 7R0M). Images were generated using PyMol version 2.5.2.

(Table 1). Adagrasib is also being compared to docetaxel as a second-line therapy in advanced-stage NSCLC (NCT04685135).

Adagrasib can also penetrate the blood–brain barrier. At a dose of 100 mg/kg BID in mice, adagrasib led to near complete tumor regression of intracranially implanted LU99-Luc and NCI-H23-Luc *KRAS*^{G12C} NSCLC cells with reductions in p-ERK and proliferation (KI-67).[67] In two stage IIIA and IV, NSCLC patients from the KRYSTAL-1[6,68] (NCT03785249) trial, adagrasib monotherapy led to the disappearance of or reduced the size of three brain lesions relative to baseline, respectively, supporting the use of adagrasib to treat brain metastases.[67] The data from KRYSTAL-1[6,68] are forthcoming and future developments should be watched closely.

JDQ443. Novartis Pharmaceuticals (Basel, Switzerland) first reported their KRAS^{G12C} covalent and irreversible inhibitor, JDQ443 in October 2021 (Figure 2).[69] This compound binds to the KRAS switch-II pocket with unique interactions not found in sotorasib- and adagrasib- KRAS^{G12C} binding.[69] Antitumor activity in mouse tumor xenografts was comparable to the leading clinical compounds, and in mouse, rat, and dog, JDQ443 was well tolerated and orally bioavailable. Combinations with SHP2 inhibitors enhanced KRAS^{G12C} occupancy *in vivo* and improved antitumor activity over combinations with MEK or CDK4/6 inhibitors.[70]

The phase I/II KontRASt-01 trial studies JDQ443 in advanced-stage solid tumor patients. The experimental arms include combinations of JDQ443 with a SHP2 inhibitor, JDQ443 with the anti-PD1 antibody tislelizumab, or JDQ443 triple combination (NCT04699188). Two patients in this trial were administered 200 mg JDQ443 BID (21-day cycle) or 200 mg JDQ443 BID (21-day cycle) with 20 mg QD 2 weeks on/1 week off TNO155 (SHP2i); each reduced the target lesion sizes by ~30.4% and ~44.2%, respectively, *versus* baseline.[70] At the time of cutoff, Grade 3 treatment-related adverse events (TRAEs) occurred in 10.3% of 39 patients with no Grade 4 or 5 TRAE

observed, and the ORR across all dose levels in 20 NSCLC patients was 30% (6/20), and 43% (3/7) at the recommended dose of 200 mg BID.[71]

The phase III KontRASt-02 trial was initiated in March 2022 comparing JDQ443 with docetaxel in an estimated 360 treated advanced-stage NSCLC patients previously treated with one platinum-based chemotherapy and one immune checkpoint inhibitor (NCT05132075).

GDC-6036. Genentech (South San Francisco, CA, USA) has also developed their own KRAS direct inhibitor, GDC-6036. GDC-6036 has greater potency and selectivity *versus* sotorasib and adagrasib *in vitro* and *in vivo*, with median IC_{50} values in the sub-nanomolar range.[72] A phase I trial for advanced-stage *KRAS*^{G12C}-positive solid tumors has been initiated. This trial includes 342 participants and combinations of GDC-6036 with atezolizumab, cetuximab, bevacizumab, erlotinib, inavolisib, and their own SHP2 inhibitor, GDC-1971 (NCT04449874). As a monotherapy, confirmed ORR reached 46% (26/57) patients.[73] The international phase II/III B-FAST study also includes an arm evaluating GDC-6036 *versus* docetaxel (NCT03178552) with forthcoming data.

Additional promising clinical compounds

Several other G12Ci exist, but the clinical assessments of many of them are not as mature as those for sotorasib, adagrasib, JDQ443, and GDC-6036. After these, the next three compounds with the most data are listed below in alphabetical order.

BI 1823911. The method of development for BI 1823911 (Boehringer Ingelheim, Ingelheim am Rhein, Germany) is undisclosed, but it was reported to possess comparative antiproliferative activity to sotorasib and adagrasib in a panel of 2 CRC, 12 NSCLC, and 1 PDAC cell lines with *KRAS*^{G12C} mutations.[74] MIA PaCa-2 and CRC cell-derived xenografts and PDX were used to demonstrate similarity in tumor response between BI 1823911, sotorasib, and adagrasib. Of note, the NSCLC SW1573 cell-derived xenograft was resistant to BI 1823911(60 mg/kg),[74] similarly to ARS-1620 (200 mg/kg),[10] sotorasib (*in vitro* $IC_{50} > 7.5\,\mu M$)[12,47] and adagrasib (100 mg/kg).[75] Combinations with the SOS1:KRAS inhibitor, BI 1701963, led to tumor regression in 9/9 NSCLC NCI-H2122 cell-derived xenografts, whereas monotherapy led to regression in either 1/8 (BI 1823911) or 0/8 (BI 1701963) mice.

A phase I trial of BI 1823911 was initiated in August 2021 to investigate its combination with SOS1:KRAS inhibitors (NCT04973163) in prior-treated advanced-stage solid tumors.

D-1553. InventisBio (Shanghai, China) reported the discovery of D-1553.[76] Activity was observed in NSCLC and PDAC cell-derived xenografts, and combinations with a MEKi, SHP2i, or other cytotoxic agents led to tumor regression.

