Current Advances and Trends in *KRAS* Targeted Therapies for Colorectal Cancer

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ABSTRACT

Kirsten Rat Sarcoma (*KRAS*) gene somatic point mutations is one of the most prominently mutated proto-oncogenes known to date, and accounts for approximately 60% of all colorectal cancer cases. One of the most exciting drug development areas against colorectal cancer is the targeting of undruggable kinases and kinase-substrate molecules, although whether and how they can be integrated with other therapies remains a question. Current clinical trial data have provided supporting evidence on the use of combination treatment involving MEK inhibitors and either one of the PI3K inhibitors for patients with metastatic colorectal cancer to avoid the development of resistance and provide effective therapeutic outcome rather than using a single agent alone. Many clinical trials are also ongoing to evaluate different combinations of these pathway inhibitors in combination with immunotherapy for patients with colorectal cancer whose current palliative treatment options are limited. Nevertheless, continued assessment of these targeted cancer therapies will eventually allow patients with colorectal cancer to be treated using a personalized medicine approach. In this review, the most recent scientific approaches and clinical trials targeting *KRAS* mutations directly or indirectly for the management of colorectal cancer are discussed.

Introduction

Colorectal cancer continues to be one of the most frequent causes of cancer death in developed countries, and is on the rise in developing countries especially among Asian nations. Ranked third worldwide behind lung and stomach cancers in terms of mortality rate, colorectal cancer's overall curative rate has also not improved over the last decade with a constant 5-year survival rate of 64% (1). To date, somatic point mutations of the Kirsten Rat Sarcoma (*KRAS*) gene account for approximately 30% to 50% of all colorectal cancer cases (2, 3), making it the most prominently mutated proto-oncogene known to date.

Ras was the first discovered superfamily of small G proteins comprising of three proto-oncogenes (*KRAS, HRAS, NRAS*) and four 21 kDa subfamily proteins (KRAS4A, KRAS4B, NRAS, HRAS; refs. 4, 5). The Ras gene superfamily is mainly involved upstream of the RAF–MEK–ERK MAPK cascade and PI3K-AKT cell signaling pathway, which have an important role in cell proliferation, differentiation, and survival (6). KRAS exists as either a guanosine triphosphate (GTP)-bound active state or guanosine diphosphate (GDP)-inactive state in the GTP hydrolysis cycle. This is regulated by both switch I and switch II domains, which undergo complex conformational changes during nucleotide exchange along the inner plasma membrane. The wild-type (wt) KRAS protein is temporarily activated following RTK signal transduction, but a mutated KRAS protein will forces downstream signaling pathways to be activated all of the time, thereby promoting a tumorigenic cellular circuit that is often associated with targeted therapy resistance (7, 8). Most of these findings support the tumor suppressor function of wt KRAS (9–11), and its dysregulation can be further enhanced by gain of guanine nucleotide exchange factors (GEF) function or loss of downstream GTPase activating proteins (GAP) negative regulators (12).

The activating point mutation of the *KRAS* oncogene on codon 12 (exon 2) is the initiating event in the majority of colorectal cancer cases (83%; ref. 13). Point mutations can also occur at codon 13 (exon 2; 14%) and occasionally at codon 61 (exon 3; 2%). These allelic mutations are located in close proximity of the GTP binding site, thus interfering with GTPase activity. The most clinically frequent substitutions are aspartate (G12D, 36%) followed by valine (G12V, 23%), both of which are found to occur on codon 12 (14). These positions are crucial for KRAS to function as a GTPase and RAF kinase activator. Even though RAS genes share up to 90% amino acid sequence identity with near-identical structural and biochemical properties, they are nevertheless differentially expressed and mutated with different frequencies in different cancers (15, 16). This has been seen in the case of *NRAS* mutations, which are found mostly in cutaneous malignant melanoma on the codon 61, whereas *KRAS* mutations are found mostly in pancreatic ductal and colorectal adenocarcinomas on codons 12 and 13 (17, 18). These suggest that different RAS mutations exert distinct oncogenic properties in a tissue-specific context due to yet unknown reasons.

Current approaches involving anti-*EGFR* therapies as well as nonspecific cytotoxic chemotherapeutic regimens such as FOLFOX [folinic acid (FA), fluorouracil (5-FU), and oxaliplatin] and FOLFIRI [FA, 5-FU, and irinotecan hydrochloride] in treating patients with colorectal cancer harboring mutated (mt) *KRAS* genes have apparently shown little benefit. Indirect alternative approaches such as the disruption of KRAS posttranslational modification using farnesyltransferase inhibitors have also failed to demonstrate efficacy in *KRAS*-driven colorectal cancer trials (19, 20). To date, most of these clinically approved conventional regimens are often accompanied by dose-related toxicities, drug resistance, and undesirable physiological side effects, thus creating the need for other target-specific therapeutic alternatives. In this scoping review, the most recent targeted approaches and clinical trials tackling *KRAS* mutations either directly or indirectly in the clinical management of colorectal cancers are discussed and are summarised in **Fig. 1**.

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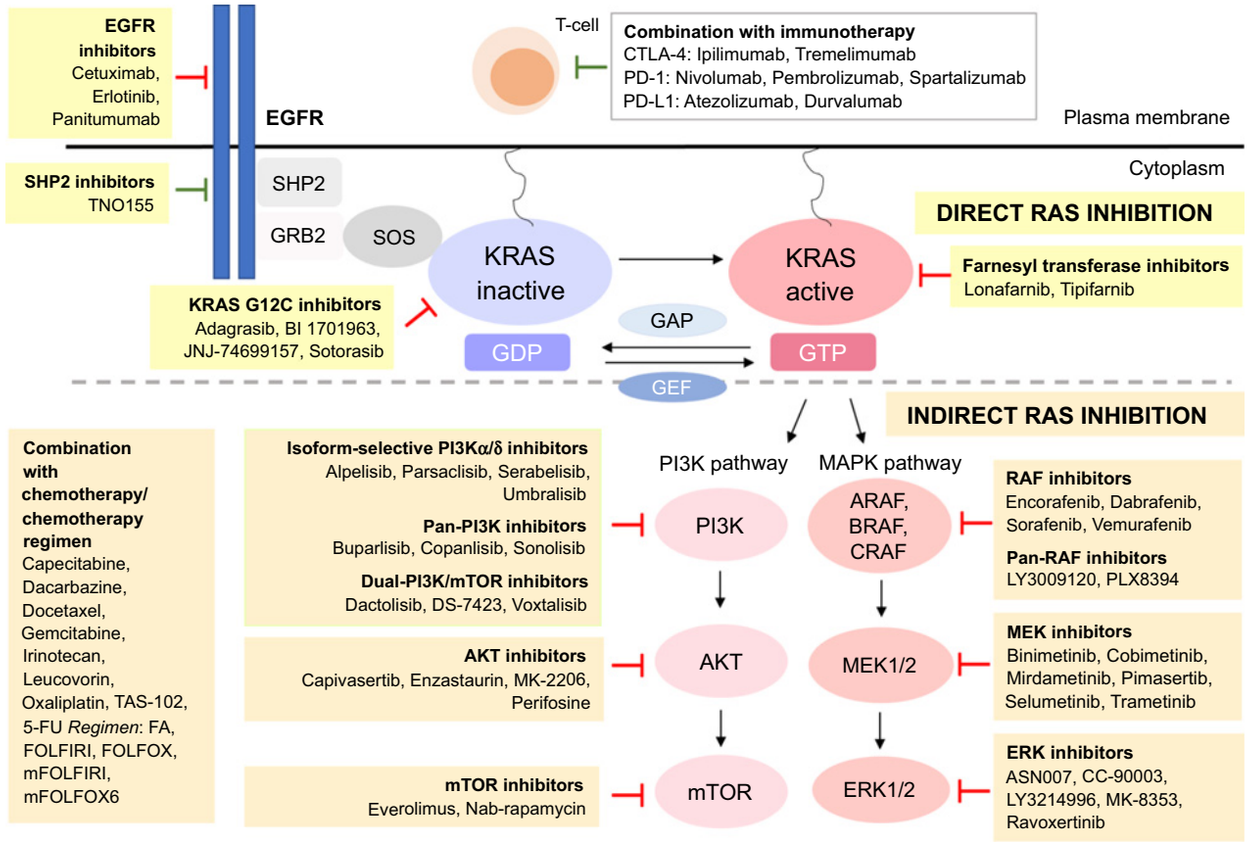

Figure 1.

