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KRAS inhibition in metastatic colorectal cancer – An update

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Abstract

About half of colorectal cancers harbor mutations in the *KRAS* gene. The presence of these mutations is associated with worse prognosis and, until now, the absence of matched targeted therapy options. In this review, we discuss clinical efforts to target KRAS in colorectal cancer from studies of downstream inhibitors to recent direct inhibitors of $KRAS^{G12C}$ and other KRAS mutants. Early clinical trial data, however, suggest more limited activity for these novel inhibitors in colorectal cancer compared to other cancer types, and we discuss the role of receptor tyrosine kinase signaling and parallel signaling pathways in modulating response to these inhibitors. We also review the effect of *KRAS* mutations on the tumor-immune microenvironment and efforts to induce an immune response against these tumors.

Introduction

The Kirsten rat sarcoma virus (*KRAS*) gene is the most commonly mutated oncogene in cancer, and its activation promotes tumor proliferation and survival. *KRAS* mutations occur in 45% of colorectal cancer (CRC) and is a key driver in CRC oncogenesis.[1] Mutant *KRAS* is frequent in microsatellite stable right-sided CRC and is associated with poor prognosis (Figure 1).[2–4] *KRAS* mutations preclude response to epidermal growth factor receptor (EGFR) inhibitors and testing for *KRAS* mutations is recommended for all patients with metastatic CRC.[5,6] Successful targeting of KRAS has been elusive until the recent development of $KRAS^{G12C}$ inhibitors. In this review, we will discuss progress in direct KRAS inhibition, highlight other therapeutic approaches for *KRAS* mutant CRC, and summarize recently completed and ongoing clinical trials (Tables 1 and 2).

Inhibition of signaling downstream from KRAS

The role of KRAS in oncogenic signaling of CRC is shown in Figure 2. Early clinical efforts used selective inhibitors of downstream effectors to target signaling in *KRAS* mutant CRC

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but were unable to achieve meaningful clinical activity. In the phase 1 expansion for the MEK inhibitor trametinib, for example, no objective responses were seen in 28 patients with metastatic CRC, including 12 patients with *KRAS* mutant CRC.[7] Based on preclinical studies suggesting that, in *KRAS* mutant CRC, ERK signaling depends on KRAS activation, while phosphatidylinositol 3-kinase (PI3K) activation results from receptor tyrosine kinase (RTK) signaling, particularly IGF-IR, or *PIK3CA* mutation, combination therapy against ERK and PI3K signaling was tested.[8,9] The combination of the MEK inhibitor binimetinib plus the PI3K inhibitor buparlisib was not tolerated at continuous dosing in the phase 1 trial and responses were not seen in the CRC patients enrolled.[10] The narrow therapeutic index of MEK/ERK inhibitors likely limited the ability to sufficiently inhibit ERK signaling in these studies. The complexity of intracellular interactions among different signaling mediators also precluded success by release of negative regulatory holds on ERK signaling and reactivation of other oncogenic pathways.

Cell cycle proteins have also been targeted to inhibit *KRAS* mutant CRC. Gene expression profiling of CRCs from 55 patients showed that cell cycle and mitosis pathways were significantly upregulated in *KRAS* mutant tumors.[11] MEK and CDK4/6 inhibitors synergize to downregulate expression of cell cycle proteins and increase tumor regression in *KRAS* mutant CRC cell lines and patient-derived xenograft models[11,12]. This led to a randomized phase 2 clinical trial (ACCRU-GI-1618) of binimetinib (MEK inhibitor) and palbociclib (CDK4/6 inhibitor) versus TAS-102 in refractory metastatic *KRAS/NRAS* mutant CRC (NCT03981614). Median progression-free survival (PFS) of 2.1 months was seen in each arm, and median overall survival (OS) was also not improved (7.7 months in experimental arm, 6.6 months in TAS-102 arm).[13] Polo-like kinase 1 (PLK1) is a serine/threonine-protein kinase that promotes cell cycle progression. In *KRAS* mutant irinotecan resistant patient-derived xenograft models, PLK1 inhibitor (onvansertib) re-sensitized tumors to irinotecan.[14] An objective response rate (ORR) of 36% was seen in phase 1/2 clinical trial of onvansertib with FOLFIRI + bevacizumab in patients with *KRAS* mutant metastatic CRC, with plans for a follow-up randomized, phase 2 trial of this combination versus FOLFIRI + bevacizumab alone.[15]

Direct KRAS inhibition

$KRAS^{G12C}$ inhibitors:

KRAS^{G12C} is present in about 3% of metastatic CRC.[16] The potential to target KRAS has been revolutionized with selective KRAS^{G12C} inhibitors that bind to a pocket in the switch II region exposed in GDP-bound RAS. These drugs take advantage of the cysteine residue in the mutant protein and the intrinsic GTPase of KRAS^{G12C}, which is relatively higher than for other KRAS mutants.[17] The two KRAS^{G12C} inhibitors furthest along in clinical development are sotorasib (AMG510) and adagrasib (MRTX849).