InventisBio is collaborating with Merck Sharp & Dohme (Kenilworth, NJ, USA) on a phase I/II trial to study D-1553 as a monotherapy or with standard-of-care agents in previously treated solid tumors (NCT04585035). In this China-based trial, there was no dose-limiting toxicity and an ORR of 40.4% (21/52) and DCR 90.4% (47/52) in NSCLC *KRAS*^{G12C} patients.[77] In another phase I/II study based in China, ORR reached 37.8% (28/74) and DCR 91.9% (68/74) in a 100% Asian and 11.4% (9/79) female population.[78]

LY3537982. Eli Lilly (Indianapolis, IN, USA) reported their covalent KRAS^{G12C} inhibitor, LY3537982.[79] IC_{50} values of KRAS-GTP loading and inhibiting p-ERK in NCI-H358 cells were 3.35 nM and 0.65 nM, respectively, which were lower than values for sotorasib and adagrasib,[12,47] and LY3537982 was predicted to have a >90% clinical target occupancy. A phase I trial is ongoing for patients with *KRAS*^{G12C} solid tumors at any stage (NCT04956640). LY3537982 is being investigated as a monotherapy or in combination with CDK4/6, AURKA, EGFR, ERK inhibitors, or PD-1 or anti-EGFR antibodies. This comes after the failure of their earlier G12Ci, LY3499446 (NCT04165031).

Other KRAS^{G12C} inhibitors in development. There are several other G12C inhibitors under clinical investigation, but these have little data available and are subject to scrutinization in the future. All G12Ci under clinical investigation are summarized in Table 1.

Resistance to KRAS G12Ci

Studies investigating oncogene driver-mutant tumors and their respective treatments have highlighted the need to understand mechanisms of therapeutic resistance, as this invariably occurs

during treatment. *KRAS*^{G12C}-mutant cancers are no exception to this rule.[9–20] Sharma *et al.* broadly defined resistance to immunotherapy into three separate but interrelated phenomena: primary, adaptive, and acquired.[80] For this review, we refer to primary resistance as intrinsic resistance, and define acquired resistance mechanisms as more permanent tumor changes like mutations or cell transformation. Here, for the sake of conceptualization, we consider intrinsic, adaptive, and acquired resistance as three separate phenomena that are fundamentally different, although there is likely overlap between them.[80]

Intrinsic resistance

To translate to the clinical perspective, we define intrinsic resistance as resulting in either progressive disease (PD) or stable disease (SD) according to the response evaluation criteria in solid tumors (RECIST) V1.1.[81] Here, we relate intrinsic resistance to the tumor cell independence from the driver oncogene which may have initiated the tumor. Intrinsic resistance has been observed in *KRAS*-mutant cell lines since at least 1997, when knockdown of *KRAS via* transfection of a plasmid containing an antisense gene fragment in cancer cell lines resulted in non-uniform growth suppression.[82] *KRAS* dependence was analyzed more systematically in 2009 using *KRAS*-directed shRNA.[83] Here, after shRNA treatment, there was a wide variety of *KRAS* dependence in *KRAS*-mutant cancer cell lines irrespective of *KRAS* zygosity, and lines were classified as *KRAS*-dependent or independent based on the expression of the apoptotic marker cleaved caspase-3. Cell lines that were more mesenchymal in nature or cells undergoing epithelial-to-mesenchymal transition (EMT) were more likely to be *KRAS* independent.[83] Indeed, the initiation of EMT in *KRAS*-dependent NCI-H358 cells induced by TGFβ1 led to characteristic traits of *KRAS*-independency (E-cadherin loss and gain of vimentin expression).[83]

Shao and colleagues demonstrated that upon shRNA knockdown of *KRAS* in CRC and PDAC cell lines that were survival dependent on *KRAS*, Yes-associated protein 1 (YAP1) signaling led to independence from PI3K/AKT and MAPK pathway activity for survival, and induction of EMT.[84] In addition, withdrawing doxycycline in engineered mice containing a doxycycline inducible *KRAS*^{G12D} transgene and conditional *TP53* null alleles led to tumor relapse where *KRAS*^{G12D} was absent and *YAP1* was amplified.[85] Thus, both YAP1 amplification and signaling lead to *KRAS*-independent tumor growth, possibly through EMT.

KRAS protein expression is correlated with *KRAS* dependency,[83] and both KRAS protein expression and GTP activity are associated with sotorasib sensitivity.[12] However, although NCI-H358 and NCI-H23 LUAD cell lines are both G12Ci sensitive,[9–13] they were categorized as *KRAS* dependent and *KRAS* independent, respectively.[83] Importantly, cell lines resistant to sotorasib had GSEA enrichments of EMT gene sets.[12] While evidence points to EMT, the relationship of EMT and G12Ci intrinsic resistance has not been conclusively demonstrated *in vivo*.

Adaptive resistance

We consider adaptive resistance as short-term changes in gene and protein expression. These adaptive mechanisms have been widely reported for KRAS-directed therapies (prior to G12Ci)[53,84,54,86] and to KRAS^{G12C} inhibitors themselves.[9–14] Like intrinsic resistance, we define adaptive resistance as resulting in either PD or SD according to RECIST 1.1.[81] However, the mechanisms to reach these tumor response criteria may be different (Figure 3). For that reason, while intrinsic resistance is primary resistance, we consider adaptive (and acquired) resistance as secondary resistance mechanisms.

Epithelial-to-mesenchymal transition. The role of EMT in adaptive G12Ci resistance is better understood than in intrinsic resistance.[83,87] Historical *KRAS*-dependent signatures[53,83,86] and those developed post-G12Ci treatment using RNA-seq[12] and protemics[13] both have EMT genes significantly represented. Artificial means of inducing EMT *via* TGFβ treatment confirmed these findings in NCI-H358[12,13] and LU65[12] cell lines. Upon EMT induction and the acquisition of mesenchymal features, both lines became significantly less sensitive to ARS-1620 and sotorasib, with little effect on MAPK signaling. *KRAS* oncogene addiction *via* EMT is facilitated through YAP1/Tead2 signaling,[84,85] which can regulate *FGFR1*.[88] Indeed, in a recent autopsy case of a LUAD patient rapidly resisting sotorasib, transcriptome analysis cited one of the seven gene sets upregulated in five of six post-treatment (relative to two pre-treatment) lymph node