Clinical development of *KRAS* targeted therapies for colorectal cancer. EGFR tyrosine kinase receptors are activated upon ligand binding and subsequently auto-phosphorylated for the adaptor protein GRB2, which binds to the guanine nucleotide exchange factor-Son of Sevenless (GEF-SOS) as the exchange factor for K-ras. Inhibition of SOS or SHP2 decreases the exchange rate of GDP-GTP, thereby reducing GTP-bound RAS. Mutant RAS proteins result in persistent activation of various intracellular signalling cascades, including both PI3K/AKT and MAPK/ERK pathways, and serve to activate critical oncogenic signalling in human cancers. A number of strategies have been developed to directly or indirectly inhibit RAS. Direct Ras inhibitions are shown in yellow boxes, whereas indirect Ras inhibitions are shown in orange boxes. All data were compiled from ClinicalTrials.gov. ARAF, A-RAF proto-oncogene serine; BRAF, B-RAF proto-oncogene serine; CRAF, C-RAF proto-oncogene serine; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FOLFIRI, FA, 5-FU and irinotecan; FOLFOX, chemotherapy regimen of FA, 5-FU, and oxaliplatin; GEF, guanosine exchange factor; GRB2, growth factor receptor bound protein 2; mFOLFIRI, modified FOLFIRI; mFOLFOX6, 5-FU, leucovorin, and oxaliplatin; PD-L1, programmed cell death-ligand 1; SHP2, cytoplasmic Src homology 2-containing protein tyrosine phosphatase-2; SOS, son of sevenless.

Direct Targeted Therapies for *KRAS* Colorectal Cancer

KRAS has been long considered to be undruggable, in part because of lacking picomolar affinity targetable binding pockets for drugs on their smooth surfaces. However, recent laboratory and clinical advancements in drug discovery reveal opportunities to bring an end to *KRAS*-driven cancers. In 1997, Taveras and colleagues (21) developed the first ras nucleotide exchange inhibitor SCH-53239, which binds to ras-GDP protein to prevent Ras activation. However, the low toxicity and poor metabolic stability of the inorganic functional group of hydroxylamine may not be an ideal drug compound. Since then, more compounds were developed to target the RAS protein towards guanosine substrates by inhibiting the RAS–RAF interaction (22–24), Ras–SOS interaction (25, 26), or Ras–effector interaction (27). Again, the development of these compounds has been disappointing due to low potency. Perhaps the full-length Ras's hidden structure is the key to combat the untargetable KRAS.

New insights into the structure and function of KRAS proteins reveal opportunities to target the activating substitution of oncogenic Ras, and are provided in **Table 1**. In 2013, Ostrem and colleagues (28) were the first to identify compounds that selectively target cysteine-12 in mt KRAS G12C without inhibiting wt KRAS. These compounds were used to lock the mt KRAS G12C in its inactive GDP-bound state by binding to a pocket near the nucleotide-binding site and preventing GTP recharging. A similar direct targeted approach led to the development of adagrasib, ARS-1620, ARS-853, SML-8–73–1, and sotorasib that acts as KRAS G12C allele-specific inhibitors with promising therapeutic potential (29–33).

Moving forward, Amgen and Mirati Therapeutics reported promising early clinical data evaluating two novel covalent KRAS G12C inhibitors, sotorasib (NCT03600883) and adagrasib (NCT03785249) in patients with *KRAS* G12C mutation solid tumors (34–36). Sotorasib appears to be safe and well-tolerated at the doses and time courses reported to date (33). An improved version of ARS-1620, JNJ-74699157 was developed by Janssen Research and Development and Wellspring Biosciences under clinical evaluation (NCT04006301).

Table 1. Direct targeted strategies for mutated *KRAS* colorectal cancer in clinical trials.

Agents	Targets	Trial phase	Clinical trial
KRAS G12C			
Adagrasib - *MRTX-849* [Array BioPharma, Mirati Therapeutics, Novartis]			
±Pembrolizumab ±Cetuximab/Afatinib	±PD-1 ±EGFR	I/II	NCT03785249*
+TNO155	+SHP2	I/II	NCT04330664*
BI 1701963 [FORMA Therapeutics, Boehringer Ingelheim]			
±Trametinib	±MEK1/2	I	NCT04111458*
+Irinotecan	+Chemo	I	NCT04627142*
JNJ-74699157 - *ARS-3248* [Janssen Biotech & Wellspring Biosciences]			
Monotherapy	+KRAS G12C	I	NCT04006301
Sotorasib - *AMG510* [Amgen, Carmot Therapeutics]			
Monotherapy	+KRAS G12C	I	NCT03600883*

Abbreviations: CDK, cyclin-dependent kinase; Chemo, chemotherapy; ChemoR, chemotherapeutic regimen; PD-L1, programmed cell death-ligand 1; Italized, other names; [], developer; +, drug included in the treatment arms; ±, drug included in one of treatment arms; *, clinical trial recruitment. Compiled from Clinical Trials.gov.

Novartis and Mirati Therapeutic had recently paired up to recruit *KRAS* G12C mutation advanced solid tumor patients to evaluate the combination efficacy of MRTX849 and Src homology region 2-containing protein tyrosine phosphatase 2 (SHP2) inhibitor TNO155 in a phase I/II trial (NCT04330664). Until recently, a pan-KRAS inhibitor, BI 1701963, that targets both KRAS G12C and son of sevenless 1 (SOS1), has entered clinical trials for patients with *KRAS* mutated solid tumors (NCT04111458; ref. 37) and colorectal cancer (NCT04627142; ref. 38). Another phase I trial with anti-PD-L1 antibody Avelumab (NCT04449874) was also recently approved for *KRAS* G12C mutated metastasis colorectal cancer (mCRC).

A direct targeted approach for another type of mt KRAS protein was carried out with a few promising KRAS-G12D selective targeting compounds. Welsch and colleagues (39) have developed a pan-RAS inhibitor, compound 3144 to target multiple adjacent sites on KRAS G12D protein near D38, A59, and Y32 sites. This compound was found to inhibit tumor growth in xenograft mouse cancer models. Another group identified KRpep-2d peptide that selectively inhibits protein–protein interactions (PPI) between KRAS G12D and SOS1 protein after screening a random T7 phage display peptide library (40). Computational analyses of the mt KRAS G12D protein structure make it feasible to develop KRAS allosteric ligand KAL-214040358 that selectively binds to the P110 site KRAS G12D (41). These findings suggest a new potential site for binding small molecule inhibitors of mt KRAS G12D.

Plasma membrane association inhibitors—FTI, GGTI, and PDEδ

Early approaches to disrupt Ras protein localization to the plasma membrane once seemed to be a promising prospects for pharmacologic interventions using farnesyl transferase inhibitors (FTI). Preclinical mouse studies using FTIs showed significant antitumor activity with no toxicity stimulated the start of clinical trials (42, 43). However, colorectal cancer clinical outcomes from phase II and III trials testing FTIs were disappointing (19, 20). This failure was predicted years earlier when Whyte and colleagues (44) found that *KRAS* and *NRAS* can alternatively be geranylgeranylated without farnesylation, a process known as alternative prenylation. Since then, more studies are focused on using combination therapy of FTIs and geranylgeranyl transferase inhibitors (GGTI) to suppress KRAS activity. *In vivo* study of the L-778, 123, a dual FTI and GGTI demonstrated dose-limiting toxicity; however, this compound's clinical pharmacodynamic assessment did not inhibit KRAS prenylation in human patient samples (45). Until recently, a novel FGTI-2734, dual FT, and geranylgeranyl transferase-1 (GGT-1) inhibitor was developed by Kazi and colleagues (46). This compound was found to inhibit tumor growth of mt KRAS in xenograft animal models and showed promising drug potential for *KRAS* cancers. Since most discoveries suggest that FTIs are not effective in managing *KRAS*-mutated cancers, researchers have subsequently shifted their attention towards using FTIs for *HRAS*-mutated cancers.

