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Review Article

Targeting *KRAS*: Crossroads of Signaling and Immune Inhibition

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ABSTRACT

Mutations of *RAS* are commonly seen in human cancers, especially in lung, colorectal, and pancreatic adenocarcinoma. Despite huge effort for decades, targeting *RAS* mutations has been "undruggable" because of the molecular instability of RAS protein inhibition. However, the recent discovery of the *KRAS* G12C inhibitor paved the way to expand therapeutic options for patients with cancer harboring the *KRAS* G12C mutation. At the same time, the successful development of immune checkpoint inhibitors (ICIs) drastically changed the paradigm of cancer treatment and resulted in a better understanding of the tumor immune microenvironment in patients with *KRAS*-mutant cancer. This review describes the following: the clinical characteristics of cancer with *KRAS* mutation; successful development of the *KRAS* G12C inhibitor and its impact on the tumor immune microenvironment; and potential new avenues such as the combination strategy using *KRAS* inhibitor and ICI, with preclinical and clinical rationales for overcoming resistance to inhibition of *KRAS* to improve therapeutic efficacy for patients with cancer harboring *KRAS* mutations.

Keywords: *KRAS* mutation, *KRAS* G12C inhibitor, immune checkpoint inhibitor, tumor immune microenvironment

INTRODUCTION: *RAS* MUTATIONS IN CANCER

The *RAS* family of oncogenes, including Kirsten rat sarcoma viral oncogene homolog (*KRAS*), neuroblastoma rat sarcoma viral oncogene homolog (*NRAS*), and Harvey rat sarcoma viral oncogene homolog (*HRAS*), is the most frequently mutated gene family and accounts for approximately 30% of mutations in cancer cells.[1,2] *RAS* genes encode GTPases, which act as a gatekeeper to switch on and off RAS proteins, and thus, controls the downstream signaling pathways.[3] Therefore, RAS proteins are well known to have an important role in cell differentiation, division, proliferation, and survival by regulating its downstream pathways such as RAF-MEK-ERK (MAPK) and PI3K-PTEN-AKT pathways.[4] Hence, enormous effort was made to develop therapeutic options targeting these pathways, resulting in the

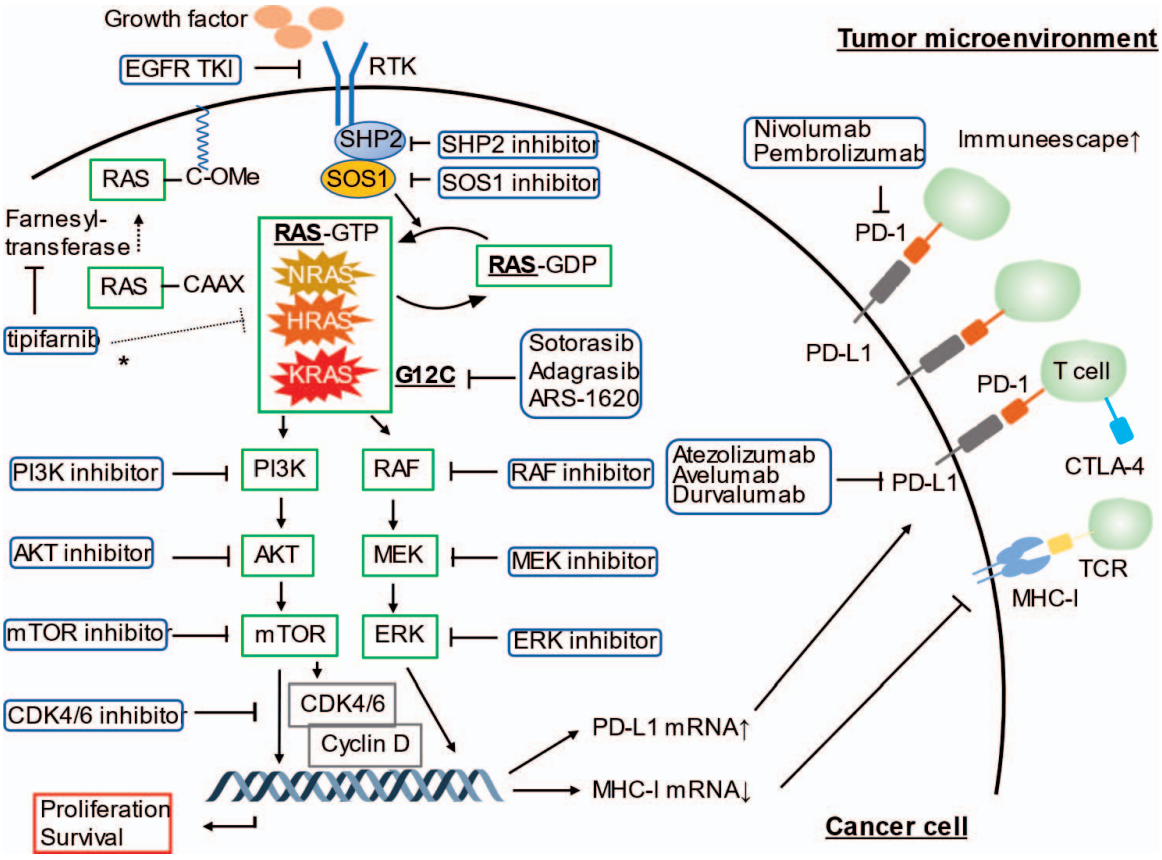

Figure 1. A schema of the RAS-RAF-MEK-ERK pathway, the immune microenvironment in *RAS*-mutant cancer, and potential therapeutic strategies targeting *RAS*-mutant cancer. Oncogenic RAS signaling promotes PD-L1 expression through stabilization of PD-L1 mRNA, leading to immune escape in the tumor microenvironment. The inhibitors of the RAS-RAF-MEK-ERK pathway and the RAS-PI3K-AKT-mTOR pathway are potential agents to improve survival outcomes in patients with *RAS* mutations. *Tipifarnib is a farnesyltransferase inhibitor and demonstrated encouraging efficacy (objective response rate: 55%) in patients with head and neck squamous cell carcinoma harboring *HRAS* mutations.