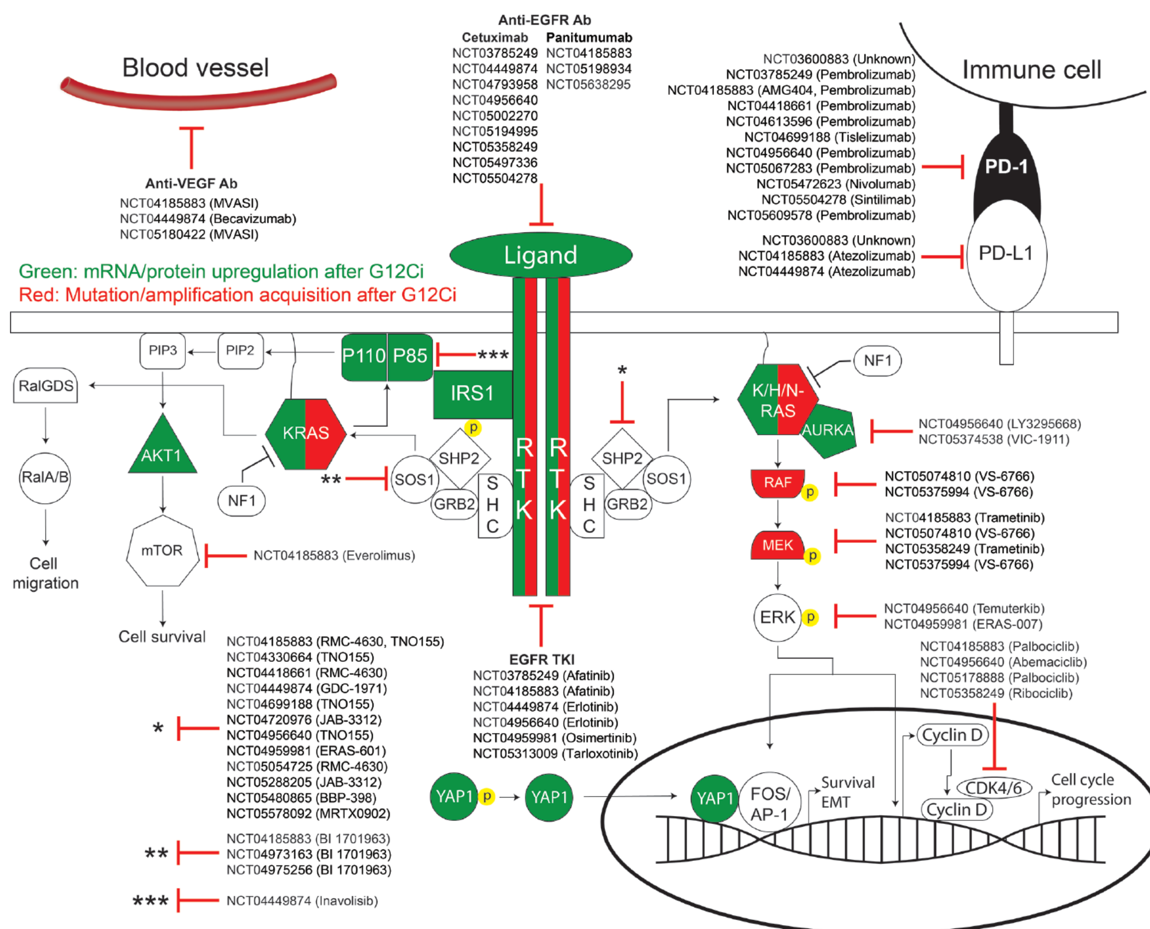

Figure 3. Detailed schematic of adaptive and acquired GDP-KRAS^{G12C} inhibitor resistance. The MAPK and PI3K/AKT pathways are shown here. Any protein in green indicates mRNA or protein upregulation after GDP-KRAS^{G12C} inhibitor (G12Ci) treatment, while any protein in red indicates any secondary mutation or copy number amplification that occurred after G12Ci treatment. Clinical trials investigating G12Ci combination therapies with targeted molecules against specific proteins are listed here. This schematic fails to address the resistance mechanisms including but not limited to histologic transformation, genetic rearrangements, immunomodulation, or global proteome changes in metabolic programming.

metastases as EMT.[20] In these post-treatment samples, significant upregulation of signaling pathways involving YAP1 was observed.

Epithelial cells seemingly have separate adaptive resistance programs *versus* mesenchymal cells. In epithelial-like cancer cells, ErbB2/3 is upregulated after ARS-1620 treatment,[11,40] and reactivates the MAPK and PI3K/AKT pathways after MEK inhibition[53] and ARS-1620 treatment.[13] Relating to EMT, gene expression patterns in 59 *KRAS*^{G12C} LUAD tumors identified a significant negative correlation of a TGFβ-EMT gene signature and *ErbB2/3* gene expression.[13] While ErbB2/3 are suggested receptor tyrosine kinases (RTKs) to target in combination with G12Ci, they are not the only RTKs responsible for adaptive resistance.

RTK/RTK ligand upregulation and SHP2. Treatment with KRAS^{G12C} inhibitors can alter phospho-RTK and RTK/RTK ligand expression profiles in NSCLC, CRC, and PDAC cell lines.[9,11,40,89] Some RTKs and their signaling intermediates have been assessed in detail. *EGFR*, *FGFR*, and *AXL* were identified in NCI-H358 and MIA PaCa-2 cells as dependencies in genome-wide knockout screens after ARS-1620 treatment.[89] Unfortunately, RTK/RTK ligand expression profiles are different in each cell line, obscuring any attempt to derive a universal adaptive resistance strategy used by cancer cell lines.[40,75] Instead, groups decided to focus heavily on a more common node in KRAS signaling.