In 2013, the discovery of the farnesyl-binding pocket of phosphodiesterase 6 delta (PDEδ) provided an alternative therapeutic window against mt *KRAS* cancers. PDEδ inhibitors have been developed to disrupt KRAS localization of the plasma membrane by altering KRAS4B membrane trafficking and cellular activity, thereby impairs KRAS/PDEδ interaction. The first reported PDEδ inhibitor, deltarasin was identified to inhibit the prenyl-binding pocket of PDEδ with nanomolar affinity selectively and reduce tumor growth *in vitro* and *in vivo* (47). An improved PDEδ inhibitor, deltazinone 1 was subsequently reported with improved selectively and lesser cytotoxicity (48). Klein and colleagues (49) recently reported that PDEδ inhibitors specifically inhibit proliferation and survival of *KRAS* colorectal cancer cell lines and can be exploited for therapeutic intervention for mt *KRAS* cancers.

Indirect Targeted Therapies for *KRAS* Colorectal Cancer

PI3K inhibitors

Several PI3K inhibitors have been developed and tested in preclinical studies and early clinical trials. Three main categories of PI3K inhibitors have been developed, including isoform-selective, pan-PI3K, and dual pan-PI3K inhibitors. Several clinical trials are still ongoing with isoform-selective PI3K inhibitors (p110α, β, δ, or γ) in patients with advanced solid tumors, hematologic malignancies, and lymphomas (50–53). Because of hematopoietic cell-restricted expression of p110δ and p110γ, the PI3Kδ and PI3Kγ isoform-selective inhibitors were found to achieve greater therapeutic efficacy in such hematologic malignancies rather than solid tumors (54, 55). More recent work has shown that the inhibition of either p110α or p110β leads to PI3K signaling's reactivation via p110β or p110α, respectively, limiting its efficacy in the clinic (56, 57). These data suggest that pan-PI3K inhibitors or combination therapy of PI3Kα and PI3Kβ inhibitors may require maximal PI3K pathway inhibition.

Notably, a PI3Kα inhibitor, alpelisib has entered phase Ib/II trial (NCT01719380) combined with a BRAFi encorafenib, and anti-*EGFR* mAb cetuximab in patients with mCRC has demonstrated promising results (58). Petra pharma has initiated a phase Ib/II trial

Table 2. Indirect targeted strategies for mutated *KRAS* colorectal cancer in clinical trials.

Agents		Targets		Trial phase	Clinical trial
Isoform-selective PI3K					
PI3Kα					
Alpelisib - *BYL719* [Novartis]					
±Alpelisib	+Cetuximab	±PI3Kα	+EGFR	Ib/II	NCT01719380
+Encorafenib		+BRAF			
Serabelisib - *INK1117, MLN1117, TAK-117* [Intellikine, Petra Pharma, Takeda]					
+Canagliflozin		+SGLT2		Ib/II	NCT04073680
PI3Kδ					
Parsaclisib - *INCB050465* [Incyte Corporation, Innovent Biologics]					
+Pembrolizumab	±Itacitinib	+PD-1	±JAK1	I	NCT02646748
Umbralisib - *TGR-1202* [TG Therapetics]					
±Nab-paclitaxel	±Gemcitabine	+PI3Kδ/CK1-epsilon	±Tubulin	I	NCT02574663
±FOLFOX	±Bevacizumab	±ChemoR	±VEGF-A		
Pan-PI3K - PI3Kα/β/δ/γ, mTOR, Vps34					
Buparlisib – *BKM120, NVP-BKM120* [Array BioPharma, Novartis]					
+Sonidegib		+SMO		Ib	NCT01576666
Monotherapy		PI3Kα/β/δ/γ		Ia	NCT01068483
+Panitumumab		+EGFR		I/II	NCT01591421
+mFOLFOX6		+ChemoR		I	NCT01571024
+Irinotecan		+Chemo		I	NCT01304602
Monotherapy		PI3Kα/β/δ/γ		II	NCT01501604
Copanlisib – *Aligopa, BAY 80–6946* [Bayer, Bristol-Myers Squibb]					
+Nivolumab		+PD-1		Ib/II	NCT03735628*
+Nivolumab		+PD-1		I/II	NCT03711058*
Sonolisib – *PX-866* [Cascadian Therapeutics, Oncothyreon]					
+Cetuximab		+EGFR		I/II	NCT01252628
Dual-PI3K/mTOR – PI3Ks, mTOR					
Dactolisib – *BEZ235, NVP-BEZ235* [Novartis]					
+MEK162		+MEK		I	NCT01337765
DS-7423 [Daiichi Sankyo]					
Monotherapy		+PI3K/mTOR		I	NCT01364844
Voxtalisib – *SAR245409, XL765* [EMD Serono, Exelixis, Sanofi]					
+Pimasertib		+MEK1/2		Ib	NCT01390818
AKT – AKT1/2/3					
Capivasertib - *AZD5363* [Astex Therapeutics, AstraZeneca, Cancer Research Technology]					
Monotherapy		AKT1/2/3		I	NCT01353781
±Capivasertib	+Olaparib	±AKT1/2/3	+PARP	II	NCT02576444
±Adavosertib	±Ceralasertib	±WEE1	±ATR		
MK-2206 [Merck]					
Monotherapy		AKT1/2/3		II	NCT01802320
+Selumetinib		+MEK1/2		II	NCT01333475
Monotherapy		AKT1/2/3		II	NCT01186705
±MK-2206	+Dalotuzumab	±AKT1/2/3	+IGF-1R	I	NCT01243762
±MK-0752	±Ridaforolimus	±γ-secretase	±mTOR		
Enzastaurin – *LY317615* [Denovo Biopharma]					
Monotherapy		+AKT/PKCβ		II	NCT00192114
+FA +5-FU	+Bevacizumab	+ChemoR	+VEGF-A	II	NCT00612586
+Irinotecan	+Cetuximab	+Chemo	+EGFR	II	NCT00437268
Perifosine – *KRX-0401, NSC639966* [Aeterna Zentaris, Keryx Biopharmaceuticals]					
+Capecitabine		+Chemo		I	NCT01048580
±Perifosine	+Capecitabine	±AKT/PI3K	+Chemo	II	NCT00398879
±Perifosine	+Capecitabine	±AKT/PI3K	+Chemo	III	NCT01097018
mTOR – mTORC1					
Everolimus – *RAD001, 42-O-(2-Hydroxyethyl)rapamycin, Afinitor, Zortress* [Novartis]					
±Everolimus	+Spartalizumab	±mTORC1	+PD-1	Ib	NCT02890069*
±LCL161	±Panobinostat	±IAP	±HDAC		
±QBM076	±Siremadlin	±CXCR2	±p53-Mdm2		
Monotherapy		mTORC1		II	NCT00419159
+Panitumumab	+Irinotecan	+EGFR	+Chemo	Ib/II	NCT01139138
+Tivozanib		+VEGF		I/II	NCT01058655
+Bevacizumab	+FOLFOX	+VEGF-A	+ChemoR	I/II	NCT01047293
+Cetuximab	+Irinotecan	+EGFR	+Chemo	I/II	NCT00522665
+Linsitinib		+IGF-1R		I	NCT01154335
+Bevacizumab		+VEGF-A		II	NCT00597506
+Cetuximab	+Irinotecan	+EGFR	+Chemo	I	NCT00478634

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Table 2. Indirect targeted strategies for mutated *KRAS* colorectal cancer in clinical trials. (Cont'd)