Early data suggest response rates of 7–20% for KRAS^{G12C} inhibitor monotherapy in patients with metastatic *KRAS*^{G12C} CRC. The phase 1 clinical trial of sotorasib had a 7% response rate (3 of 42 patients), 73.8% disease control rate (DCR) and median PFS of 4.0 months.[18] The subsequent phase 2 trial of sotorasib at 960 mg daily reported objective response in 6 out of 62 (9.7%) patients but did not meet the primary endpoint of ORR of

20%. DCR was 82.3%; median PFS and OS were 4 and 10.6 months, respectively. The phase 2 CRC cohort of the KRYSTAL-1 study of adagrasib had an ORR of 19% (in 8 patients) and DCR of 86% (in 37 patients) among 43 evaluable patients. Median PFS was 5.6 months.[19] This clinical activity of $KRAS^{G12C}$ inhibitors in metastatic CRC is lower than that seen in metastatic non-small cell lung cancer, for which sotorasib has been granted accelerated approval by FDA based on results of phase 2 trial.[20]

Several research groups have shown reactivation of RAS signaling through RTKs to be a key mechanism of resistance to $KRAS^{G12C}$ inhibition in CRC.[21–23] Amadeo et al showed that in $KRAS^{G12C}$ CRC cell lines, $KRAS^{G12C}$ inhibition results in transient ERK inhibition, after which there is phospho-ERK rebound. Compared to non-small cell lung cancer cells, the $KRAS^{G12C}$ CRC cells have high basal RTK activation and are more responsive to growth factor stimulation, which induces higher level of phospho-ERK. The combination of cetuximab (EGFR inhibitor) and sotorasib resulted in sustained inhibition of phospho-ERK, increased cell death rate, and inhibited growth of patient derived CRC organoids and xenografts.[21] Ryan et al showed that multiple RTKs can drive feedback reactivation of wild-type RAS (including NRAS and HRAS) after $KRAS^{G12C}$ inhibition and provided evidence for co-inhibition of convergent nodes such as SHP2 or MEK to overcome adaptive resistance.[22,23] Clinical trials of $KRAS^{G12C}$ and SHP2 inhibitors are ongoing.

Consistent with the preclinical studies, clinical outcomes of patients with $KRAS^{G12C}$ metastatic CRC improved with co-targeting of $KRAS^{G12C}$ and EGFR in ongoing trials. Results reported from the phase 1b (n=40) cohort of sotorasib with panitumumab combination demonstrated ORR of 30% and DCR of 93%.[24] Median PFS was 5.7 months. Similarly, in the KRYSTAL-1 study phase 1b expansion cohort, the addition of cetuximab to adagrasib improved ORR to 46% (13 patients) and DCR to 100% among 28 evaluable patients; median PFS was 6.9 months. Treatment related adverse events of grade 3/4 were seen in 16% of patients.[19] These results have paved the way for registrational phase 3 clinical trials in patients with $KRAS^{G12C}$ metastatic CRC. The KRYSTAL-10 trial (NCT04793958) randomizes patients to adagrasib (600 mg twice a day) with cetuximab (500 mg/m2 every 2 weeks) versus chemotherapy (mFOLFOX6 or FOLFIRI +/− anti-VEGF/VEGFR) after progression on first line fluoropyrimidine-based doublet regimen. CodeBreaK300 (NCT05198934) is comparing the combination of sotorasib (960 mg daily in Arm A and 240 mg daily in Arm B) with panitumumab (6mg/kg every 2 weeks) versus chemotherapy (Trifluridine and Tipiracil or Regorafenib) in the third-line setting. In contrast with the higher efficacy of this combination, co-inhibition of $KRAS^{G12C}$ and MEK with sotorasib and trametinib combination among 18 patients with $KRAS^{G12C}$ metastatic CRC achieved ORR and DCR of 11% (2 patients) and 83% (15 patients), respectively, and is not being further pursued. [25] Further combination regimens that are being investigated in metastatic CRC in the CodeBreaK101 study include triplet regimens, such as, sotorasib + trametinib + panitumumab, sotorasib + panitumumab + chemotherapy, and sotorasib + bevacizumab + chemotherapy.

