

REVIEW

## Strategies to tackle *RAS*-mutated metastatic colorectal cancer

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The *RAS* oncogene is among the most commonly mutated in cancer. *RAS* mutations are identified in about half of patients diagnosed with metastatic colorectal cancer (mCRC), conferring poor prognosis and lack of response to anti-epidermal growth factor receptor (EGFR) antibodies. In the last decades, several investigational attempts failed in directly targeting *RAS* mutations, thus *RAS* was historically regarded as ‘undruggable’. Recently, novel specific *KRAS*<sup>G12C</sup> inhibitors showed promising results in different solid tumors, including mCRC, renewing interest in this biomarker as a target. In this review, we discuss different strategies of *RAS* targeting in mCRC, according to literature data in both clinical and preclinical settings. We recognized five main strategies focusing on those more promising: direct *RAS* targeting, targeting the mitogen-activated protein kinase (MAPK) pathway, harnessing *RAS* through immunotherapy combinations, *RAS* targeting through metabolic pathways, and finally other miscellaneous approaches. Direct *KRAS*<sup>G12C</sup> inhibition is emerging as the most promising strategy in mCRC as well as in other solid malignancies. However, despite good disease control rates, tumor response and duration of response are still limited in mCRC. At this regard, combinational approaches with anti-epidermal growth factor receptor drugs or checkpoint inhibitors have been proposed to enhance treatment efficacy, based on encouraging results achieved in preclinical studies. Besides, concomitant therapies increasing metabolic stress are currently under evaluation and expected to also provide remarkable results in *RAS* codon mutations apart from *KRAS*<sup>G12C</sup>. In conclusion, based on hereby reported efforts of translational research, *RAS* mutations should no longer be regarded as ‘undruggable’ and future avenues are now opening for translation in the clinic in mCRC.

**Key words:** *RAS*, *KRAS*, sotorasib, adagrasib, colorectal cancer

### INTRODUCTION

Colorectal cancer (CRC) is the second most common cause of cancer-related death in the Western world.<sup>1</sup> Five-year relative overall survival (OS) is around 14% for those patients diagnosed with metastatic CRC (mCRC).<sup>1</sup> In this setting, molecular alterations occurring in Kirsten rat sarcoma virus (*KRAS*), neuroblastoma *RAS* viral oncogene homolog (*NRAS*), and B-Raf proto-oncogene (*BRAF*) significantly worsen disease prognosis.<sup>1</sup> In particular, *RAS* and *BRAF* mutations confer more aggressive tumor biology, shorter OS in particular in microsatellite stable (MSS) mCRC, and are negative predictive factors for response to milestone anti-epidermal growth factor receptor (EGFR) therapy