Agents		Targets		Trial phase	Clinical trial
Nab-rapamycin – *ABI-009, FYARRO, Nab-sirolimus, TARZIFYX* [Abraxis BioScience]					
+Nivolumab		+PD-1		I/II	NCT03190174*
+Bevacizumab	+FOLFOX	+VEGF-A	+ChemoR	I/II	NCT03439462*
RAF - BRAF					
Encorafenib – *LGX818* [Array Biopharma, Merck, Novartis, Ono Pharmaceutical, Pfizer, Pierre Fabre]					
±Encorafenib	+Cetuximab	±BRAF	+EGFR	III	NCT02928224
±Binimetinib	±FOLFIRI	±MEK1/2	±ChemoR		
+Binimetinib	+Cetuximab	+MEK	+EGFR	II	NCT03693170
+Cetuximab		+EGFR		III	NCT04607421
±Irinotecan/Leucovorin/Oxaliplatin/5-FU		±ChemoR			
+Cetuximab	±Alpelisib	+EGFR	±PI3Kα	Ib/II	NCT01719380
+Binimetinib	+Nivolumab	+MEK	+PD-1	I/II	NCT04044430*
Monotherapy		BRAF		I	NCT01436656
+Cetuximab	+Nivolumab	+EGFR	+PD-1	I/II	NCT04017650*
+Cetuximab	+WNT974	+EGFR	+PORCN	Ib/II	NCT02278133
Dabrafenib – *DRB436, GSK2118436, Tafinlar* [GlaxoSmithKline, Novartis]					
±Dabrafenib	+Panitumumab	±BRAF	+EGFR	I/II	NCT01750918
±Trametinib	±5-FU	±MEK1/2	±Chemo		
+Spartalizumab	+Trametinib	+PD-1	+MEK1/2	II	NCT03668431*
Sorafenib – *BAY43–9006, Nexavar* [Bayer, Genentech, Merck, Onyx]					
+Capecitabine		+Chemo		II	NCT01471353
Monotherapy		BRAF/CRAF/PDGF/VEGF		II	NCT01715441
±Irinotecan		±Chemo			
+Capecitabine	+Radiation	+Chemo	+Radiation	I/II	NCT00869570
+Cisplatin	+Pemetrexed	+Chemo		I	NCT00703638
+Bevacizumab		+VEGF-A		II	NCT00826540
+Cetuximab		+EGFR		II	NCT00326495
±Sorafenib		±BRAF/CRAF/PDGF/VEGF		II	NCT00865709
+mFOLFOX6		+ChemoR			
+FOLFIRI		+ChemoR		I	NCT00780169
+Irinotecan		+Chemo		I/II	NCT00989469
+Bevacizumab	+mFOLFOX6	+VEGF-A	+ChemoR	I	NCT00779311
Vemurafenib – *PLX4032, RG7204, RO5185426, Zelboraf* [Genentech, Plexxikon, Roche]					
±Vemurafenib	+Cetuximab	±BRAF	+EGFR	II	NCT02164916
+Irinotecan		+Chemo			
+Cetuximab	+Irinotecan	+EGFR	+Chemo	I	NCT01787500
+Cetuximab	+FOLFIRI	+EGFR	+ChemoR	II	NCT03727763*
+Monotherapy		+BRAF		I	NCT00405587
+Panitumumab		+EGFR		I	NCT01791309
Pan-RAF – ARAF, BRAF, CRAF					
LY3009120 – *DP-4978* [Deciphera Pharmaceuticals, Eli Lilly and Company]					
Monotherapy		ARAF/BRAF/CRAF		I	NCT02014116
PLX8394 [Plexxikon]					
+PLX8394	+Cobicistat	+BRAF	+CYP3A4	I/IIa	NCT02428712*
MEK – MEK1, MEK2					
Binimetinib – *ARRY-162, ARRY-438162, MEK-162, MEKTOVI* [Array BioPharma, Novartis, Ono Pharmaceutical, Pfizer, Pierre Fabre, Plexxikon]					
±Binimetinib	±Palbociclib	±MEK1/2	±CDK4/6	II	NCT03981614*
±TAS-102		±Chemo			
+mFOLFIRI		+ChemoR		I	NCT02613650*
±Binimetinib	+Cetuximab	±MEK1/2	+EGFR	III	NCT02928224
±Encorafenib	±FOLFIRI	±BRAF	±ChemoR		
+Bevacizumab		+VEGF-A		II	NCT03475004*
+Pembrolizumab		+PD-1			
+Cetuximab	+Encorafenib	+EGFR	+BRAF	II	NCT03693170
+Panitumumab		+EGFR		Ib/II	NCT01927341
+Dactolisib		+PI3Ks/mTOR		I	NCT01337765
+Encorafenib	+Nivolumab	+BRAF	+PD-1	I/II	NCT04044430*
Monotherapy		MEK1/2		I	NCT00959127
±Monotherapy	+Pembrolizumab	±MEK1/2	+PD-1	I	NCT03374254*
±5-FU	±Leucovorin	±ChemoR			
±Oxaliplatin	±Irinotecan				
+Nivolumab	±Ipilimumab	+PD-1	±CTLA-4	Ib/II	NCT03271047
±Binimetinib	±Mirdametinib	±MEK1/2		I	NCT02510001
+Cirzotinib		+c-MET/ALK			
+FOLFOX		+ChemoR		I	NCT02041481

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Table 2. Indirect targeted strategies for mutated *KRAS* colorectal cancer in clinical trials. (Cont'd)

Agents		Targets		Trial phase	Clinical trial
Cobimetinib – *Cotellic, GDC-0973, XL-518* [Bristol-Myers Squibb, Exelixis, Genentech, InxMed, Roche]					
±Cobimetinib	±Atezolizumab	±MEK1/MAP2K1	±PD-L1	III	NCT02788279
±Regorafenib		±VEGFR2-TIE2			
+Atezolizumab	+Bevacizumab	+PD-L1	+VEGF-A	I	NCT02876224
+Ravoxertinib		+ERK1/2		Ib	NCT02457793
±Cobimetinib	+Atezolizumab	±MEK1/MAP2K1	+PD-L1	II	NCT03340558
Mirdametinib – *PD-0325901, PD-325901* [Pfizer, AstraZeneca, Bioensis, SpringWorks Therapeutic]					
+Dacomitinib		+Pan-HER		I/II	NCT02039336*
±Mirdametinib	±Binimetinib	±MEK1/2		I	NCT02510001
+Cirzotinib		+c-MET/ALK			
Monotherapy		MEK1/2		I/II	NCT00147550
Pimasertib – *AS-703026, EMD-1036239, MSC1936369B* [Santhera Pharmaceuticals, Merck Serono, Sanofi]					
+Voxtalisib		+PI3K/mTOR		I	NCT01390818
Selumetinib – *ARRY-142886, AZD-6244, NSC-748727* [Array BioPharma, AstraZeneca, Merck]					
±Dacarbazine	±Docetaxel ±Erlotinib	±Chemo		I	NCT00600496
±Temsirolimus		±EGFR	±mTOR		
+Cyclosporin A		+Calcineurin		I	NCT02188264
+Cetuximab		+EGFR		I	NCT01287130
+Durvalumab	±Tremelimumab	+PD-L1	±CTLA-4	I	NCT02586987
+Capecitabine	±Cediranib	+Chemo	±VEGFR2	I	NCT01160926
+MK-2206		+AKT1/2/3		II	NCT01333475
+Irinotecan		+Chemo		II	NCT01116271
Trametinib – *GSK-1120212, JTP-74057, Mekinist* [Amgen, GlaxoSmithKline, Merck, Novartis, Pfizer]					
+TAS-102		+Chemo		I	NCT03317119
+Ribociclib		+CDK4/6		Ib/II	NCT02703571
+Spartalizumab		+PD-1		I	NCT02900664
+Nivolumab	±Ipilimumab	+PD-1	±CTLA-4	I/II	NCT03377361
±Trametinib	±Dabrafenib	±MEK1/2	±BRAF	II	NCT01750918
+Panitumumab	±5-FU	+EGFR	±Chemo		
+Panitumumab		+EGFR		II	NCT02399943
+Siremadlin		+p53-Mdm2		I	NCT03714958*
+Durvalumab		+PD-L1		II	NCT03428126
+Ruxolitinib		+JAK1/2		I	NCT04303403*
±Trametinib	+Panitumumab	±MEK1/2	+EGFR	II	NCT03087071*
+Dabrafenib	+Spartalizumab	+BRAF	+PD-1	II	NCT03668431*
+Lapatinib		+EGFR/HER2		I/II	NCT02230553*
ERK – ERK1, ERK2					
ASN007 [Asana BioSciences]					
Monotherapy		ERK1/2		I	NCT03415126
CC-90003 [Celgene]					
Monotherapy		ERK1/2		I	NCT02313012
LY3214996 [Eli Lilly and Company]					
±Abemaciclib	±Cetuximab	±CDK4/6	±EGFR	I	NCT02857270*
±Encorafenib	±Gemcitabine	±BRAF	±Chemo		
±Midazolam	±Nab-paclitaxel	±GABA	±Tubulin		
±Abemaciclib	+Cetuximab	±CDK4/6	+EGFR	Ib/II	NCT04616183*
MK-8353 – *SCH-900353* [Schering-Plough, Merck Sharp & Dohme]					
+Pembrolizumab		+PD-1		Ib	NCT02972034
Monotherapy		ERK1/2		I	NCT01358331
Ravoxertinib – *GDC-0994, RG-7842* [Array BioPharma, Genentech]					
+Cobimetinib		+MEK1/MAP2K1		Ib	NCT02457793
Monotherapy		ERK1/2		I	NCT01875705