A mechanism of intrinsic resistance to targeted therapy may be co-occurrence of other genomic alterations in the tumor that sustain oncogenic signaling through the same or different pathways when the driver oncogene is inhibited.[26] Over a quarter of patients

with *KRAS*^{G12C} CRC have activating alterations in the PI3K/mTOR pathway, and 8% of patients have other likely pathogenic co-alterations in ERK signaling (e.g., mutations in *BRAF*, *RAF1*, *HRAS*, *NRAS*, *MAP2K1*, *PTPN11*, and other mutations in *KRAS*).[27] Data from the adagrasib monotherapy and combination cohorts showed no association between *PIK3CA* mutation status and response, but analysis was limited by the small sample size.[19] Furthermore, CRISPR screens in lung and pancreatic cancer models treated with KRAS^{G12C} inhibitor have revealed collateral dependencies on genes in cell cycle, RTKs that promote target engagement upstream of KRAS, and parallel PI3K signaling pathway.[28] Hence, combinations of sotorasib with everolimus (mTOR inhibitor), sotorasib with palbociclib (CDK 4/6 inhibitor), and adagrasib with palbociclib are also being investigated in advanced solid tumors. Other KRAS^{G12C} inhibitors in development include GDC-6036 (alone and in combination with inhibitors of EGFR, VEGF, PI3Kα, SHP2), JDQ443 (alone and with co-inhibition of SHP2, EGFR, MEK, CDK4/6, anti-PD-1), JAB21822 (alone and with cetuximab), LY3537982 (alone and with co-inhibition of SHP2, EGFR, ERK1/2, AurA, CDK4/6, anti-PD-1).

Early studies of progression samples suggest multiple resistance alterations can emerge with KRAS^{G12C} inhibition in CRC and primarily converge to reactivate ERK signaling. Data from the 74 gene circulating tumor DNA assay (Guardant360) from baseline and progression samples collected from 45 CRC patients treated with sotorasib in CodeBreaK100 study [29,30] revealed detectable acquired genomic alterations in 32 patients (71%), involving RTK genes in 27% (including *EGFR*, *ERBB2*, *KIT*, *ROS1*, *FGFR1*, *FGFR1*, *MET* and *PDGFRA*), cell cycle genes in 22%, DNA damage repair genes in 22%, and secondary RAS alterations in 16%. Genomic mechanisms of acquired resistance were also evaluated in 10 patients with metastatic CRC treated with adagrasib monotherapy on KRYSTAL-1 study using next generation sequencing of tumor tissue and or circulating tumor DNA.[31] Six patients had at least one putative resistance mechanism and five patients had multiple resistance alterations. Secondary *KRAS* mutations within the drug binding pocket (H95Q, H95R) were noted in two patients, other activating *KRAS* alterations in three and *MAP2K1* alterations in 4 patients. *KRAS*^{G12C} amplification, *NRAS*^{Q61K}, *BRAF*^{V600E}, and likely oncogenic *PIK3R1*^{S361fs} and *PTEN*^{N48K} mutations were noted in one patient each. Three patients developed acquired gene fusions (*EML4-ALK* rearrangement; *CCDC6-RET* fusion; and multiple fusions involving *FGFR3*, *BRAF*, and *RAF1*). A functionally distinct KRAS^{G12C} inhibitor, RM-018, that forms a tricomplex (RM-018, cyclophilin A, GTP-bound KRAS^{G12C}), was able to overcome resistance due to acquired *KRAS*^{Y96D} mutation affecting the switch-II binding pocket in cell lines from KRAS^{G12C} lung and pancreatic cancers.[32]

KRAS^{G12D} inhibitors:

KRAS^{G12D} mutation is present in 12% of metastatic CRC and is the most common *KRAS* mutation in CRC. The switch II pocket of KRAS^{G12D} molecule lacks a reactive residue that could form a potent covalent bond with an inhibitor, thereby limiting efforts at targeting it. Another challenge is a lower intrinsic GTP hydrolysis rate with *KRAS*^{G12D} mutation than with *KRAS*^{G12C}.[17] Recently, MRTX 1133 was developed as a noncovalent KRAS^{G12D} inhibitor that occupies the switch II pocket and extends three substituents (piperazine,

pyrrolidine and naphthyl groups) for binding with a picomolar affinity. Binding of MRTX 1133 prevented the formation of $KRAS^{G12D}$/GTP/RAF1 complex, inhibited signaling in cell lines and also showed *in vivo* tumor regression in 04.03 xenograft model of pancreatic cancer.[33] Investigational New Drug application for MRTX 1133 is planned in the second half of 2022.

$KRAS^{G12x}$ inhibitors:

Targeting of other *KRAS* G12 mutations, similarly, has been challenging due to the lack of a deep reactive binding pocket. $KRAS^{G12A}$ or $KRAS^{G12R}$ have about 40–80 fold decrease in GTP hydrolysis rate[17]. RMC-6236 has been developed to prevent interaction of activated RAS with downstream proteins by forming a non-covalent high affinity complex with intracellular chaperone protein, cyclophilin A, and GTP-RAS.[34] A phase I clinical trial of RMC-6236 in patients with advanced refractory solid cancers harboring specific *KRAS* mutations (G12A, G12D, G12R, G12S, G12V) is ongoing (NCT05379985).