AKT: protein kinase B; CDK: cyclin-dependent kinase; CTLA-4: cytotoxic T-lymphocyte–associated antigen 4; EGFR: epithelial growth factor receptor; ERK: extracellular signal regulated kinase; GDP: guanosine diphosphate; GTP: guanosine triphosphate; HRAS: Harvey rat sarcoma virus oncogene; KRAS: Kirsten rat sarcoma viral oncogene homologue; MEK: mitogen-activated protein kinase; MHC-1: major histocompatibility class I; mRNA: messenger RNA; mTOR: mammalian target of rapamycin; NRAS: neuroblastoma rat sarcoma virus oncogene; PD-1: programmed cell death 1; PD-L1: programmed death-ligand 1; PI3K: phosphatidylinositol 3-kinase; RAF: rapidly accelerated fibrosarcoma; RAS: rat sarcoma virus oncogene; RNA: ribonucleic acid; RTK: receptor tyrosine kinase; SHP2: Src homology 2 domain-containing protein tyrosine phosphatase-2; SOS1: Son of sevenless 1; TCR: T-cell receptor; TKI: tyrosine kinase inhibitor.

successful development of clinically approved inhibitors of several proteins in these pathways such as MEK, BRAF, and epidermal growth factor receptor (EGFR) inhibitors in various types of cancer (Fig. 1).[5–8] Notably, *KRAS* is the most commonly mutated *RAS* isoform, making up approximately 85% of oncogenic *RAS* mutations in all cancer types.[1] *KRAS* mutation is seen in 61–86% of pancreatic ductal adenocarcinoma, 33–41% of colorectal adenocarcinoma, and 32% of lung adenocarcinoma.[2,9,10] Mutations in *KRAS* lead to a single amino acid substitution at a codon, and the substitution usually occurs in G12, G13, or Q61.[1] G12 mutations are known to account for more than 80% of all *KRAS* mutations.[1] In addition, the pattern of mutations of codons and substitutions of amino acid varies among tumor types. Among patients with cancer harboring *KRAS* mutation, *KRAS* G12C is seen in 46% of lung adenocarcinoma, and in contrast, *KRAS* G12D mutation is observed in approximately 45% of colorectal adenocarcinoma and pancreatic ductal adenocarcinoma.[9,11,12] Beyond these cancer types, analysis of the COSMIC database (version 95) demonstrated that *KRAS* G12D mutation is commonly seen in biliary tract cancer (45%), small intestine adenocarcinoma (41%), ovarian carcinoma (36%), and endometrial carcinoma (34%), whereas *KRAS* G12C mutation is seen in fewer than 10% of these cancer types.[13] The number of each *KRAS* point mutation across common cancer types and their histology is illustrated in Table 1. Therefore, targeting mutated *KRAS* rather than their downstream molecules, such as RAF, MEK, and mTOR, sounds reasonable to improve the survival outcome in a variety of tumors (Fig. 1). However, despite the effort for the past several decades, the strategy of targeting RAS proteins had not achieved a feasible therapeutic response. This is because of limited drug-binding pockets outside of the nucleotide-binding

pocket in RAS proteins and of the high affinity of guanosine diphosphate (GTP) for RAS proteins resulting in difficulties in the development of GTP-competitive inhibitors.[14,15] Given RAS proteins are active when they are associated with the plasma membrane, another strategy inhibiting farnesyltransferase, which is involved in the process of connection of RAS proteins to the plasma membrane, was attempted with promising preclinical results but unfortunately demonstrated minimal clinical activity.[16–18] However, a recent study revealed that tipifarnib, another farnesyltransferase inhibitor, demonstrated antitumor activities against *HRAS* mutated head and neck squamous cell carcinoma both in patient-derived xenograft (PDX) models and in a phase II clinical trial.[19,20] The breakthrough of the strategy to inhibit *KRAS* mutations was driven by the discovery of small molecules that can bind to the acquired cysteine residue in *KRAS* G12C covalently leading to the clinical development of KRAS G12C inhibitors.[21]