Abbreviations: ATR, ataxia telangiectasia and rad3 related; BRAF, B-Raf proto-oncogene serine/threonine-protein kinase; CDK4/6, Cyclin-dependent kinases 4/6; Chemo, chemotherapy; ChemoR, chemotherapeutic regimen; CK1, casein kinase; c-MET, tyrosine-protein kinase Met; CRAF, C-Raf proto-oncogene serine/threonine-protein kinase or Raf-1; CYP3A4, cytochrome P450 3A4; CXCR2, CXC chemokine receptor 2; HDAC, histone deacetylase; IAP, Inhibitor of apoptosis protein; IGF-1R, Insulin like growth factor type 1 receptor-1; MAP2K1, dual specificity mitogen-activated protein kinase kinase 1; mFOLFIRI, modified FOLFIRI; mFOLFOX6, 5-FU, leucovorin, and oxaliplatin; mTORC1, mammalian target of rapamycin complex 1; p53-Mdm2, tumor suppressor p53-mouse double minute 2 homolog; PORCN, protein-serine O-palmitoleoyltransferase porcupine; RAF, RAF proto-oncogene serine/threonine-protein kinase; SGLT2, sodium/glucose co-transporter 2; SMO, smoothene; TAS-102, trifluridine and tipiracil hydrochloride; TIE2, angiopoietin-1 receptor; italized, Other names; [], developer; +, drug included in the treatment arms; ±, drug included in one of treatment arms; *, clinical trial recruitment. Compiled from ClinicalTrials.gov.

(NCT04073680) to evaluate the efficacy of PI3K-α inhibitor serabelisib and sodium/glucose cotransporter 2 (SGLT2) inhibitor canagliflozin, which used to treat type 2 diabetes, for advanced solid tumors patients. Another type of PI3K-δ inhibitor parsaclisib was tested in combination with a programmed cell death protein 1 (PD-1) inhibitor pembrolizumab and compared with pembrolizumab and a JAK1 inhibitor itacitinib in patients with advanced solid tumors, which currently ongoing in a phase I trial (NCT02646748). A dual inhibitor of PI3K-δ and casein kinase (CK) 1-epsilon umbralisib was tested alone and in combination therapy with either nanoparticle albumin-bound tubulin inhibitor Nab-paclitaxel, chemotherapy drug gemcitabine, regimen FOLFOX or VEGF-A inhibitor bevacizumab for relapsed solid tumors patients. This phase I trial (NCT02574663) is completed and the results are pending.

Pan-PI3K inhibitors (**Table 2**) targets all the four PI3K kinase isoforms of class I PI3K (α, β, δ, and γ) in cancer. On the basis of clinical trial data, it is unlikely that pan-PI3K inhibitors have significant activity as single agents in patients with solid tumors (59–61). The key drawbacks of pan-PI3K inhibitors are the large spectrum of drug-related side effects, including anorexia, fatigue, nausea, and hyperglycemia, limiting the use of such agents at therapeutic doses. Hence, combination trials of pan-PI3K inhibitors (e.g., buparlisib, sonolisib, pictilisib) with other agents in solid tumors are required to improve the efficacy of the agents (62–66). However, a pan-PI3K inhibitor, sonolisib combined with anti-*EGFR* antibody cetuximab demonstrated disappointing results in mCRC patients in a phase II trial (NCT01252628; ref. 62). Ongoing phase I/II trials (NCT03735628 and NCT03711058) are currently recruiting patients with advanced colorectal cancer to explore the safety and efficacy of combination therapy of copanlisib with anti-PD-1 antibody nivolumab. To date, only one pan-PI3K inhibitor, buparlisib has progressed into phase III trial (NCT01633060) for metastatic breast cancer.

To further enhance inhibition of the PI3K–AKT–mTOR pathway, dual PI3K–mTOR inhibitors (**Table 2**) were developed to target all PI3K catalytic isoforms and mTORC1/2 by binding to ATP binding cleft. Thus, these drugs should effectively turn off this pathway and result in tumor regression. A preclinical study demonstrated growth-inhibitory activity in the colorectal cancer cell xenograft tumor model when treated with the dual PI3K–mTOR inhibitor dactolisib (67). Besides, dactolisib and LY3023414 were found significant treatment responses in mutated *APC* and *PIK3CA* mice with 53% and 24% reduction in the median lumen occlusion, respectively (68). The dual PI3K–mTOR inhibitor dactolisib was shown to inhibit tumor growth in the genetically engineered sporadic colorectal cancer mouse model (69). Although dactolisib demonstrated significant tumor regression in the preclinical model, the clinical efficacy in advanced solid malignancies patients has been unsatisfactory (70–73). Another two dual PI3K–mTOR inhibitors DS-7423 and voxtalisib showed disappointing results in the phase I trials (NCT01364844 and NCT01390818) for patients with advanced solid tumor (74, 75). However, it remains unclear whether dual PI3K–mTOR inhibitors are tolerable at effective doses that inhibit either PI3K and mTOR isoforms or completely inhibit one or more of the possible targets.

AKT inhibitors

Most AKT inhibitors (**Table 2**) block AKT 1, 2, and 3, either interfering with ATP or binding partially to the ATP-binding site. Although targeting selectivity of AKT was predicted higher than PI3K inhibition, the evidence of clinical results is unsatisfactory. Adverse side effects of AKT inhibitors were included severe rash and hyperglycemia (76–78). A promising oral AKT/PI3K inhibitor, perifosine combined with a chemotherapy drug capecitabine demonstrated clinical benefits for mCRC in the phase I and II trial (NCT01048580 and NCT00398879; ref. 79). However, the phase III trial (NCT01097018) did not demonstrate better overall survival in patients with refractory colorectal cancer (80). A dual potent AKT/PKCβ inhibitor, Enzastaurin, was again shown no clinical benefits on mCRC in a phase II trial (NCT00612586; ref. 81). Two phases II clinical trials (NCT00192114 and NCT00437268) of enzastaurin for patients with colorectal cancer were completed while the results are pending.

Another attempt to inhibit AKT was an oral allosteric AKT1/2 inhibitor, MK-2206. A phase II monotherapy trial (NCT01802320) for patients with recurrent mCRC was shown no significant therapeutic benefits (82). In 18 patients, 9 patients in cohort A had progressive disease whereas two cohort B patients had stable disease for 1.8 months. When combined with a MEK1/2 inhibitor selumetinib in a phase II trial (NCT01333475), patients with advanced colorectal cancer showed no objective responses (83). Two clinical trials (NCT01186705 and NCT01243762) with MK-2206 were terminated early due to insufficient patients are recruited to reach the preliminary conclusions (77). Capivasertib, developed by AstraZeneca, was reported to inhibit all AKT isoforms and AGC family kinase *in vivo* assays (84). Davies and colleagues (85) reported capivasertib was susceptible to wt RAS, PI3KCA, or PTEN mutated cell lines. A phase I trial of capivasertib (NCT01353781) in solid tumor patients demonstrated antitumor activity with intermittent dosing (86) and now undergoing phase II trial as combinatorial therapy (NCT02576444).

mTOR inhibitors

Most mTOR inhibitor (**Table 2**) monotherapy in clinical trials for patients with *KRAS* mCRC has not demonstrated therapeutic benefits (87–89). Ineffective mTOR inhibitors were found contributed to drug resistance to colorectal cancer, which correlated to the elF4E-binding protein 1 (4E-BP1) kinase (90). Because of the minimal effectiveness of a single agent, more studies are focused on the combination of mTOR inhibitor with upstream receptor inhibitors. Recent findings on combination therapy of mTOR inhibitors and other drugs have demonstrated promise in patients with colorectal cancer. For example, the oral derivative of rapamycin, everolimus that specifically inhibits mTORC1 is the most advanced in the clinic. In a phase I/II study, the combined therapy of everolimus with modified FOLFOX-6 chemotherapy regimen and VEGF-A inhibitor bevacizumab reported prolonged progression-free survival up to 6 months in 96% of patients with mCRC as first-line treatment (NCT01047293; ref. 91). This result was showed a statistically significant progression-free survival improvement than the previous study conducted by Saltz and colleagues (92). An expanded cohort phase II trial (NCT00597506) for refractory mCRC using everolimus and combination with the VEGF-A inhibitor bevacizumab demonstrated tolerable toxicity (93). In addition, a multicenter phase II trial (NCT01058655) of everolimus with another type of *VEGF* inhibitor tivozanib was reported well-tolerated in treating 50% of the patients with refractory mCRC (94).