Pan-RAS inhibitors:

BI-3406 and BI 1701963 are small molecule inhibitors that bind the catalytic site of SOS1 and prevent interaction of SOS1 with RAS-GDP, which reduces activation to RAS-GTP. In preclinical models of *KRAS* mutant tumors, these agents also attenuate MEK inhibitor-induced feedback reactivation of RAS signaling, synergized with $KRAS^{G12C}$ inhibitor, and potentiated irinotecan-induced DNA damage.[35,36] BI 1701963 is currently in phase I clinical development as monotherapy and in combination with MEK inhibitor (trametinib) in *KRAS* mutant advanced solid tumors (NCT04111458).[36]

Immunotherapy

The immune microenvironment of *KRAS* mutant CRC is immunosuppressive. An analysis of immune signatures using The Cancer Genome Atlas (TCGA) RNA-seq and the Koo Foundation Sun Yat-Sen Cancer Center microarray datasets showed decrease in Th1-centric co-ordinate immune response cluster in *KRAS* mutant CRC. [37] There was reduced expression of cytotoxic T cells, neutrophils, interferon gamma pathway, STAT1 and CXCL10 in *KRAS* mutant samples as compared to *KRAS* wild-type samples. *KRAS* mutant CRC also have lower expression of immune inhibitory molecules (*C*TLA4*, PDL1, PDL2*, LAG3, and TIM3) and CD4.[38] Similarly, another TCGA analysis showed significantly lower levels of IL6/JAK/STAT3, IFN-γ, complement, and IL2/STAT5 in $KRAS^{G12C}$ as compared to $KRAS^{nonG12C}$ and $KRAS^{wild\text{-}type}$ CRC patients.[16] Liao et al showed in mouse models of CRC with doxycycline-inducible oncogenic KRAS that mutant KRAS expressing tumors showed *de novo* resistance to anti-PD-1 therapy.[39] This was mediated by suppression of interferon regulatory factor 2, which results in increased expression of CXCL3, which bind CXCR2 and promote infiltration of myeloid derived suppressor cells in the tumor microenvironment. A phase I/II trial of SX-628 (CXCR1/2 inhibitor) in combination with nivolumab is ongoing in refractory RAS mutant MSS CRC (NCT04599140).

Ebert et al showed that MEK inhibition improved intratumoral CD8+ cell infiltration and increased anti-tumor activity in combination with anti-PD-L1 *in vivo*.[40] Canon et al also showed that in CT26 *KRAS*^{G12C} mutant CRC model, sotorasib increased intratumoral cytotoxic T-cell infiltration and pro-inflammatory cytokines including interferon gamma signaling.[41] Similarly, adagrasib was shown to increase MHC class 1 expression and effector cell infiltration while decreasing immune inhibitory cytokines and myeloid-derived suppressor cells in CT26 mice expressing KRAS^{G12C} [42]. Marked *in vivo* synergy between KRAS^{G12C} inhibitor and anti-PD-1 was shown by both groups. Clinically, the combination of sotorasib with anti-PD-1 (pembrolizumab or atezolizumab) in non-small cell lung cancer resulted in grade 3/4 transaminitis in about half of the patients. A low dose sotorasib lead-in strategy before the combination treatment appears to be better tolerated and is being further investigated.[43] The phase 3 clinical trial of atezolizumab and cobimetinib and of atezolizumab monotherapy versus regorafenib in microsatellite stable CRC did not meet the primary end point of improved OS in the combination arm.[44] About half of the patients (99 out of 183) in the combination arm were *KRAS* mutant.

Cancer vaccines:

Various vaccination strategies, including mRNA and peptide vaccines, are under investigation to potentiate T cell sensitization to mutant KRAS neoantigens. A phase 1 clinical trial is ongoing using mRNA-5671/V941 vaccine alone or combined with anti-PD-1 (pembrolizumab) in patients with advanced or metastatic microsatellite stable CRC with one of the four *KRAS* mutations (G12D, G12V, G13D or G12C) and HLA types of HLA-A11:01 or HLA C08:02 (NCT03948763). Another phase 1 trial of pooled mutant-KRAS peptide vaccine with nivolumab and ipilimumab in microsatellite stable CRC is ongoing (NCT04117087). TG02 is a neoantigen peptide cancer vaccine developed by Targovax that contains eight synthetic peptides representing fragments of the most frequent RAS mutant peptides seen in rectal cancer. A phase 1b clinical trial of TG02 alone or in combination with pembrolizumab was conducted in locally advanced primary or recurrent KRAS codon 12 or 13 (exon 2) mutant CRC (NCT02933944). TG02 doses were administered along with granulocyte–macrophage colony-stimulating factor before pelvic surgery. The trial was terminated early. Out of the 6 patients enrolled, 4 had TG02 specific immune response assessed by delayed type hypersensitivity and 3 had systemic presence of TG02 specific T cells.