UNIQUE ROLE OF *KRAS* G12C MUTATION IN CANCER

Among patients with *KRAS*-mutant cancer, the prevalence of *KRAS* G12C mutation is higher in patients with non–small-cell lung cell carcinoma (NSCLC), accounts for approximately 25–46% of all *KRAS* mutations in NSCLC.[9,12,22,23] Therefore, clinical and molecular characteristics of *KRAS* G12C mutations were mainly derived from NSCLC. Among *KRAS* G12 mutations, G12C is frequently seen in former or current smokers than never smokers and is relatively more frequent in women than in men. In contrast, G12D mutation is the most common mutation in never smokers among patients with NSCLC with the *KRAS* mutation.[24] A recent analysis of a large series of patients with metastatic *KRAS*-mutant NSCLC revealed that G12C had a higher tumor mutation burden (TMB) and programmed cell death-ligand 1(PD-L1) expression, although overall survival from diagnosis was similar for G12C and non-G12C mutations.[23] In addition, in early-stage lung cancer, G12C mutation is also associated with an increase in higher TMB and recurrence of early-stage lung adenocarcinoma after resection.[25] On the other hand, lower TMB is accompanied by G12D mutation, suggesting different immunogenicity in each *KRAS* G12 mutation.[26] In NSCLC, TMB could be a potential biomarker to predict clinical benefit with anti-PD-L1 inhibitor, or atezolizumab, and therefore, the *KRAS* mutational status might become a predictor for immune checkpoint blockade therapy.[27]

Another cancer type that commonly accompanies *KRAS* mutation is colorectal cancer, and *KRAS* G12C mutation occurs in approximately 8% of metastatic colorectal cancers with *KRAS* mutation.[28] A recent comprehensive study[29] analyzed patients with colorectal cancer molecularly and clinically elucidated that the progression-free survival of patients with *KRAS* G12C mutation was poorer than those with *KRAS* non-G12C mutation, suggesting innate resistance to chemotherapy for this subpopulation in colorectal cancer. However, a robust description of clinical features of G12C mutation not only in colorectal cancer but also in other cancer types remains scarce, and further understanding of pathophysiological features, clinical characteristics such as responsiveness to systemic therapy, and prognosis of *KRAS* G12C mutations across a variety of cancer types, is needed.

CLINICAL ACTIVITY OF *KRAS* G12C INHIBITION IN CANCER

After the discovery of the small molecules covalently binding to the cysteine residue of the *KRAS* G12C mutation, several molecules inhibiting this mutation have been developed and investigated for their clinical applications preclinically and clinically. The first agent was ARS-1620, which selectively induced tumor regression in patient-derived tumor xenografts with *KRAS* G12C mutation in vivo.[30] Although ARS-1620 had little potency to continuously inhibit *KRAS* G12C because of the small size of the switch II pocket in KRAS G12C protein, it has been served as an important tool to investigate the potential therapeutic efficacy of the *KRAS* G12C inhibitor.[31] The successful clinical development of *KRAS* G12C inhibition was achieved by sotorasib (AMG 510) and adagrasib (MRTX849), with results of the recent phase 1 and 2 trials mainly for patients with NSCLC and colorectal cancer.[32–35] Sotorasib was the first agent that showed promising clinical efficacy in patients with advanced solid tumors with *KRAS* G12C through the phase 1 clinical trial. In this study, 129 patients were enrolled, among which 59 were with NSCLC, 42 with colorectal cancer, and 28 with other tumors. Approximately 32% of patients with NSCLC had an objective response and 88% had disease control. On the other hand, among patients with colorectal cancer, only 7% had a confirmed response, and 74% achieved disease control.[32] This disconcordance of response rate (RR) suggests different mechanisms of primary resistance to sotorasib among cancer types. Responses were also seen in other types of tumors, such as pancreatic and endometrial cancers, and malignant melanoma. The efficacy and safety of sotorasib for lung cancers were confirmed through the subsequent phase 2 trial. This study enrolled 126 patients with NSCLC among which most (81.0%) were previously treated with systemic chemotherapy or immune checkpoint inhibitors (ICIs), and demonstrated 37.1% of objective RR with 11.1 months of the median duration of response, leading to the U.S. Food and Drug Administration approval of sotorasib for patients with NSCLC harboring *KRAS* G12C mutations.[33] Adagrasib, another KRAS G12C inhibitor, initially demonstrated suppression of the downstream MAPK pathway and tumor regression across multiple