When testing everolimus with the chemotherapy drug irinotecan and anti-*EGFR* antibody panitumumab as a second-line treatment (NCT01139138), this combination treatment fails to translate clinical benefits for patients with wt *KRAS* mCRC (95). However, the replacement of another *EGFR* inhibitor cetuximab in the same combination treatment demonstrated clinical benefits in refractory mCRC patients (NCT00478634 and NCT00522665; refs. 96, 97). In a phase I trial (NCT01154335), the maximum tolerated dose of everolimus and an insulin-like growth factor 1 receptor (IGF-1R inhibitor) linsitinib

combination treatment was determined, and no clinical activity was observed in patients with refractory mCRC (98). Novartis is currently recruiting patients with CRC across seven countries for a phase I trial (NCT02890069) using everolimus in combination with a PCR001 checkpoint inhibitor, whereas Aadi is recruiting advanced or mCRC patients for phase I/II trial (NCT03439462) as first-line therapy to test the nanoparticle albumin-bound sirolimus, Nab-rapamycin in combination with chemotherapy regimen modified FOLFOX-6 and VEGF-A inhibitor bevacizumab. Besides, Aadi collaborated with Sarcoma Oncology Research Centre recruiting advanced sarcoma and solid tumors patients to evaluate the combination efficacy of Nab-rapamycin and a PD-1 inhibitor nivolumab (NCT03190714). Combinatorial therapeutic strategies using downstream PI3K pathway inhibitors effectively treat primary and mCRCs, rendering the pathway a promising therapeutic target for *KRAS* colorectal cancer.

MAPK signaling pathways

Even though direct RAS inhibitors are still the best option for *KRAS* targeted therapies, inhibitors of the RAS/RAF/MEK/ERK (MAPK) signaling pathway may provide an alternative solution for future colorectal cancer treatments to overcome upstream drug inhibitors resistance. In this section, the latest findings and advancements related to MAPK pathway inhibitors are reviewed.

RAF inhibitors

The first-generation BRAFi that target BRAF kinases have been extensively clinically investigated in patients with $BRAF^{V600E}$ mCRC, such as vemurafenib, dabrafenib, encorafenib, and sorafenib (**Table 2**). In 2015, Kopetz and his research team (99) reported patients with $BRAF^{V600E}$ colorectal cancer treated with vemurafenib in a phase I study (NCT00405587) did not benefit from monotherapy. However, when vemurafenib was tested in combination therapy, it improved the outcome of patients with colorectal cancer with promising efficacy (100–102). Shanghai Changzheng Hospital is currently recruiting patients with $BRAF^{V600E}$ advanced colorectal cancer for phase II trial (NCT03727763) to investigate the effects of vemurafenib, cetuximab, and FOLFIRI combinatorial therapy. Another type of BRAFi dabrafenib demonstrated promising activity in patients with $BRAF^{V600E}$ colorectal cancer. For example, an EGFRi panitumumab was added in the combinatorial therapy with dabrafenib and MEK1/2 inhibitor trametinib in a phase I/II clinical trial (NCT01750918; ref. 103). Notably, the addition of EGFRi was shown to overcome the resistance to MAPK pathway inhibition in patients with colorectal cancer. Thus, van Brummelen and colleagues (104) suggested BRAF status as a predictive biomarker for response to anti-*EGFR* treatment. A phase II study (NCT03668431) of dabrafenib and MEK1/2 inhibitor trametinib combined with an anti-PD-1 mAb spartalizumab is currently undergoing for patients with $BRAF^{V600E}$ mCRC. Among the BRAFi, the most promising drug is encorafenib, which is currently undergoing phase III trials. The encorafenib treatment for patients with mt $BRAF^{V600E}$ mCRC has been tested in combination with anti-PD-1 (nivolumab), EGFRi (cetuximab), MEK inhibitor (binimetinib), PI3Kα inhibitor (alpelisib), and other cytotoxic agents (5-FU, bevacizumab, capecitabine, FA, irinotecan, leucovorin, or oxaliplatin), which demonstrated promising clinical benefits (58, 105–107).

A promising oral dual Raf/platelet-derived growth factor (PDGF)/*VEGF* inhibitor, sorafenib demonstrated clinical benefits in combination trials (NCT00989469, NCT01471353, NCT01715441, and NCT00826540) with either irinotecan, capecitabine, or bevacizumab in pretreated patients with *KRAS* mCRC (89, 108, 109). Besides, von Moos and colleagues (110) reported that patients with advanced *KRAS*-mutated colorectal cancer benefited from the combination of sorafenib and neoadjuvant radiotherapy (NCT00869570). The maximum tolerated dose of the combination between sorafenib and chemotherapy drugs pemetrexed and cisplatin was determined in a phase I trial (NCT00703638) for advanced solid tumors (111), which recommended further studies. Sorafenib combined with another EGFRi cetuximab in a phase II trial (NCT00326495) also showed no clinical benefit in 30 patients with pretreated *KRAS* mutated mCRC (112). Similar clinical outcome was observed for patients with mCRC treated with a combination of sorafenib and chemotherapy regimens (NCT00865709 and NCT00779311; ref. 113). To date, a total of forty clinical trials are currently in the recruiting phase for patients with leukemia and hepatocellular cancer to test the combination of sorafenib with various chemotherapy regimens or immune checkpoint inhibitors (ICI). These efforts are progressively pointing towards underexplored pathways and targets for potential encorafenib combination designs.

Despite first-generation BRAFi providing improved clinical benefit in some patients with $BRAF^{V600E}$ mCRC, drug resistance to BRAF inhibition persists. This effect can be explained as BRAFi drives the binding of BRAF-CRAF heterodimers, resulting in the recovery of MEK-ERK signaling which drives tumor progression (114). To overcome the BRAF-selective inhibition, second-generation pan-RAF inhibitors were developed to inhibit both BRAF and CRAF isoforms, preventing paradoxical MAPK pathway activation when a RAS mutation is present. The pan-RAF inhibitors, LY3009120 and PLX8394 showed significant activity in *BRAF, NRAS*, or *KRAS* colorectal cancer xenografts models (115, 116). Recently, PLX8394 demonstrated promising results in a phase I/II trial (NCT02428712) with a cytochrome P450 3A4 (CYP3A4) inhibitor cobicistat and is currently recruiting patients for the phase II trial (117). However, a phase I study (NCT02014116) of the LY3009210 capsule was terminated early due to no clinical efficacy being observed in patients with advanced colorectal cancer (118).

MEK inhibitors

In comparison to RAF inhibitors targeted strategy, MEK inhibitors were developed to target MEK-dependent cell signaling pathways because MEK kinase harbors fewer mutations. The most promising MEK inhibitors are binimetinib and trametinib, which are now ongoing in several phase II/III trials, as shown in **Table 2**.

Binimetinib

Binimetinib is an oral inhibitor of MEK1/2 developed by Array Biopharma. The binimetinib monotherapy study (NCT00959127) reported no clinical benefits for patients with *KRAS* or *BRAF* mutated mCRC (119). When combining binimetinib with chemotherapy regimen FOLFOX in phase I trial (NCT02041481), Cho and colleagues (120) reported manageable toxicity profiles and promising antitumor activity in patients with advanced mCRC. Hence, multiple clinical trials are currently recruiting patients with mCRC to evaluate the combination efficacy of binimetinib with *BRAF, EGFR*, or other chemotherapy regimens (NCT03693170, NCT02928224, and NCT02613650). The results for a nonrandomized phase Ib/II study (NCT01927341) of binimetinib and anti-*EGFR* antibody panitumumab in patients with mCRC are still pending. Because of the clinical benefits of immunotherapy, more researchers are currently focusing on combining binimetinib with anti-PD-1 antibody nivolumab or

anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody ipilimumab (NCT03271047, NCT04044430).