Adoptive T cell therapies:

Adoptive T cell therapy involves ex vivo expansion of patient's tumor infiltrating T cells that have specificity for neopeptides generated by somatic mutations in the patient's tumor followed by autologous transfusion of these T cells. Tran et al published a case report of a patient with *KRAS*^{G12D} metastatic CRC who received a polyclonal infusion of HLA-C*08:02–restricted tumor-infiltrating lymphocytes targeting four neopeptides of KRAS^{G12D} and achieved objective response in all lung metastases.[45] On progression of one of the lung metastasis after 9 months, loss of chromosome 6 haplotype that encoded the HLA-C*08:02 allele was observed in the tumor. Maoz et al reported that (18.4%) of patients (687 of 3734) with CRC have at least one copy of HLA-C*08:02, and 2.3 % of patients (85 of 3734) have HLA-C*08:02 and *KRAS*^{G12D}.[46] HLA-A*11:01-restricted T-cell receptors

specifically targeting $KRAS^{G12D}$ and $KRAS^{G12V}$ neo-peptides have also been reported.[47] Another strategy to enhance neopeptide recognition by T cells is to genetically modify patient's tumor infiltrating lymphocytes by transduction of an HLA-restricted murine T-cell receptor that specifically recognizes mutant RAS neopeptides using a retroviral vector, followed by adoptive T cell transfer.[47] This strategy is being investigated in a phase 1/2 clinical trial in patients with HLA-A*11:01 positive metastatic or unresectable RAS^{G12D} or RAS^{G12V} cancers, including CRC (NCT03745326, NCT03190941).

Additional Strategies to target KRAS mutant colorectal cancer

Novel non-direct KRAS inhibition treatment strategies for *KRAS* mutant CRC are detailed in Table 2, such as, inhibition of autophagy, induction of apoptosis, inhibition of metabolism and reversal of mesenchymal phenotype.

Conclusions

In conclusions, there has been encouraging progress in development of novel therapeutics for *KRAS* mutant CRC, which include mutation specific KRAS inhibitors as well as immunotherapeutic approaches. Further research is needed to elucidate mechanisms of resistance to targeted therapy for combination development and to effectively immunomodulate the tumor microenvironment.

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Conflict of Interest Statement

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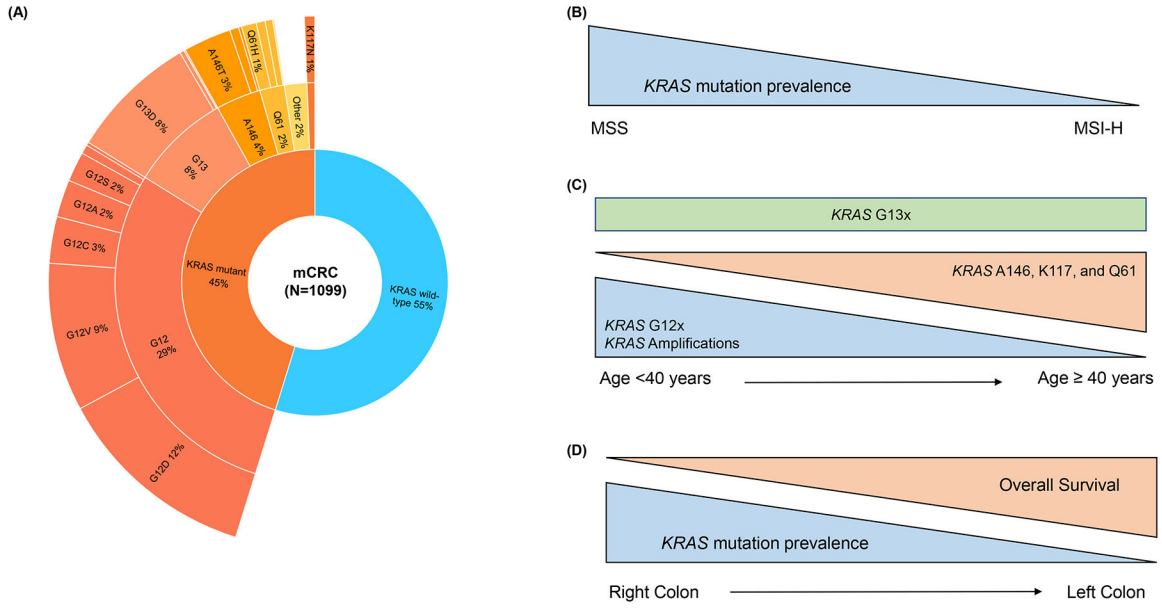


Figure 1.