Table 1. The overview of each point *KRAS* mutation across cancer types

	***KRAS* Mutation and Cancer Type**																				
	G12							**G13**							**Q61**						
Location	**12A**	**12C**	**12D**	**12R**	**12S**	**12V**	**Others**	**13A**	**13C**	**13D**	**13R**	**13S**	**13V**	**Others**	**61H**	**61K**	**61L**	**61P**	**61R**	**Others**	**Others**	**Total**
Anus	**2**	**1**	**6**			**4**															**1**	**14**
Anal squamous cell carcinoma	2	1	6			4															1	14
Biliary tract	**41**	**60**	**428**	**34**	**72**	**181**	**10**		**7**	**47**	**4**	**3**	**3**	**3**	**20**		**5**		**1**	**6**	**29**	**954**
Bile duct carcinoma	38	55	331	24	55	164	10		5	37	2	3	3	2	17		5		1	6	25	783
Bile duct carcinoma intraductal papillary neoplasm	2		16			2			1	1					2						3	27
Bile duct intraepithelial neoplasia			13			1																14
Gallbladder carcinoma	1	5	68	10	17	14			1	9	2			1	1						1	130
Breast	**9**	**13**	**25**	**10**	**6**	**22**	**1**		**1**	**17**					**2**	**1**	**4**				**41**	**152**
Carcinoma	9	13	25	10	6	22	1		1	17					2	1	4				41	152
Central nervous system	**8**	**1**	**10**	**2**	**1**	**1**				**1**					**1**		**1**		**1**	**3**	**10**	**40**
Glioma	8	1	10	2	1	1				1					1		1		1	3	10	40
Cervix	**5**	**13**	**55**	**3**	**21**	**26**				**18**				**1**	**4**			**2**	**1**		**16**	**165**
Carcinoma	5	13	55	3	21	26				18				1	4			2	1		16	165
Colorectal	**271**	**420**	**1781**	**54**	**293**	**1116**	**9**		**33**	**1031**	**14**	**2**	**9**	**10**	**59**	**16**	**15**	**1**	**14**		**278**	**5426**
Anorectal adenocarcinoma			5	1	1	1				2					2		1					13
Colon adenocarcinoma	207	326	1328	38	207	838	3		19	762	7	1	5	5	41	13	6	1	7		201	4015
Rectal adenocarcinoma	64	94	448	15	85	277	6		14	267	7	1	4	5	16	3	8		7		77	1398
Endometrium	**74**	**59**	**241**	**3**	**20**	**157**		**2**	**12**	**78**		**1**	**2**	**3**	**6**		**4**				**49**	**711**
Carcinoma	74	59	241	3	20	157		2	12	78		1	2	3	6		4				49	711
Esophagus	**1**	**3**	**16**	**1**	**3**	**7**			**1**	**10**									**1**		**30**	**73**
Adenocarcinoma	1	2	8		3	5			1	8											18	46
Squamous cell carcinoma		1	8	1		2				2									1		12	27
Germ cell tumor	**7**	**5**	**10**	**7**	**4**	**31**				**2**		**1**	**1**	**1**	**1**		**1**	**1**	**5**		**19**	**96**
Extragonadal	2	1	3		2	8				2					1				5		13	37
Testicular	5	4	7	7	2	23						1	1	1			1	1			6	59
Hematopoietic and lymphoid	**77**	**34**	**284**	**33**	**60**	**102**	**4**	**2**	**15**	**288**	**1**	**1**		**9**	**72**	**3**	**6**	**13**	**13**	**1**	**277**	**1295**
Hematopoietic neoplasm	36	15	144	17	32	49	2	1	9	129				7	24	1	5	5	2		107	585
Lymphoid neoplasm	41	19	140	16	28	53	2	1	6	159	1	1		2	48	2	1	8	11	1	170	710
Kidney		**5**	**8**		**1**	**7**				**5**		**1**			**1**	**1**	**1**				**2**	**32**
Carcinoma		5	8		1	7				5		1			1	1	1				2	32
Liver		**4**	**19**			**4**			**2**	**6**		**1**			**6**						**43**	**85**
Carcinoma		4	19			4			2	6		1			6						43	85
Lung	**550**	**2578**	**1342**	**106**	**206**	**1543**	**42**	**4**	**206**	**212**	**7**	**6**	**7**	**6**	**64**	**13**	**20**	**2**	**11**	**4**	**58**	**6987**
Adenocarcinoma	355	1425	715	48	107	879	28	3	108	129	2	4	4	2	38	5	13		4		38	3907
Bronchioloalveolar adenocarcinoma	10	42	69	2	3	47		1	3													177
Invasive mucinous adenocarcinoma	1	11	22	1	3	26				1											1	66
Large cell carcinoma	9	52	17	2	5	38	1		5	3	1				8	1	3				1	146
Mixed adenosquamous carcinoma	1	18	8		3	5	1			7					2							45
Neuroendocrine tumor		2	3	1	2	8				1											4	21
Non–small cell carcinoma	149	915	434	44	72	480	10		87	64	4	1	3	4	12	5	3	1	5	1	5	2299
Pleomorphic carcinoma	9	16	5			10			1													41
Sarcomatoid carcinoma	3	37	14		1	17	1			2						1						76
Squamous cell carcinoma	11	54	55	7	10	31	1		2	5		1			3	1	1	1	2	3	9	197
Undifferentiated carcinoma	2	6		1		2									1							12