Extracellular signals feed into RTKs which are then relayed through adaptor proteins that can

activate the phosphatase SHP2.[90] This phosphatase can recruit the GRB2:SOS1 complex to the plasma membrane to facilitate RAS activation[90–92] and promotes RAS activation itself by dephosphorylating the Src phosphorylation sites of Y32 and Y64 on KRAS.[93] Knockout of *SHP2* significantly delayed development of pancreatic intraepithelial neoplasias and the course of atypical adenomatous hyperplasia–adenoma–adenocarcinoma in *KRAS*^{G12D} mice, suggesting an indispensable role for *SHP2* in tumor initiation and progression.[92] ShRNA targeting SHP2 impacted NCI-H2122 cell fitness *in vitro* and *in vivo*,[75] and relevant to KRAS^{G12C} inhibitors, a genome-wide CRISPR/Cas9 screen identified SHP2 as a dependency in *KRAS*^{G12C} cancer cells treated with ARS-1620.[89] Finally, SHP2 inhibition in LUAD NCI-H1944 cell-derived xenografts stopped *in vivo* tumor growth with a total loss of p-ERK.[94] Thus, SHP2 is a key common node in the KRAS signaling axis and may be better suited as a target in combination with a G12C inhibitor compared to individual RTKs.

RAS activation and upregulation. KRAS^{G12C} inhibitors very potently reduce pools of KRAS-GTP.[4,37] In CRC and NSCLC cell lines, NRAS-GTP and HRAS-GTP have been shown to be upregulated (possibly by RTKs) by fivefold within 48 h of ARS-1620 or sotorasib treatment leading to MAPK signaling rebound[11] and G12Ci adaptive resistance.[95] This illuminates a potential RTK-driven mechanism of MAPK pathway reactivation that is KRAS^{G12C} independent, although it is not widely reported.[11,40,95] In this scenario, inhibiting SHP2 is sufficient to dampen NRAS-GTP increases and resultant MAPK signaling after G12Ci treatment, supporting its feasibility in overcoming several adaptive resistance programs.

Protein upregulation of KRAS is observed in cell lines 48–72 h after G12Ci treatment.[9–12,14,40,75] Cell lines treated with G12Ci and subjected to single-cell RNA-seq and trajectory inference analyses have informed us of at least two distinct cell fates including cells with inhibited KRAS signaling or cells that have dampened and subsequent rapid reactivation of KRAS signaling.[9] Translation of new, drug-free KRAS^{G12C} through EGFR and Aurora Kinase A signaling was noted as a major contributor to adaptive G12Ci resistance in these models.[9] In this scenario, *KRAS*-dependent cells continue to activate KRAS signaling after G12Ci treatment through these mechanisms. More broadly, the determinant of the adaptive resistance measures induced in response to targeted agents may be linked the oncogenic dependence of cells, and this may be heterogeneous within a tumor, complicating efforts to identify viable G12C combinations.[9]

Proteomic adaptation. A focus on the expression patterns of individual genes and proteins helped choreograph the identification of targets that may be leveraged to sensitize tumors to G12C inhibitors.[13] Quantitative global and phospho-proteomic isobaric-tag-based mass spectrometry analyses have also been utilized to understand broad changes after G12Ci administration.[14] In the PDAC MIAPaCa-2 and the *KRAS*-dependent[83] LUAD NCI-H358 cell lines, increased amino acid, fatty acid, and lipid metabolism and TCA cycle were noted in cell lines at 24 h and 7 days relative to control, while decreases in cell cycle regulation, transcription, translation, and mRNA processing occurred in the same timeframe.[14] The contribution of these broad adaptive changes in the context of G12Ci resistance requires further exploration.

Acquired resistance

Tumors undergo partial or complete responses to therapies[81] and sometime later may relapse or regrow leading to disease progression. In this scenario, we postulate that the tumor develops G12Ci resistance by acquiring permanent alterations, for example, mutational changes or histologic transformation that supersede KRAS as the driver mechanism for tumor cell growth. In the context of lung cancer, *EGFR*-mutant tumors may overcome EGFR-tyrosine kinase inhibitors (TKI), erlotinib and gefitinib, by developing T790M mutations.[96] Similarly, *ALK*-rearranged NSCLC have been shown to develop secondary L1152R mutations after treatment with the ALK inhibitor, crizotinib.[97] Before the discovery of the switch-II pocket in GDP-KRAS^{G12C}, it was not known whether *KRAS*-mutant tumors also developed secondary mutations leading to resistance, whereas *KRAS* copy number changes were better understood, at least for pancreatic cancer.

In a cohort of 38 mouse *KRAS*^{G12D} PDAC tumors, chromothripsis-induced changes led to the identification of *KRAS* as the most amplified locus globally.[87] Here, the copy number of *KRAS* was referred to as gene dosage, with four distinct groupings of tumors: (i) focal gain, (ii) arm-level

gain, (iii) loss of $KRAS^{WT}$ (copy number neutral), and (iv) no change. Tumors with increased *KRAS* gene dosage (groups i–iii) had increased metastatic potential, while tumors with no change (group iv) were largely non-metastatic. Indeed, a LUAD case study including pre- and post-sotorasib patient metastases identified a decrease in $KRAS^{G12C}$ mutant allele frequency (MAF) in post-treatment samples as well as an inverse correlation of $KRAS^{G12C}$ MAF with G12Ci resistance pathway activation while loss of G2/M cycle checkpoints positively correlated with $KRAS^{G12C}$ MAF.[20] This is plausible considering how $KRAS^{WT}$ impairs mutant-KRAS upon heterodimerization in LUAD by sacrificing MAPK pathway output for increased resistance to MEK inhibition, whereas mutant-KRAS homodimerization increases MAPK pathway output while also increasing sensitivity to MEK inhibitors.[98] Thus, while *KRAS* MAF and zygosity fail to predict G12Ci antitumor activity,[10,75] they may be important determinants in predicting likelihood of acquired resistance.