Frequency and clinical associations reported for *KRAS* alterations in metastatic colorectal cancer (mCRC)

A) Frequencies of *KRAS* alterations in mCRC at Memorial Sloan Kettering Cancer Center.[1] Dataset was obtained through the cBioPortal for Cancer Genomics.

B) *KRAS* mutations are more frequent in microsatellite stable (MSS) CRC as compared to microsatellite instability-high (MSI-H) CRC.[3]

C) In a CRC dataset from Foundation Medicine (N = 13,336), there was higher prevalence of *KRAS* G12 mutations and *KRAS* amplifications in younger patients; more frequent *KRAS* A146, K117, and Q61 mutations in older patients; and no difference in *KRAS* G13 alterations by age. Analysis used age as a continuous variable as well as used age 40 as a stratification cut-off.[3]

D) Prevalence of *KRAS* mutations decreases sharply from right to left-sided colon cancer but starts rising again in rectal cancer.[2] Right-sided CRC is independently associated with worse overall survival as compared to left-sided CRC.[2] Specific *KRAS* mutations are also associated with poor prognosis.[4]

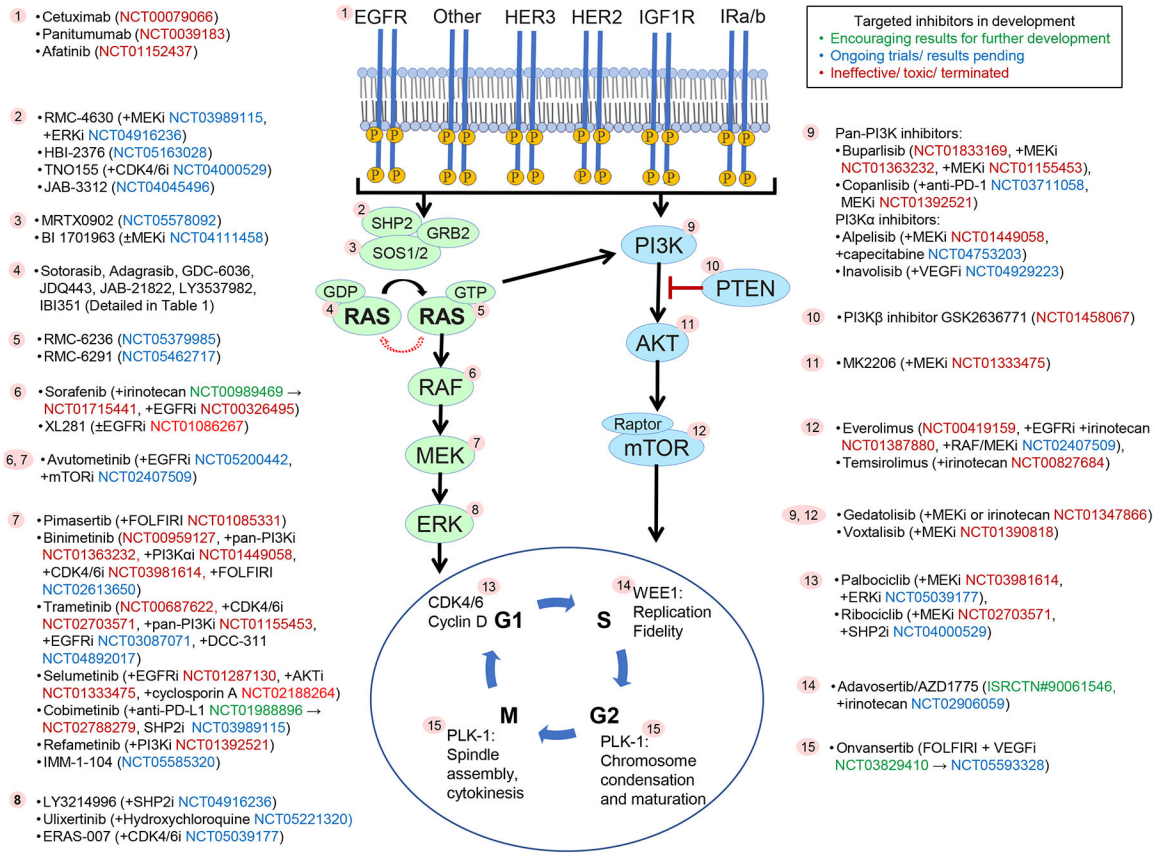


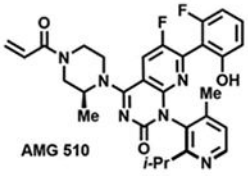
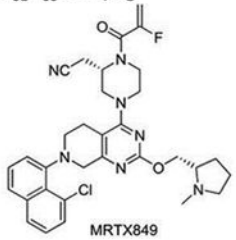
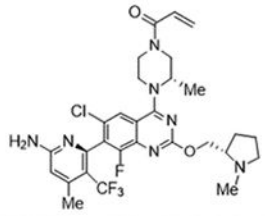
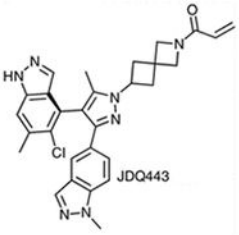
Figure 2.