Another type of PD-1 inhibitor, pembrolizumab was reported safe and tolerable in mCRC when treated together with mFOLFOX7 or FOLFIRI (121). Merck and Array Biopharma are currently recruiting mCRC patients for phase I trial (NCT03374254) of binimetinib and PD-1 inhibitor pembrolizumab or chemotherapy. VEGF-A inhibitor bevacizumab to binimetinib and pembrolizumab combination treatment demonstrated clinical benefits in patients with refractory colorectal cancer (122). This phase II trial (NCT03475004) is currently recruiting patients for the next stage of the study. Pfizer initiated a phase I trial (NCT01337765) to evaluate the combination efficacy of binimetinib and a dual PI3K/mTOR inhibitor dactolisib, but no results have been reported. Another MET combination treatment (MErCuRIC1) is under investigation using a dual tyrosine-protein kinase Met (c-MET)/anaplastic lymphoma kinase (ALK) inhibitor crizotinib and another MEK inhibitor mirdametinib in a phase I trial (NCT02510001) for patients with colorectal cancer with wt and mt RAS harboring aberrant c-MET expression. A phase II trial (NCT03981614) employing a combination of binimetinib and cyclin-dependent kinases CDK4/6 inhibitor palbocicib or chemotherapy trifluridine and tipiracil hydrochloride (TAS-102) is also recruiting *KRAS* and *NRAS* mt mCRC.

Trametinib

Trametinib is the first FDA-approved MEK inhibitor for $BRAF^{V600E}$ metastatic melanoma with the trade name Mekinist. In a phase II trial (NCT01750918), Trametinib was shown to overcome the MAPK resistance in $BRAF^{V600E}$ mutated metastatic or patients with refractory colorectal cancer in combination with BRAFi dabrafenib and EGFRi panitumumab (103). Without adding the RAF inhibitor in the combination treatment of trametinib and panitumumab, Alshammari and colleagues (123) reported no clinical benefits were observed in wt advanced colorectal cancer in an ongoing phase II trial (NCT02399943). NCI is currently recruiting patients with stage IV colorectal cancer for a phase II trial (NCT03087071) to evaluate the efficacy of trametinib and EGFRi panitumumab compared with panitumumab monotherapy. Notably, a promising FDA-approved trifluorothymidine/thymidine phosphorylase inhibitor, TAS-102 was added into the combination treatments with trametinib for patients with chemotherapy-resistant RAS-mutated (PI3KCA/PTEN-wt)-specific mCRC (NCT03317119).

Novartis is focused on developing trametinib immunotherapy combinations for patients with mCRC. A group of advanced CRC patients was recruited from nine different countries to test trametinib's efficacy combined with PD-1 inhibitor spartalizumab in a phase I trial (NCT02900664). With the addition of BRAFi dabrafenib into immunotherapy combinations with the PD-1 inhibitor spartalizumab, Novartis is currently recruiting $BRAF^{V600E}$ mCRC patients for a phase II trial (NCT03668431). A collaborative study between Novartis and Bristol-Myers Squibb have entered into phase I/II clinical trials (NCT03377361) to evaluate the safety, tolerability, and efficacy of trametinib in combination with PD-1 ICI nivolumab and CTLA-4 inhibitor Ipilimumab as a potential treatment option for patients with advanced mCRC. Another type of PD-L1 inhibitor durvalumab was found to be ineffective in mCRC patients with MSS (124). Unfortunately, this phase II trial (NCT03428126) was discontinued after completing the first stage of the study. Furthermore, other phase I trials exploring combinations of trametinib plus JAK1/2 inhibitor ruxolitinib (NCT04303403), p53-MDM2 inhibitor siremadlin (NCT03714958), or dual *EGFR*/HER2 inhibitor lapatinib (NCT02230553) are currently recruiting patients with CRC.

Selumetinib

In 2020, the oral MEK1/2 inhibitor selumetinib was approved by FDA for pediatric patients and currently under phase I/II study for patients with colorectal cancer with different drug combinations, such as AKT inhibitor, EGFRi, immunotherapies, and chemotherapies. The first selumetinib clinical trial (NCT01160926) was terminated early because the combination with chemotherapy drug capecitabine does not respond well in patients with locally advanced rectal cancer, even on the lowest possible dose cohort. Another early termination of a clinical trial (NCT01116271) combined with a different chemotherapy drug irinotecan was reported (125). Hence, AstraZeneca evaluated another selumetinib chemotherapy combination with docetaxel, dacarbazine, or EGFRi erlotinib or mTOR inhibitor temsirolimus in patients with advanced solid tumor. This clinical trial (NCT00600496) is currently ongoing and clinical benefits were reported in each targeted drug (126, 127). A phase II pilot study (NCT01333475) combining an AKT inhibitor MK-2206 showed no objective responses in advanced colorectal cancer (83).

Conversely, combination therapy with an anti-*EGFR* cetuximab antibody reported manageable safety profile and are well-tolerated in patients with *KRAS* mutated refractory mCRC (NCT01287130; ref. 128). Patients with colorectal cancer were included as one of the advanced refractory solid tumors patients groups to test the efficacy of the combination of selumetinib and FDA-approved immunotherapy PD-L1 checkpoint inhibitor durvalumab or CTLA-4 inhibitor tremelimumab. This multicenter trial (NCT02586987) was completed by AstraZeneca and the results are pending. Another first-in-human clinical trial (NCT02188264) is currently ongoing to evaluate the efficacy of both selumetinib and an immunosuppressant drug cyclosporin A in patients with advanced mCRC. Recently, Krishnamurthy and colleagues (129) reported that these combination treatments were well tolerated and suggested for patients with advanced solid tumor.

Unfortunately, intolerable toxicity remains an issue for the three MEK1/2 inhibitors (cobimetinib, mirdametinib, and pimasertib), when administered as monotherapy (NCT00147550) or combination therapy (NCT03340558, NCT02788279, NCT02876224, NCT02457793, NCT02039336, NCT02510001, NCT01390818; refs. 75, 130–133). However, one phase Ib trial (NCT02876224) reported an acceptable safety profile and manageable adverse effects with the combination treatment of cobimetinib, PD-L1 inhibitor atezolizumab and VEGF-A inhibitor bevacizumab in patients with mCRC, indicating that mutated RAS responded better than wt RAS colorectal cancer patients (134).

ERK inhibitors

Targeting the third tier of MAPK cascade, ERK was first assume have no additional clinical benefits. However, the resistance mechanism of RAF and MEK drugs was reported due to ERK1/2 reactivation (135), blocking ERK1/2 directly may overcome the current limitations of upstream RAF or MEK inhibitors. Thus, researchers are gaining renewed interest in developing ERK inhibitors, which can be combined with upstream BRAF and MEK inhibitors to multi-target in the MAPK cascade. Several ERK inhibitors (**Table 2**) are in the trial phase for RAF/RAS-mutated or BRAF/MEK inhibitors-resistant tumors, but it remains unclear if these agents exhibit more clinical benefits compared with MEK inhibitors.

One of the inhibitors is ravoxertinib, a potent dual inhibitor of ERK1/2. Ravoxertinib has shown promising efficacy in the phase I

trial (NCT01875705) for patients with advance pancreatic adenocarcinoma and mt *BRAF* colorectal cancer (136). Unfortunately, the combination of ravoxertinib and MEK inhibitor cobimetinib shows adverse drug reactions for patients with mCRC in a phase Ib dose-escalation study (NCT02457793; ref. 131). LY3214996 is another potent ERK1/2 inhibitor that has demonstrated antitumor activity in a preclinical study (137). The first-in-human trial in patients with advanced cancer demonstrated an acceptable safety profile and further support LY3214996 as monotherapy or in combination treatment (138). Two clinical trials (NCT02857270 and NCT04616183) are currently recruiting patients with metastatic cancer to evaluate the combinatorial treatment, including combination with gamma-aminobuturic acid (GABA) agonist midazolam, CDK4/6 inhibitor abemaciclib, nanoparticle albumin-bound tubulin inhibitor Nab-paclitaxel, chemotherapy drug gemcitabine, BRAFi encorafenib, or EGFRi cetuximab.