Table 1 continues on next page

Table 1. Continued

	KRAS Mutation and Cancer Type																						
	G12							G13							Q61								
Location	**12A**	**12C**	**12D**	**12R**	**12S**	**12V**	**Others**	**13A**	**13C**	**13D**	**13R**	**13S**	**13V**	**Others**	**61H**	**61K**	**61L**	**61P**	**61R**	**Others**	**Others**	**Total**
Melanoma	**3**	**4**	**12**	**4**	**8**	**11**	**2**		**1**	**14**				**1**	**1**	**2**	**7**	**1**	**5**		**35**	**111**
Malignant melanoma	3	4	12	4	8	11	2		1	14				1	1	2	7	1	5		35	111
Ovary	**40**	**36**	**278**	**35**	**14**	**264**		**4**	**9**	**48**	**1**		**2**	**1**	**9**		**3**				**23**	**767**
Carcinoma	34	25	243	30	14	231		4	7	43	1		2	1	9		3				21	668
Others	6	11	35	5		33			2	5											2	99
Pancreas	**82**	**160**	**3097**	**835**	**99**	**2169**	**21**	**3**	**5**	**73**	**5**	**7**	**2**	**4**	**102**	**9**	**15**	**1**	**40**	**2**	**51**	**6782**
Ductal carcinoma	79	133	2760	738	85	1867	16	3	5	56	5	5	2	4	87	9	8	1	26	2	42	5933
Dysplasia-in situ neoplasm	2	17	281	77	12	244	1			17		2			11		6		14		4	688
Neuroendocrine tumor	1		3	3		2	4								1						5	19
Osteoclast-like giant cell carcinoma		2	8	3	2	7																22
Pancreatic intraepithelial neoplasia (PanIN)		8	45	14		49									3		1					120
Prostate	**2**	**10**	**23**	**3**	**3**	**41**				**23**		**3**			**1**	**7**	**4**		**1**		**19**	**140**
Adenocarcinoma	2	10	23	3	3	41				23		3			1	7	4		1		19	140
Salivary gland			**1**							**1**				**1**			**1**				**6**	**10**
Carcinoma			1							1				1			1				6	10
Skin		**7**	**13**	**1**	**1**	**6**									**1**		**1**				**24**	**54**
Carcinoma		7	13	1	1	6									1		1				24	54
Small intestine	**15**	**13**	**94**	**4**	**11**	**37**				**40**			**1**	**1**	**2**		**1**				**10**	**229**
Adenocarcinoma	15	13	94	4	11	37				40			1	1	2		1				10	229
Soft tissue	**3**	**15**	**21**			**4**	**1**		**1**	**33**		**3**	**1**				**1**				**2**	**85**
Angiosarcoma		6	11			1			1	11		2										32
Leiomyosarcoma		4	2			1	1			1			1									10
Pleomorphic sarcoma			1			2				18												21
Rhabdomyosarcoma	3	5	7							3		1					1				2	22
Stomach	**13**	**12**	**97**		**8**	**29**			**2**	**61**		**11**	**1**	**1**	**4**	**3**	**2**		**3**		**30**	**277**
Adenocarcinoma	13	12	97		8	29			2	61		11	1	1	4	3	2		3		30	277
Thyroid	**4**	**20**	**38**	**30**	**20**	**22**		**1**	**1**	**24**	**2**	**11**			**2**	**8**	**3**	**3**	**40**		**10**	**239**
Anaplastic carcinoma	2	12	15	3	9	6				11		4				1			11		1	75
Follicular carcinoma	1	3	3	1	3	5				7		1				1		2	10			37
Medullary carcinoma				24	2	4				1		1			2	2	2		3		5	46
Mixed papillary and follicular carcinoma			8									4										12
Papillary carcinoma	1	5	12	2	6	7		1	1	5	2	1				4	1	1	16		4	69
Unknown primary	**4**	**14**	**9**	**2**		**12**	**2**		**1**	**5**					**1**			**1**			**3**	**54**
Carcinoma	4	14	9	2		12	2		1	5					1			1			3	54
Upper aerodigestive tract	**3**	**7**	**31**	**1**	**7**	**2**			**2**	**8**		**1**	**1**			**1**		**1**	**1**		**22**	**88**
Adenocarcinoma		1	19		1	1			1	5			1									29
Squamous cell carcinoma	3	6	12	1	6	1			1	3		1				1		1	1		22	59
Urinary tract	**10**	**20**	**44**	**11**	**7**	**32**				**13**	**1**				**3**	**2**	**3**				**21**	**167**
Adenocarcinoma	1		7	2	1	8				7							1				5	32
Carcinoma, unclassified	2	7	11	3		6				2					1	1	1				5	39
Transitional cell carcinoma	7	13	26	6	6	18				4	1				2	1	1				11	96
Total	**1224**	**3514**	**7983**	**1179**	**865**	**5830**	**92**	**16**	**299**	**2058**	**35**	**52**	**30**	**42**	**362**	**66**	**98**	**26**	**137**	**16**	**1109**	**25,033**

Data were extracted from the COSMIC database (version 95 released Mar 31, 2022). The number of tumors with a point mutation on the *KRAS* isoform is indicated in the table. Subtypes of tumors with the number of fewer than 10, benign tumors, and tumors with undetermined primary sites and unclear histology were removed from this analysis.
Bold values are the totals by location, mutation, and cancer type.
KRAS: Kirsten rat sarcoma viral oncogene homologue.

tumor types in cell lines and PDX models.[36] Subsequently, preliminary results from the KRYSTAL-1 trial showed promising efficacy of adagrasib mainly in patients with NSCLC (RR, 45%; disease control rate [DCR], 96%) and colorectal cancer (RR, 22%; DCR, 87%).[34,35] For heavily pretreated *KRAS* G12C-mutated advanced pancreatic cancer, encouraging clinical activity was demonstrated by sotorasib (RR, 21% [$n = 8$ of 38]; DCR, 84% [32 of 38]), and adagrasib (RR, 50% [5/10]; DCR, 100% [10/10]), respectively.[37,38] Sotorasib monotherapy also demonstrated meaningful clinical activity in other gastrointestinal tumors, such as biliary, appendiceal, and gastro-esophageal junction cancer (RR, 35% [6/17]; DCR; 100% [17/17]).[38]