A major roadblock in understanding resistance to G12Ci is the heterogeneity of tumors. Even within cell lines sensitive to G12Ci, resistant subclones after G12Ci treatment have been observed,[9] and outgrowth of clones with G12Ci resistance mutations have also been noted.[15–19] The earliest work exploring resistance to G12Ci *via KRAS* secondary mutations came in 2016 from Lito and colleagues,[99] even before sotorasib and adagrasib were known. $KRAS^{A59G}$ mutations displaced the Q61 residue essential for GTP hydrolysis, and $KRAS^{G12C/A59G}$ HEK293 or NCI-H358 double mutant cells were resistant to ARS-853. Other G12C co-mutations in residues impacting GTP hydrolysis, GTPase activity, and nucleotide exchange including *Y40A*, *Q61L*, *Y64A*, *N116H*, and *A146V* also led to ARS-853 resistance.[99] More recently, Koga and colleagues used N-ethyl-N-nitrosourea mutagenesis to identify secondary mutations in sotorasib- or adagrasib-resistant $KRAS^{G12C}$-transduced Ba/F3 cells.[15] While several mutations including *KRAS V8E*, *G13D*, *A59S/T*, *R68M/S*, *M72I*, *Q61L*, *Q99L*, and *Y96D/S* were observed, only *A59S* and *Y96D* were shared between both sotorasib- and adagrasib-resistant cells. Sotorasib or adagrasib treatment suppressed P-ERK and p-S6 in Ba/F3 cells with various secondary *KRAS* mutations, but not with *Y96D/S*. IC_{50} values of NCI-H358 cells with retrovirally introduced *Y96D/S* were 30 times higher to both G12Ci than parental NCI-H358 cells, while cells with *A59T* were much more sensitive.[15] Several of the mutations listed above have been found through independent saturation mutagenesis screening[100] and in patient tumors Table 2.[18,19]

In 38 $KRAS^{G12C}$ patients (27 NSCLC, 10 CRC, and 1 appendiceal cancer) treated with adagrasib, circulating tumor DNA (ctDNA) analysis detected 17 (10 NSCLC, 6 CRC, and 1 appendiceal cancer) cases of acquired secondary *KRAS* mutations including *G12D/V/R/W*, *G13D*, *Q61H*, *R68S*, *H95D/R*, and *Y96C*, and multiple mutations could be found in individual patients.[19] Additional alterations along the MAPK axis were found in *NRAS*, *MEK1*, *EGFR*, and several gene fusions were confirmed. There is also at least one instance of a patient who acquired an *ERBB2* amplification as a means of sotorasib resistance, identified by fluorescence *in situ* hybridization.[17] *MET* amplifications have also been identified as an acquired G12Ci resistance mechanism.[16] A combination of SHP2i + G12Ci were able to overcome both *MET* amplification in sotorasib-resistant NCI-H23 cell lines and xenografts[16] and *ERBB2* overexpression in NCI-H358 or Calu-1 cell lines and xenografts,[17] suggesting the feasibility of this combination.

cfDNA from a metastatic $KRAS^{G12C}$ NSCLC patient treated with adagrasib identified secondary *KRAS, NRAS*, *BRAF*, and *MEK* mutations,[18] many of which overlapped with the other studies.[15,19,99] In this patient, secondary mutations within *KRAS* including *Y96D* arose *in trans* with $KRAS^{G12C}$, although wild-type *KRAS* was retained.[18] Until these studies, $KRAS^{Y96D}$ mutations were unknown. It is now understood that Y96 interacts with sotorasib and adagrasib through hydrogen bonds that are abolished with a *Y96D* mutation.[101] *Y96D*-mutants sustained MAPK pathway activity and IC_{50} of sotorasib and adagrasib in NCI-H358 were increased by >100-fold.[18] While $KRAS^{G12C}$ co-mutation with *KRAS G13D*, *A59S*, *R68S*, *H95D/Q/R*, and *Q99L* was resistant to either sotorasib or adagrasib, *Y96*-mutations were the only ones resistant to both.[15,18,19] Indeed, mutations in residues that impact GTP hydrolysis and nucleotide binding like *G13D*, *A59S*, *K117N*, and *A146P* were less resistant to G12Ci compared to mutations which prevent drug binding like *G12R* or *Y96C*.[19,99] While Tsai and colleagues did not observe any secondary *KRAS* or MAPK pathway resistance mutations to G12Ci by whole-exome sequencing

Table 2. Observed alterations leading to GDP-KRAS^{G12C} inhibitor resistance for all indications.

Gene	Mutation	Context of the alteration	Observing studies (PMID)
KRAS	A146V	Preclinical	26841430
	A59G/S/T	Preclinical	26841430; 33971321; 35471904
	C12F	Preclinical	35471904
	D69P	Preclinical	35471904
	D92R	Preclinical	35471904
	F156L	Preclinical	35471904
	G12C AMP**	Clinical	34161704
	G12R/V/W	Clinical	34161704; 33824136
	G13D/E	G13D: preclinical and clinical; G13E: preclinical	33824136; 34161704; 33971321; 35471904
	H95D/N/R/Q/V	H95D/R: preclinical and clinical; H95Q: clinical; H95N/V: Preclinical	35471904; 34161704
	M72I	Preclinical	33971321; 35471904
	N116H	Preclinical	26841430
	Q61H/L	Q61L: preclinical; Q61H: clinical	26841430; 33971321; 34161704
	Q99F/L/W	Preclinical	35471904; 33971321
	R68L/M/S	R68L/M: preclinical; R68S: Preclinical and clinical	33971321; 35471904; 34161704
	S65W	Preclinical	35471904
	T58I	Preclinical	35471904
	V8A/E/L	Preclinical	33971321; 35471904
	V9Y	Preclinical	35471904
	Y40A	Preclinical	26841430
	Y64A	Preclinical	26841430
	Y96C/D/H/S	Y96C: preclinical and clinical; Y96D: clinical; Y96H/S: preclinical	35471904; 34161704; 33824136; 33971321
AKAP9-BRAF	Fusion	Clinical	34161704
BRAF	V600E	Clinical	34161704; 33824136
CCDC6-RET	Fusion	Clinical	34161704
EGFR	A289V	Clinical	34161704
EML4-ALK	Fusion	Clinical	34161704
ERBB2	Gain	Clinical	34715459
FGFR3-TACC3	Fusion	Clinical	34161704