KRAS in oncogenic signaling.

Binding of ligands (e.g., growth factors, cytokines) to receptor tyrosine kinases activates guanine nucleotide exchange factors (e.g., SOS1/2), which mediates conversion of inactive GDP-bound RAS to active GTP-bound RAS. GTP-bound RAS promotes cancer growth mainly via activating ERK signaling and, to a lesser extent, via activating the phosphatidylinositol 3-kinase (PI3K) and other oncogenic pathway. An intrinsic GTPase of RAS inactivates it. Activating mutations of RAS are most frequent in the KRAS isoform. Signaling mediators of RAS against which targeted inhibitors have been / are in clinical development for *KRAS* mutant metastatic colorectal cancer are marked with numbers. Targeted drugs along with combination drugs/regimens (if applicable) and respective clinical trial numbers are shown next to each number. Clinical trials colors indicate encouraging results for further development (green), ongoing trials (blue) and ineffective/toxic regimens (red).

Table 1.

Ongoing and recent clinical trials of KRAS inhibitors for KRAS mutant colorectal cancer..

KRAS Inhibitor	Combination Drug	Phase	Cancer Type	Trial ID
KRAS G12C Inhibitor				
AMG510 (Sotorasib) $C_{30}H_{30}F_2N_6O_3$  AMG 510	AMG 404 (Anti-PD-1); Trametinib (MEK inhibitor); RMC-4630 (SHP2 inhibitor); TNO155 (SHP2 inhibitor); Everolimus (mTOR Inhibitor); Palbociclib (CDK4/6 Inhibitor)	1b/2	Advanced solid cancers including CRC	NCT04185883
	MVASI (bevacizumab-awwb) + FOLFIRI or FOLFOX; Panitumumab (Anti-EGFR) +/- FOLFIRI; Trametinib (MEK Inhibitor) + Panitumumab		CRC	
	Panitumumab (EGFR inhibitor) vs Chemotherapy (TAS-102 or regorafenib)	3	CRC	NCT05198934
MRTX849 (Adagrasib) $C_{32}H_{35}ClFN_7O_2$  MRTX849	Cetuximab (anti-EGFR)	1b	CRC	NCT03785249
	TNO155 (SHP2 inhibitor)	1/2	NSCLC/ CRC	NCT04330664
	Palbociclib (CDK4/6 inhibitor)	1/1b	Advanced solid cancers including CRC	NCT05178888
	Cetuximab (anti-EGFR) vs Chemotherapy (mFOLFOX6 or FOLFIRI)	3	CRC	NCT04793958
	MRTX0902 (SOS1 inhibitor)	1/2	Advanced solid cancers including CRC	NCT05578092
GDC-6036 $C_{29}H_{32}ClF_4N_7O_2$ 	Cetuximab (anti-EGFR)		CRC	NCT04449874
	Bevacizumab (anti-VEGF); Inavolisib (PI3Kα inhibitor); GDC-1971 (SHP2 inhibitor)	1/2	Advanced solid cancers including CRC	
JDQ443 $C_{29}H_{28}ClN_7O$  JDQ443	TNO155 (SHP2 inhibitor); Tislelizumab (anti-PD-1); TNO155 + Tislelizumab	1/2	Advanced solid cancers including CRC	NCT04699188
	Trametinib (MEK inhibitor), Ribociclib (CDK4/6 inhibitor), Cetuximab (anti-EGFR)	1b/2	NSCLC/ CRC	NCT05358249
JAB-21822 Undisclosed formula and structure	Cetuximab (anti-EGFR)	1/2	CRC	NCT05002270
LY3537982 Undisclosed formula and structure	Abemaciclib (CDK4/6 inhibitor); Erlotinib (EGFR inhibitor); Pembrolizumab (anti-PD-1); Temuterkib (ERK 1/2 inhibitor); LY3295668 (Aurora kinase A inhibitor); Cetuximab (anti-EGFR); TNO155 (SHP2 inhibitor)	1	Advanced solid cancers including CRC	NCT04956640
IBI351 Undisclosed formula and structure	-	1	Advanced solid cancers including CRC	NCT05005234
	Cetuximab (anti-EGFR)	1b/3	CRC	NCT05497336
RMC-6291 Undisclosed formula and		1	Advanced solid cancers including	NCT05462717

Abbreviation: CRC: colorectal cancer.

Table 2:

Novel non-direct KRAS inhibition treatment strategies for *KRAS* mutant colorectal cancer.