Although these KRAS G12C inhibitors demonstrated meaningful clinical efficacy for *KRAS G12C*-mutated cancer, most patients with a response eventually became refractory after initiation of these treatments.[33] The mechanisms of primary and acquired resistance to these KRAS G12C inhibitors have been gradually explored. Skoulidis et al[39], reported sotorasib had similar RR among patients with *KRAS* G12C NSCLC with *STK11* comutation (RR with *STK11* comutation, 40.0%; without *STK11* comutation, 39.1%). Considering *STK11* mutation is associated with poor clinical outcome in general, sotorasib for *KRAS* G12C/*STK11* mutations may be an ideal option. In contrast, comutation with *KEAP1* was found to have lower RR (RR with *KEAP1* comutation, 20.0%; without *KEAP1* comutation, 44.0%).[39] Similarly, the KRYSTAL-1 trial also showed favorable RR in patients with NSCLC harboring *STK11* mutation but lower RR in those with *KEAP1* mutation.[34] These results suggest certain co-genomic alterations are associated with better or worse outcomes for patients treated with *KRAS* G12C mutation. Several trials combining KRAS G12C inhibitors with other therapy, such as chemotherapy, molecular-targeted therapy including EGFR inhibitors and cyclin-dependent kinase (CKD) 4/6 inhibitors, and ICIs, are ongoing to overcome the resistance and enhance the therapeutic efficacy. Several possible mechanisms of resistance to inhibition of *KRAS* G12C have been proposed recently in preclinical and clinical studies. Activation of bypass MAPK downstream pathway, epithelial-to-mesenchymal transition, activated proliferative signaling in cancer cells, and diminished antitumor immunity, were suggested as mechanisms of resistance to *KRAS* G12C inhibition, and a better understanding of resistance mechanisms is needed to explore promising treatment options to overcome resistance to inhibition of *KRAS* G12C.[40]

MECHANISM OF RESISTANCE TO ICIs IN *KRAS*-MUTANT LUNG CANCER

Recent advances in immunotherapy, including ICIs and adoptive cell therapies (ACT) such as chimeric antigen receptor therapy and T-cell receptor (TCR) therapy, have revolutionized the paradigm of cancer treatments, and along with the rapid progress of these treatments for a variety of types of cancer, the mechanisms of resistance and strategies to overcome resistance to immunotherapy have also been elucidated. The cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) inhibitor and the programmed cell death 1 (PD-1) and PD-L1 inhibitors were successfully developed and incorporated into the clinical setting, and the mechanisms of resistance have been revealed through both preclinical and clinical studies using these drugs. Resistance mechanisms are primarily categorized into innate and extrinsic resistance; and several mechanisms were reported, such as lack of neoantigen expression, activated or altered cell signaling pathways, an increase in immunosuppressive cells including regulatory T cells and myeloid-derived suppressor cells, activation in epithelial-mesenchymal transition, angiogenesis, and gut microbiome changes.[41–44] Among cancers harboring *KRAS* mutations, NSCLC was the main tumor type that the strategy with the use of ICI became successful.[45–48] Therefore, mechanisms of resistance to ICIs in *KRAS*-mutant cancer have been gradually reported through analyses of patients with NSCLC treated with ICI-containing regimens. Several studies revealed that patients with NSCLC or gastrointestinal tumors harboring *KRAS* mutation respond better to ICIs than those with the wild type or other oncogenic driver mutations, including *EGFR* and *ALK* mutations. Indeed, mutations in the RAS-MAPK pathway and TP53 were reported as potential positive predictors of the efficacy of the checkpoint blockade strategy.[49,50] However, most of this proportion shows primary or acquired resistance to checkpoint blockade, and therefore, the risk stratification and identification of promising therapeutic options are needed.[49,51,52] A study that evaluated *KRAS*-mutant lung adenocarcinoma treated with PD-1 axis inhibitors revealed that *STK11/LKB1* alterations are one of the major mechanisms of primary resistance when patients are categorized into three subgroups: *STK11/KLB1* comutations, *TP53* comutations, and *KRAS* mutation alone.[53–55] *STK11/LKB1* deficiency was also reported to be associated with the accumulation of neutrophils with T-cell suppression, T-cell exhaustion around the tumor microenvironment, and reduced PD-L1 expressions on the surface of tumor cells.[56] Loss of *LKB1* also results in suppression of stimulator of interferon genes (STING) expression that usually has a pivotal role in antigen presentation mainly by regulating dendric cells, and in priming of CD8+ T cells.[57] However, recent data showed *STK11/LKB1* alterations are not specific as a negative predictor for response to ICI, rather could be a poor prognostic biomarker across different therapies.[58] Overactivation of the RAS/RAF/MAPK pathway contributes to resistance to ICIs through its inhibitory effect on T-cell recruitment and function by producing the vascular endothelial growth factor and other immunosuppressive cytokines, and through the reduction of major histocompatibility complex (MHC) class I expression on

Table 2. Ongoing clinical trials evaluating the combination of *KRAS* inhibitors with immune checkpoint inhibitors

KRAS G12C Inhibitor	Immune Checkpoint Inhibitor	Phase	Cancer Type	Study Description	ClinicalTrials.gov Identifer
Sotorasib (AMG 510)	Pembrolizumab (PD-1 inhibitor)	I/II	Advanced solid tumors with *KRAS* G12C mutation	Sotorasib activity in subjects with advanced solid tumors with *KRAS* p.G12C mutation (CodeBreak 101)	NCT04185883
Sotorasib (AMG 510)	Anti-PD-1/PD-L1 inhibitors	I/II	Advanced solid tumors with *KRAS* G12C mutation	A Phase 1/2 study evaluating the safety, tolerability, PK, and efficacy of AMG 510 in subjects with solid tumors with a specific *KRAS* Mutation (CodeBreaK 100)	NCT03600883
Adagrasib (MRTX849)	Pembrolizumab (PD-1 inhibitor)	II	NSCLC with *KRAS* G12C mutation	Phase 2 trial of MRTX849 plus pembrolizumab for NSCLC with *KRAS* G12C mutation KRYSTAL-7	NCT04613596
Adagrasib (MRTX849)	Pembrolizumab (PD-1 inhibitor)	I/II	Advanced malignancy with *KRAS* G12C mutation	Phase 1/2 study of MRTX849 in patients with cancer having a *KRAS* G12C mutation KRYSTAL-1	NCT03785249
TNO155 (SHP2 inhibitor)	Spartalizumab (PD-1 inhibitor)	Ib	Selected malignancy including *KRAS* G12C-mutant NSCLC	Phase Ib study of TNO155 in combination with spartalizumab or ribociclib in selected malignancies	NCT04000529