(Continued)

Table 2. (Continued)

Gene	Mutation	Context of the alteration	Observing studies (PMID)
MAP2K1	E102_I103del	Clinical	34161704; 33824136
	I99_K104del	Clinical	34161704
	K57N/T	Clinical	34161704; 33824136
	Q56P	Clinical	33824136
MET	AMP**	Preclinical and clinical	34161704; 34365406
NF1	R2637*	Clinical	34161704
NRAS	Q61K/L/R	Clinical	34161704; 33824136
NRF1-BRAF	Fusion	Clinical	34161704
PIK3CA	H1047R	Clinical	34161704
PIK3R1	S361fs	Clinical	34161704
PTEN	G209V	Clinical	34161704
	N48K	Clinical	34161704
RAF1-CCDC176	Fusion	Clinical	34161704
RAF1-TRAK1	Fusion	Clinical	34161704
RET	M918T	Clinical	34161704
RIT1	P128L	Clinical	34161704
TP53	F338fs	Clinical	33824136

*Nonsense mutation.
**Amplification (AMP).

(WES) in their case report,[20] secondary mutations were identified elsewhere in patients using cfDNA[18] and ctDNA.[19] It is likely the case that the 200× WES coverage used by Tsai and colleagues[20] was not deep enough to capture the low-frequency variants,[102] and cfDNA and ctDNA detection methods are superior for this purpose.

With such a dramatic shift in G12Ci sensitivity, it is intriguing how *Y96* mutations have only been identified in a few patients thus far. As multiple resistance mutations were identified within individual patients, there may be coordination within the tumor to determine which mutation is most resistant to G12Ci and promotes tumor fitness the best. This is in line with the notion of treatment-induced clonal evolution contrary to the presence of pre-existing, drug-resistant clones within a tumor.[103] Thus, the acquisition of G12Ci-resisting *KRAS* mutations may be dictated by their ability to prevent drug binding, and more clinical data should be compiled to evaluate the prevalence of these mutations in the G12Ci-treated patient population.

Finally, akin to *EGFR*-mutant LUAD transforming to small-cell carcinoma on EGFR-TKIs,[104,105] two LUAD patient tumors treated with adagrasib transformed to squamous cell carcinoma without any genomic alterations as a means of acquired resistance.[19] Thus, secondary mutations or *KRAS* copy number gains may not be universal G12Ci-acquired resistance mechanisms.

Therapeutic combinations to overcome KRAS^{G12C} inhibitor resistance

Years of research and development have culminated into a growing list of covalent KRAS^{G12C} inhibitors. Despite intense validation of protein binding and antitumor efficacy, adaptive

resistance can develop in as little as a few hours. As such, potential G12Ci combination partners have been proposed to re-sensitize tumors to G12Ci and overcome resistance, and many are being investigated in clinical trials (Table 1).

Receptor TKIs. Activation of RTKs after G12Ci as a resistance mechanism to maintain MAPK signaling is well established.[9,11,40,89] Recent works have identified an increasing number of RTKs as combination partners, including EGFR, ErbB2/3, IGF1R, FGFR, and AXL in different cell lines and contexts.[10,13] EGFR, FGFR, and AXL are suggested to be dependencies in cells specifically in a G12Ci-induced state,[89] although these are cell-line dependent. Nonetheless, several clinical trials are evaluating EGFR TKI or EGFR antibodies with G12Ci (Table 1, Figure 3). Once clinical G12Ci resistance is better characterized, G12Ci + specific TKI responses may be better predicted.

SHP2 inhibitors. It is hinted that the SHP2i + G12Ci combination works well in cancer cells with an epithelial subtype, whereas RTKi + G12Ci is more effective in mesenchymal-like cancer cells.[13] However, overwhelming evidence of reductions in cell viability and KRAS signaling across several mesenchymal-like and epithelial-like pancreatic, colon, and lung cancer cell lines suggests broad activity of the SHP2i + G12Ci combination.[9,11,12,75,89] In addition, SHP2 inhibition promotes an antitumor immune program that can be leveraged with immunotherapy.[40]

PI3K inhibition is suggested to be a lethal combination with G12Ci[10,13,89] and leads to SW1573 cell-derived xenograft tumor regression,[10] despite SW1573 NSCLC cells being intrinsically resistant to G12Ci monotherapy.[13] However, SW1573 possess a PIK3CA^{K111E} gain-of-function allele, possibly rendering them *KRAS* independent.[40] While PI3Ki + G12Ci fails to account for the MAPK pathway reactivation that comes with G12Ci adaptive resistance, SHP2i + PI3Ki + G12Ci can,[12] and this triple combination reduced viability in this cell line.[13] Unfortunately, the regimen is unlikely to be evaluated in the clinic due to the high toxicity of PI3Ki combinations observed clinically.[106,107]