Mechanism	Combination Drug	Phase	Cancer Type	Trial ID	Status
Immunotherapy					
Cancer Vaccines					
mRNA-5671/V941 vaccine	Alone or with Pembrolizumab (anti-PD-1)	1	Microsatellite Stable CRC; *KRAS* G12D, G12V, G13D or G12C; HLA-A11:01 or HLA C08:02	NCT03948763	Completed 2022
Pooled Mutant KRAS-Targeted Long Peptide Vaccine	Nivolumab and ipilimumab	1	Microsatellite Stable CRC with *KRAS* mutation	NCT04117087	Ongoing
Adoptive T cell therapies					
Peripheral blood lymphocytes transduced with HLA-A11:01-restricted anti-KRAS G12D murine T cell receptor	Preparative lymphodepletion (cyclophosphamide, fludarabine), post-infusion high dose aldesleukin (IL-2).	1/2	Advanced solid cancers including CRC; *KRAS* G12D; HLA-A11:01	NCT03745326	Ongoing
Peripheral blood lymphocytes transduced with HLA-A11:01-restricted anti-KRAS G12V murine T cell receptor	Preparative lymphodepletion (cyclophosphamide, fludarabine), post-infusion high dose aldesleukin (IL-2).	1/2	Advanced solid cancers including CRC; KRAS G12V; HLA-A11:01	NCT03190941	Ongoing
Stimulators of Innate Immunity					
SX-682 (inhibits chemokine receptors CXCR1/2, thereby decreasing myeloid- derived suppressor cells in tumor microenvironment)	Nivolumab	1/2	*RAS* mutated, MSS advanced CRC	NCT04599140	Ongoing
Magrolimab (anti-CD47, promotes phagocytosis of cancer cells by macrophages)	Cetuximab	1/2	Advanced solid cancers, with *KRAS* mutant CRC cohorts	NCT02953782	Completed 2020
REOLYSIN® (Reovirus Type 3 Dearing replicates selectively in Ras-transformed cells causing cell lysis, and stimulates antigen presenting cells)	FOLFIRI and Bevacizumab	1	*KRAS* mutant metastatic CRC	NCT01274624	Completed 2018
Imprime PGG (soluble beta-1,3/1,6 glucan which binds to complement receptor 3 on innate immune cells)	Cetuximab	2	*KRAS* mutant metastatic CRC	NCT00912327	Completed 2012
Lenalidomide (activates natural killer cells)	Cetuximab	2	Advanced *KRAS* mutant CRC	NCT01032291	Terminated due to lack of efficacy
Autophagy inhibitors					
DCC-3116 (ULK1/2 inhibitor)	Trametinib (MEK inhibitor)	1/2	Advanced solid cancers with RAS/MAPK pathway mutations *(KRAS, NRAS, NF1*, or *BRAF)*, with a CRC cohort	NCT04892017	Ongoing
Hydroxychloroquine	Ulixertinib (ERK inhibitor)	2	Advanced gastrointestinal cancers with MAPK mutations *(KRAS, NRAS, HRAS, BRAF non-V600, MAP2K1/2* or *ERK1/2)*, with a CRC cohort	NCT05221320	Ongoing
Apoptosis inducers					

Mechanism	Combination Drug	Phase	Cancer Type	Trial ID	Status
Immunotherapy					
ABBV-621/Eftozanermin (TRAIL receptor agonist)	FOLFIRI ± bevacizumab (VEGF inhibitor)	1	Advanced cancers, with KRAS-mutant CRC cohort	NCT03082209	Completed 2022
HDM201 (MDM2 inhibitor)	Trametinib (MEK inhibitor)	1	*RAS/RAF* mutant and *TP53* wild-type advanced colorectal cancer	NCT03714958	Ongoing
RGX-202–01 (inhibits creatine transporter SLC6a8)	FOLFIRI ± bevacizumab (VEGF inhibitor)	1	*RAS* mutant advanced CRC	NCT03597581	Ongoing
NBF-006 (inhibits Glutathione-S-Transferase P, GST-π, thereby causing oxidative stress)	-	1	CRC, NSCLC, Pancreatic Cancer	NCT03819387	Ongoing
Conatumumab (Death Receptor 5 agonist)	FOLFIRI vs FOLFIRI alone	2	*KRAS* mutant metastatic CRC	NCT00813605	Completed 2011
Metabolism inhibitors					
Telaglenastat (Glutaminase Inhibitor)	Palbociclib (CDK4/6 inhibitor)	1b/2	Advanced solid cancers with expansion cohort in metastatic *KRAS* mutant CRC	NCT03965845	Completed 2021
Inhibition of Epithelial to Mesenchymal Transition					
TP-0903 (AXL kinase inhibitor, reverses mesenchymal phenotype)	-	1	*BRAF, KRAS*, or *NRAS* Mutant metastatic CRC	NCT02729298	Ongoing

Abbreviation: CRC: colorectal cancer.