Several other ERK inhibitors under development are ASN007, CC-90003, and MK-8353. ASN007 is an oral ERK1/2 inhibitor that has a long target residence time. In a phase I trial (NCT03415126), the MTD level and recommended phase II dose has been determined for patients with solid tumors harboring *BRAF*, MEK1, and RAS mutations (139). The irreversible ERK1/2 inhibitor CC-90003 was the first inhibitor that was terminated in a clinical trial (NCT02313012). Mita and colleagues (140) reported that the CC-90003 did not show a favorable PK profile and displayed unanticipated neurotoxicity in mt *BRAF* or *RAS* refractory metastatic patients. Monotherapy of MK-8353 phase I trial (NCT01358331) showed no clinical activity in patients with colorectal cancer with *RAS* and/or *BRAF* mutations (141). Hence, a phase I trial is ongoing to explore the combination efficacy of MK-8353 and PD-1 inhibitor pembrolizumab in patients with advanced malignancy, including colorectal cancer (NCT02972034). However, none of these compounds have entered phase III clinical trials and approved by FDA to date.

Treatments in Combination with ICIs

The clinical benefits of ICIs in colorectal cancer have primarily been limited to a subgroup of microsatellite instable (MSI/dMMR)-high patients. This subgroup of patients represents around 15% of all colorectal cancers (142). Such MSI-H colorectal cancer tumors are characterized by high numbers of tumor-infiltrating lymphocytes (TIL) and high tumor immunogenicity within the tumor microenvironment. Notably, high levels of PD-1, PD-L1, and CTLA-4 expressions were found in MSI-H group (143–145). Thus, the blocking of PD-1/PD-L1 and CD80/CTLA-4 interactions by ICIs could enhance T-cell activation and promote tumor cell killing. This therapeutic synergistic effect was reported in preclinical testing of either PD-1 or PD-L1 antibody combined with MEK inhibitor (146, 147). This tested combination treatment significantly suppresses tumor growth and increases intratumoral $CD4^+$ and $CD8^+$ T cells *in vivo*.

In 2018, the combination of low dose anti-CTLA-4 ipilimumab and anti-PD-1 antibody nivolumab or nivolumab as a single agent was approved to treat patients with refractory MSI-high mCRC (148). NICHE and CheckMate 142 phase II clinical trials are currently ongoing to investigate the combination of ipilimumab, nivolumab with COX-2 inhibitor celecoxib, MEK inhibitor cobimetinib, anti-CD38 antibody daratumumab, or anti-LAG-3 antibody BMS-986016 in patients with colorectal cancer (NCT03026140, NCT02060188). Concurrent studies have also reported that single dose of ipilimumab and two doses of nivolumab was very effective in treating dMMR tumor (149, 150). Recently, another immunotherapy, anti-PD-1 pembrolizumab was approved for MSI mCRC, with several trials ongoing (NCT03785249, NCT03475004, NCT03374254, NCT02563002). Le and colleagues (151) reported that MMR status was found to predict clinical benefit of ICI with pembrolizumab (NCT01876511), with progression free survival rates of 78%. Andre and colleagues (152) and Le and colleagues (153) further supported the use of pembrolizumab monotherapy for patients with MSI-high colorectal cancer. Conversely, the combinational treatment of pembrolizumab reported no clinical benefits in another subgroup of proficient MMR patients with colorectal cancer (NCT02981524; ref. 154). An ongoing phase II trial with anti-PD-L1 antibody Avelumab (NCT03150706) was approved for MSI-H or DNA polymerase epsilon (POLE) mutated mCRC. A few clinical trials are also ongoing for the anti-PD-L1 antibody Atezolizumab in treating patients with dMMR/MSI-H mCRC (NCT02997228, NCT03866239, NCT02912559).

Up until now, ICIs have been very successful in the subgroup of dMMR/MSI-H patients, but not in the subgroup of proficient MMR colorectal cancer, rendering these tumors insensitive to immunotherapy. Further immunotherapeutic efforts are needed to determine which subgroup of patients should receive ICI combinatorial treatment to identify those who may benefit from immune checkpoint blockade interventions. That being said, we do expect ICIs to become a standard treatment for dMMR mCRC soon.

Correlation Between *KRAS* Mutations, Genetic Heterogeneity, and Treatment Outcome in Colorectal Cancer

Among the most common mutations in *KRAS*, mutated codon 13 colorectal cancer is found frequently in poorly differentiated tumors with microsatellite-stable phenotype and significantly correlated with worst overall survival (155, 156). However, these findings are controversial with Bai and colleagues (157) and Li and colleagues (158). They reported that the *KRAS* mutated codon 12 colorectal cancers, especially G12D and G12V, are more aggressive tumor phenotypes with the worst prognosis when compared with *KRAS* mutated codon 13 colorectal cancers. Besides, other reports conflict with the correlation between *KRAS* mutations and clinical outcomes (159–161). These findings suggest that *KRAS* mutations are not created equally and have distinct mutation profiles representing different tumor clones. Even the same patients with *KRAS* mutation colorectal cancer treated with the same treatment may experience different treatment outcomes. Massive evidence on tumor heterogeneity in colorectal cancer suggests that not all *KRAS* mutations confer equivalent resistance to anti-EGFR agents (162–164). This explains why most patients with wt *KRAS* mCRC do not respond to anti-EGFR treatment, whereas a minority of them responded well (165). Because of the tumor heterogeneity in colorectal cancer, *KRAS* mutations can only partially explain the development of tumorigenesis. This study was further examined by Laurent-Puig and colleagues (166), who reported that a minor subclone of resistant cells was detected during the anti-EGFR treatment. Their findings were in line with the observation in both tissue and blood biopsy analyses in patients with mCRC treated with anti-EGFR therapy (167) and further confirmed the existence of intratumoral heterogeneity. These findings suggested that therapeutic interventions targeted specific molecular aberrations may impose an evolutionary pressure on cancer and are likely to benefit only a small percentage of patients.

To date, patients with colorectal cancer treated with chemotherapies are still highly vulnerable towards the occurrence of secondary drug resistance. The unexpected therapeutic failure can be attributed to both inter- and intratumoral heterogeneity, as the detection of tumor heterogeneity is limited by routine diagnostic testing. Current clinical practice for colorectal cancer diagnosis and treatment is still largely dependent on the status of *RAS* mutation, DNA MMR, and *BRAF* mutational status, even though prognostic or predictive biomarkers are rapidly expanding. Over the past decade, several distinct subtypes of colorectal cancers were identified based on gene expression data. For example, the colon cancer molecular subtyping divided patients into six groups, with C4 and C6 subtypes associated with a more inferior relapse-free survival rate than C1, C2, C3, and C5 subtypes (168). The consensus molecular subtyping categorized colorectal cancers into four subtypes, in which one of the subtypes, CMS4 displayed worse overall survival and relapse-free survival (169). However, this subtype was significantly associated with a positive clinical outcome when patients were treated with chemotherapy FOLFIRI. Recently, Buechler and colleagues (170) further expanded the consensus molecular subtypes into 40 gene signatures developed a ColoType assay for clinical diagnostics using formalin-fixed, paraffin-embedded tissue samples. Intriguingly, Sadanandam and colleagues (171) had proposed the colorectal cancer classification with six subtypes based on differential response to anti-EGFR cetuximab therapy. Among all the subtypes, the stem cell subtype displayed inferior disease-free survival. Hence, this subtype of patients was selected for chemotherapy FOLFIRI treatment, and clinical benefits were shown in both the adjuvant treated and metastatic patients. These findings indicate that the comprehensive genetic profiles of colorectal cancer are essential for developing a personalized treatment for patients with colorectal cancer to prevent them from evolving therapeutic resistance mechanisms.

Conclusion and Future Perspectives

Colorectal cancer is a complex dynamic disease, and its treatment strategy has greatly evolved alongside advancements in molecular therapy. Early attempts to prevent mTOR and ERK activation were initially focused on developing upstream PI3K and RAF inhibitors because the protein kinase cascade were thought to be a linear pathway. Researchers later found out that RAS cancer cells can dynamically reorganize their downstream signaling networks to restore mTOR or ERK activity and induce resistance to upstream inhibitors. It has become clear that molecular subtyping has a significant influence on the prognosis and therapeutic choices for different stages of colorectal cancer. This implies that future colorectal cancer clinical trials should consider the diverse drug resistance mechanisms, molecular subtypes of interest, and patient stratification based on prognostic and predictive biomarkers. More trials combining targeted inhibitors and immunotherapies are now being investigated. Therefore, study approaches should be designed to also consider the presence of simultaneous resistance and escape mechanism accordingly. There is also a need to identify biomarker-driven therapies in the clinic to complement the dawn of a personalized medicine approach for patients with colorectal cancer in future trials.

Authors' Disclosures

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