KRAS: Kirsten rat sarcoma viral oncogene; NSCLC: non-small-cell lung cancer; PD-1: programmed cell death 1.

tumor cells.[59–61] Oncogenic RAS signaling also promotes tumor immunoresistance and regulates cell-intrinsic PD-L1 expression by stabilizing PD-L1 messenger RNA (mRNA).[62] The PD-L1 expression through activation of the RAS pathway also mediates immune escape in cell models and human tissues of NSCLC.[63] Therefore, inhibition of the RAS signaling pathway might be beneficial to reverse the immunosuppressive tumor microenvironment, leading to improvement of susceptibility to ICIs in *KRAS*-mutant cancer (Fig. 1).

RATIONALES FOR THE COMBINATION THERAPY OF KRAS G12C INHIBITION WITH ICI

The combination strategies using ICI and molecular-targeted therapy achieved successful development in certain cancer types such as renal cell carcinoma, but ICI plus agents targeting the MAPK pathway have mixed clinical outcomes so far.[64–67] Generally, NSCLC with *KRAS* mutation responds to ICI monotherapy or ICI with chemotherapy well compared with other mutation types, but most of them become refractory afterward.[68,69] Positive predictive factors in *KRAS* mutation are high TMB and PD-L1 expression, which may lead to a better response to the ICI treatment. On the other hand, *KRAS*-mutant cancer also creates immunosuppressive conditions around the tumor microenvironment, as discussed previously, resulting in a poorer response to immunotherapy. To overcome the therapeutic limitations in this context, several rationales for combining checkpoint blockade with *KRAS* inhibition to augment the efficacy of therapy for this population were proposed through analysis of preclinical models and clinical trials. One important study using sotorasib (AMG 510) revealed that sotorasib not only led to the regression of *KRAS* G12C tumors but also created a proinflammatory tumor microenvironment resulting in a durable response in mouse models.[70] This finding supports that *KRAS* G12C inhibitor can reverse the immunosuppressive environment, making cancer cells susceptive to ICIs or other types of immunotherapies. Another pivotal study reported that adagrasib (MRTX 849) increased MHC class I protein expression and decreased immunosuppressive factors. In a mouse model with *KRAS* G12C mutation, adagrasib increased M1-type tumor-associated macrophages, dendritic cells, and infiltrative T cells, and decreased myeloid-derived suppressor cells. Of note, the combination of adagrasib with ICI was effective even after the tumor cells showed progression with either ICI therapy or adagrasib monotherapy.[71] These findings suggest that KRAS inhibition can potentially make tumor cells more susceptible to ICI therapy by producing an inflammatory tumor microenvironment. Currently, several clinical trials are ongoing to evaluate the combination strategy using KRAS G12C inhibitors with ICIs and are awaiting clinical outcomes (Table 2).

To date, the strategies combining ICIs and tyrosine kinase inhibitors (TKIs) targeting the RAS/RAF/MAPK pathway were mainly evaluated in trials for patients with *EGFR*-mutant NSCLC and *BRAF*-mutant malignant melanoma. A few studies that evaluated the safety and efficacy of inhibition of BRAF and MEK in addition to ICI revealed that this combination strategy produced a tendency of longer survival in patients with malignant melanoma but led to a significant increase in the incidence of grade 3 or more adverse events.[72–74] Among patients with NSCLC harboring *EGFR* mutation, EGFR TKIs with immune checkpoint blockade were evaluated in the first or second and beyond line settings.

Table 3. Ongoing clinical trials evaluating adoptive cell therapy and vaccine therapy for *KRAS*-mutant malignancy

Agents	Other Agents	Phase	Cancer Type	Study Description	ClinicalTrials.gov Identifier
G12V-specific TCR transduced T-cell therapy	• Cyclophosphamide and fludarabine before infusion • Anti-PD-1 inhibitor if needed	I/II	Advanced pancreatic cancer with *KRAS* G12V mutation and HLA-A*11:01 allele	Mutant *KRAS* G12V-specific TCR transduced T Cell therapy for advanced pancreatic cancer	NCT04146298
Anti-KRAS G12V murine TCR	• Cyclophosphamide and fludarabine before infusion • Aldesleukin (high-dose interleukin-2)	I/II	Advanced cancer harboring *KRAS* G12V mutation	Administering peripheral blood lymphocytes transduced with a murine t-cell receptor recognizing the G12V variant of mutated *RAS* in HLA-A*11:01 patients	NCT03190941
Anti-KRAS G12D murine TCR	• Cyclophosphamide and fludarabine before infusion • Aldesleukin (high-dose interleukin-2)	I/II	Advanced cancer harboring *KRAS* G12D mutation	Administering peripheral blood lymphocytes transduced with a murine t-cell receptor recognizing the G12D variant of mutated *RAS* in HLA-A*11:01 patients	NCT03745326
mRNA-5671 vaccine (V941)	• Monotherapy or with pembrolizumab (PD-1 inhibitor)	I	NSCLC, Pancreatic cancer, Colorectal Cancer with *KRAS* (G12D, G12V, G13D, or G12C) mutation	A study of mRNA-5671/V941 as monotherapy and in combination with pembrolizumab (V941–001)	NCT03948763