SHP2 inhibitor combinations with G12Ci are well documented to be effective in *KRAS*^{G12C} cell lines, but the same cannot be said about cancer cell lines expressing *KRAS*^{G13D} or *KRAS*$^{Q61H/R/X}$, which were resistant to SHP2i with continued expression of p-ERK.[90,94,108] As secondary *KRAS* mutations can be acquired after G12Ci,[15,18,19] it is possible that these specific *KRAS* mutations (G13D, Q61H/R/X) may occur after SHP2i + G12Ci combination treatment to escape SHP2i antitumor activity. As there are several active clinical investigations looking at this combination (Table 1), an investigation to answer this question may be warranted. Nonetheless, this combination has seen preclinical success. The combination with the SHP2 inhibitor, RMC-4630, with sotorasib was safe and tolerable in NSCLC patients, and confirmed partial responses were observed in 3/11 (27%) and 3/6 (50% – with two highest doses of RMC-4630) of pre-treated and treatment-naive patients, respectively.[60]

SOS1 inhibitors. SOS1 is a RasGEF activated by SHP2 and it facilitates RAS activation by promoting GTP binding. Downregulation of SOS1 phenocopies SHP2 inhibition,[40] suggesting an alternative to SHP2i. Indeed, SOS1 + MEK inhibition reduced MAPK pathway output and led to tumor regression in cell-derived xenografts.[109] SOS1i + sotorasib reduced phosphorylation of ERK stronger than sotorasib monotherapy in NCI-H358 cells,[109] although signal rebounded within this and other cell lines within 48–72 h.[13] Especially relevant to G12Ci-acquired resistance, sotorasib- and adagrasib-resistant *KRAS*$^{G12C/Y96D}$ NCI-H358 cells were sensitive to a combination of SOS1 and MEK inhibitors,[15] and this combination is being assessed clinically for advanced-stage *KRAS*^{G12C} solid tumors (NCT04111458).

SOS1 been implicated as a negative feedback regulator of KRAS signaling, and the GEF can activate wild-type N/HRAS *via* oncogenic KRAS.[110] SOS1 interacts with Ribosomal S6 kinase 1 (RSK1) to achieve negative regulator status, but RSK1 also complexes with the RAS GAP neurofibromin 1 (NF1) to negatively regulate wildtype KRAS, at least in pancreatic cancer.[111] It is therefore reasonable that as 7 of 14 solid cancer cell lines with aberrations in *NF1* were sensitive to SOS1 inhibitors regardless of *KRAS* mutation status,[109] a SOS1i + G12Ci combination may be useful in patients with *NF1* alterations. The SOS1i + G12Ci is under investigation in three clinical trials (NCT04585035, NCT04973163, and NCT04975256), and should shed light on whether SOS1 or SHP2 inhibitors are the superior G12Ci combination partner, or if there is no difference between the two.

Immunotherapy. *KRAS*-mutant LUAD have complex tumor immune microenvironments, and immune modulation by G12Ci has been investigated. Single-agent adagrasib or sotorasib increased CD3/4/8$^+$ T-cell tumor infiltration and decreased myeloid suppressor cell populations *in vivo*, which ultimately sensitized tumors to PD-1/PD-L1 blockade.[20,40,47,112] Resistance to sotorasib in patient lymph node metastases was linked to immunological dampening, and reductions in adaptive immune cell populations and neoantigens.[20] Combination of immune checkpoint inhibition (ICI) and G12Ci led to improved antitumor activity over single-agent G12Ci and abrogation of G12Ci resistance.[47,112] Several clinical trials are investigating PD-1/PD-L1 blockade in combination with G12C inhibitors (Figure 3, Table 1). In CodeBreak 100/101, 58 G12Ci-naïve NSCLC patients were treated with sotorasib and atezolizumab or pembrolizumab with an ORR of 29% (17/58) in all cohorts, although major hepatoxicity (Grade ⩾3 = 79% in all cohorts; any grade = 89.5%) was observed.[113] In contrast, 75 treatment-naïve *KRAS*^{G12C} advanced-stage NSCLC patients better tolerated the combination of adagrasib and pembrolizumab with grade 3–4 TRAEs occurring in 44% of patients, while increased lipase (11%) and increased alanine transaminase/aspartate transaminase (8%/9%) were all grade 3 (NCT03785249 and NCT04613596).[114] ORR was 49% (26/53) for the 53 patients with at least one on-study scan and DCR was 89%.[114] Thus, while adagrasib may be the superior combination partner for immunotherapy *versus* sotorasib in terms of tolerability, further investigations to fully explain this discrepancy are warranted.

KRAS itself can direct immunosuppression,[115] but a classification of tumors into immune 'hot' or 'cold' categories by *KRAS* co-mutations with *TP53* or *STK11*, respectively, has also been proposed.[116] Indeed, *STK11* alterations are a key contributor of intrinsic resistance to PD-1 inhibitors in *KRAS*-mutant NSCLC.[117,118] *KRAS*^{G12C}/*STK11* co-mutant LUAD patients had shorter time to next treatment, time to discontinuation, and overall survival after ICI treatment than patients without *STK11* co-mutations.[119] Meanwhile, *TP53* co-mutations increase PD-L1 expression and tumor mutation burden, which both contribute to ICI response and improved patient outcomes.[120,121] Adagrasib antitumor activity was not predicted by *STK11* or *TP53* co-mutations in preclinical studies.[75] However, patients with *STK11* co-mutations that were previously treated with ICI and/or chemotherapy had a 64%[122] (9/14 patients) ORR to adagrasib monotherapy, *versus* the 42.9% (48/112) ORR observed in all NSCLC patients.[6,64] Thus, there may be an added benefit of treating *KRAS*^{G12C}/*STK11* co-mutated patients with adagrasib after or with ICI. More work must be done to understand the role of *STK11* in G12Ci immunomodulation.

Other small-molecule combinations. mTOR signaling is activated upon ARS-1620 treatment,[13] although its downstream signaling targets p70-S6 kinase and p-S6 were partially inhibited after exposure to adagrasib.[75] The ATP-competitive mTOR inhibitor, vistusertib, in combination with adagrasib inhibited several components of the PI3K/mTOR signaling pathway and led to tumor regression in LUAD NCI-H2030 xenografts, suggesting the applicability of this combination.[75] A triple combination of mTORi + IGF1Ri + G12Ci has also been proposed.[41]

Cell cycle kinases have been supported as targets for *KRAS*-mutant tumors for several years. Large decreases in cell cycle proteomes were noted after 24 h of a single G12Ci treatment, and CDK4/6i + G12Ci reduced growth in 2D- and 3D-HCC44 cells.[14] The combination of the CDK4/6i, palbociclib, and adagrasib had profound antitumor efficacy in NCI-H2122 and in the G12Ci-resistant SW1573 cell line and xenograft tumors.[75] A combination of CDK4/6i + MEKi is being assessed in *KRAS*-mutant NSCLC (NCT02022982).

A unique tricomplex inhibitor of GTP-KRAS^{G12C} by Revolution Medicines called RM-018 was unveiled in late 2019.[123] This inhibitor creates a ternary complex with the immunophilin cyclophilin A and KRAS^{G12C}, forming non-covalent interactions within the switch-I and switch-II regions of KRAS^{G12C}, irreversibly inhibiting KRAS^{G12C} and preventing RAF binding. RM-018 monotherapy in NCI-H358 and MIA PaCa-2 cells suppressed proliferation longer than with G12Ci alone, and *in vivo* administration led to dose-dependent tumor regression.[123] Moreover, sotorasib- and adagrasib-resistant *KRAS*$^{G12C/Y96D}$ LUAD NCI-H358, PDAC MIA PaCa-2, HEK293T, and NSCLC MGH1138-1 cells were sensitive to RM-018 alone, with reductions in p-ERK and p-RSK levels and cell viability comparable to the effects of sotorasib and adagrasib in

these lines with only *KRAS*^{G12C} mutations.[18] Finally, RM-018 IC_{50} levels in *KRAS*$^{G12C/Y96D}$ NCI-H358, MIA PaCa-2, and Ba/F3 cells were 7.3 nM, 3.4 nM, and 2.8 nM, respectively, whereas these values were all >2 μM for ARS-1620, sotorasib, and adagrasib.[18] While limited experimental data on RM-018 have been released, it may be a key contender in the fight against KRAS^{G12C}.

Other novel strategies. The latest information on other types of therapies being investigated for KRAS^{G12C} including peptide/dendritic cells/mRNA cancer vaccines[42,43] (NCT03948763 and NCT04853017), adoptive T-cell therapy, PROTACs, and CRISPR/Cas9, is nicely summarized elsewhere.[124,125] While switch-II pocket inhibitors are superior to nucleotide pocket inhibitors,[126] mathematical modeling suggests the inverse to be true if secondary *KRAS* mutations that result in faster nucleotide dissociation are acquired in *KRAS*^{G12C} cells.[127] Thus, while small-molecule inhibitor combinations with G12Ci are being heavily investigated due to their widespread availability and understanding, esoteric therapies may be better suited for overcoming G12Ci resistance. At least one clinical trial evaluating a KRAS-targeted vaccine with nivolumab and ipilimumab for advanced-stage *KRAS*-mutant NSCLC patients has been initiated (NCT05254184).

Finally, as opposed to overcoming resistance, one strategy may be to prevent resistance from occurring in the first place. Multiple low dosing has been studied in *EGFR*-mutant NSCLC.[128] A combination of IC_{20} doses of EGFR TKIs + RAFi + MEKi + ERKi completely inhibited MAPK signaling and significantly reduced cell viability and proliferation in parental PC9 cells, TKI-resistant PC9 cells, and patient-derived organoids and xenografts.[128] In addition, Hayashi *et al.* evaluated alternating monotherapy with the third-generation EGFR TKI osimertinib and second-generation EGFR TKI afatinib in 46 treatment-naïve advanced *EGFR*-mutant NSCLC patients.[129] Although the trial failed to meet its primary endpoint of 77% 12-month PFS (70.2%; 95% CI, 54.2–81.5%), the median PFS in the trial was 21.3 months (95% CI, 16.3 months–not reached), hinting at a utility for alternating therapies in NSCLC. Similarly, Koga and colleagues suggested that resistance *via* acquisition of certain secondary *KRAS* mutations during sotorasib (*G13D*, *A59S/T*, *R68M*) and adagrasib (*Q99L*) treatment can be overcome by treating with the opposite G12Ci.[15] Therefore, an alternating dosing regimen of sotorasib and adagrasib may be worth investigating.

Conclusions

Targeting KRAS has been a longstanding goal for several decades. While G12Ci poised for clinical use have only been studied since 2019[3,7,8,47,57,75] and our clinical understanding of KRAS^{G12C} inhibitor resistance is in its infancy,[17–20] tremendous strides have been made in treating *KRAS*^{G12C} NSCLC patients and understanding resistance preclinically. Diverse mechanisms of adaptive and acquired resistance have been explained and numerous clinical trials investigating a wide range of different combination therapies supported by preclinical data are already underway (Table 1). Little information on G12Ci intrinsic resistance is available. As such, specific therapeutic combinations may be more effective in cases of intrinsic resistance *versus* adaptive/acquired resistance, but there is not enough information at this time to answer this question.

With the success of G12Ci pipelines, researchers are beginning to investigate inhibitors specific to other *KRAS* mutations, such as G12D[130–133] and G12V.[130,134] To date, we have seen the tip of the proverbial iceberg. It will only be a matter of time before we amass an arsenal of diverse inhibitors directed toward the once 'undruggable' KRAS protein.

Declarations

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Author contribution(s)

Joshua C. Rosen: Conceptualization; Formal analysis; Visualization; Writing – original draft; Writing – review & editing.

Adrian Sacher: Conceptualization; Supervision; Writing – original draft; Writing – review & editing.

Ming-Sound Tsao: Conceptualization; Funding acquisition; Supervision; Writing – original draft; Writing – review & editing.

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