HLA: human leukocyte antigen; *KRAS*: Kirsten rat sarcoma viral oncogene homologue; NSCLC: non–small cell lung cancer; PD-1: programmed cell death 1; PD-L1: programmed cell death-ligand 1; PK: pharmacokinetics; TCR: T-cell receptor

However, the combination of EGFR TKIs and ICIs led to a higher incidence of hepatotoxicity, interstitial lung disease, or pneumonitis with little survival benefit.[75–78] These results suggested difficulty in the development of the combination treatment using RAS/RAF/MAPK pathway inhibitors with immune checkpoint blockade. Tactics targeting *KRAS* G12C with ICIs could be a breakthrough to overcome these barriers, and ongoing trials are awaiting further safety and efficacy analysis.

Another potential strategy to enhance the efficacy of KRAS inhibitors is the use of an Src homology-2 domain-containing protein tyrosine phosphatase-2 (SHP2) inhibitor. SHP2 is a protein that mediates RAS activation downstream of receptor tyrosine kinase (RTK) and controls downstream signaling of PD-1 of T cells. SHP2 is required for the progression of *KRAS*-mutant NSCLC and thus, inhibition of SHP2 would be an ideal strategy to restore the sensitivity of *KRAS*-mutant NSCLC to MEK inhibition.[79,80] Indeed, the combination of SHP2 inhibitors and ARS-1620 (KRAS G12C inhibitor) was shown to be associated with a decrease in GTP-bound *KRAS* G12C activation, suppression of RTK-mediated MAPK reactivation, AKT and ERK pathways, and an increase in T-cell infiltration.[79–83] Several early-phase trials have just started recently to evaluate the efficacy and safety of SHP2 inhibitors in combination with ICIs such as spartalizumab (PD-1 inhibitor), cyclin-dependent kinase (CDK) 4/6 inhibitors, EGFR inhibitors, or ERK inhibitors (ClinicalTrials.gov Identifiers NCT04000529, NCT04330664, NCT04699188, NCT04670679, NCT04916236, and NCT03114319).

ADAPTIVE T-CELL THERAPY FOR *KRAS*-MUTANT CANCER

Along with the combination strategy using ICI and KRAS G12C inhibitors, several other strategies such as ACT and vaccine therapy are under investigation through early-phase clinical trials. ACT, including tumor-infiltrating lymphocyte (TIL) therapy, engineered TCR therapy, chimeric antigen receptor T-cell therapy, and natural killer cell therapy, has been recently developed to target small molecules such as tumor-specific neoantigens and clonally expressed molecules on tumor cells, leading to approval for the treatment of malignant lymphoma and multiple myeloma. In *KRAS*-mutant cancer, successful development of ACT was first identified in a metastatic colorectal cancer case harboring *KRAS* G12D mutation with an expression of HLA-C*08:02 treated with TIL.[84] HLA-A*11:01 in *KRAS* G12V and G12D mutations were also found as potential therapeutic targets, and two trials using a murine TCR recognizing these molecules were started but suspended (ClinicalTrials.gov Identifiers NCT03190941, and NCT03745326).[85] Other studies also showed potential targetable molecules such as *KRAS* G12V/HLA-A*0201 complex and *KRAS*-G12D neoantigens restricted by HLA-C*08:02 by engineering TCR-mimic antibody-drug conjugates and TIL, respectively.[86–88] These findings paved

the way to target other *KRAS* mutations rather than G12C. A tactic using mRNA vaccine encoding *KRAS* mutations was also found to induce CD-8 T-cell responses to KRAS tumor antigens in a preclinical study, and a phase I trial evaluating mRNA-5671 (V941) vaccine as monotherapy or in combination with pembrolizumab for patients with NSCLC and colorectal cancer harboring *KRAS* mutations is ongoing (ClinicalTrials.gov Identifier NCT03948763) (Table 3).

CONCLUSION

The successful clinical development of KRAS G12C inhibitor broadened treatment options for NSCLC and will possibly expand the therapeutic possibilities for other cancer types harboring *KRAS* G12C mutation. At the same time, the fact that many patients face primary or acquired resistance to this targeted therapy requires more effective strategies to augment therapeutic efficacy. This comprehensive review focusing on KRAS inhibitor and its association with the tumor immune microenvironment summarized potential strategies using the combination of KRAS inhibitor with ICIs, and other immunotherapies to overcome resistance to KRAS G12C inhibitor. In addition, there is an unmet need for patients with other *KRAS* mutations such as G12D and G12V, and further studies are necessary to bring treatment options for this population. Clinical trials using immunotherapy along with KRAS inhibition have just begun, and therefore, more investigations to evaluate clinical outcomes, reveal prognostic factors, and discover further mechanisms of resistance to these treatments, are necessary to achieve a longer duration of response and potential cure for patients with *KRAS*-mutant cancer.

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