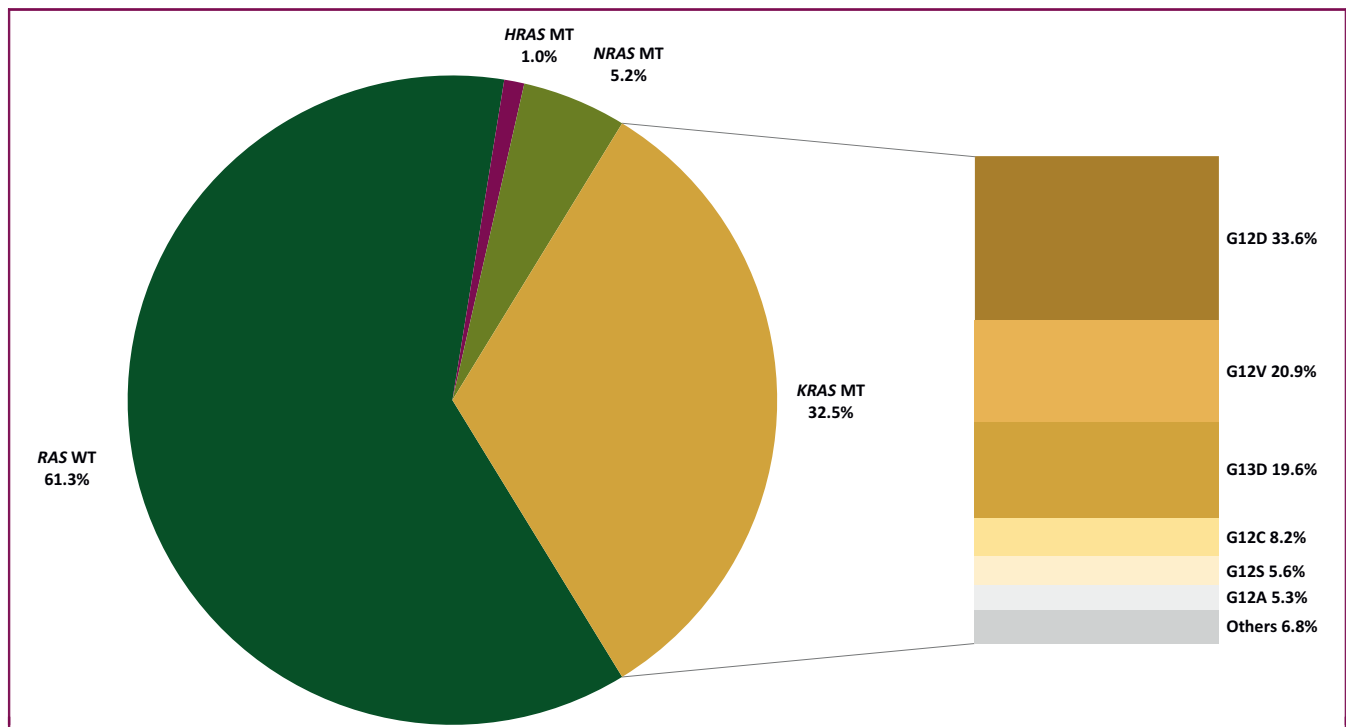
(cetuximab or panitumumab).<sup>2-6</sup> Accordingly, current clinical guidelines for *RAS* mutant (MT) MSS mCRC recommend chemotherapy (FOLFOX or FOLFIRI or FOLFOXIRI) with the addition of anti-vascular endothelial growth factor agents (bevacizumab in first line, bevacizumab or aflibercept in second line) as mainstream for early lines of treatment.<sup>7</sup> While new therapeutic strategies are emerging in other molecular subsets, like doublet or triplet combinations of anti-EGFR, anti-*BRAF*, and anti-MEK [mitogen-activated protein kinase 1 (MAPK1)] harnessing *BRAF*<sup>V600E</sup> mutations, and checkpoint inhibitor immunotherapy in microsatellite unstable (MSI) mCRC, *KRAS*/*NRAS* mutations still represent the main clinical unmet need in this disease.<sup>7-10</sup>

The *RAS* oncogene family consists of three oncogenes in humans, located in the short arm of chromosome 12, namely *KRAS*, *NRAS*, and Harvey rat sarcoma viral oncogene homolog (*HRAS*). The *RAS* family is one of the most frequently mutated across all malignancies, including CRC.<sup>11</sup> Indeed, around 40% of CRC harbors *KRAS* mutations plus an additional 4% *NRAS* mutations (and a negligible <1% prevalence of *HRAS* mutations), with >95% of them occurring in *KRAS* G12, G13, or Q61 codons.<sup>4,11,12</sup> G12

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**Figure 1. Prevalence of *RAS* mutations in metastatic colorectal cancer.**

Data were retrieved from the COSMIC database (<https://cancer.sanger.ac.uk/cosmic>) consulted on 27 February 2021. The percentage of mutated samples for point mutations regarding the '*KRAS*', '*NRAS*', and '*HRAS*' genes was collected from COSMIC v92 using auto-filtering for 'large intestinal' tissue type, and 'cecum' or 'left' or 'right' or 'colon' or 'rectum' sub-site, and 'carcinoma' histology, and 'adenocarcinoma' sub-histology. For *KRAS* mutations, the most common codon variants were also collected. Raw data are available in [Supplementary Table S1](https://doi.org/10.1016/j.esmooop.2021.100156), available at <https://doi.org/10.1016/j.esmooop.2021.100156>.

MT, mutant; WT, wild-type.

hotspot mutations account for around 68% of *KRAS* mutations in mCRC, most frequently G12D (~45%), G12V (~31%), and G12C (~11%) in MSS tumors, and predominantly G12D in MSI ones (Figure 1 and [Supplementary Table S1](https://doi.org/10.1016/j.esmooop.2021.100156), available at <https://doi.org/10.1016/j.esmooop.2021.100156>).

Prevalence of *KRAS* mutations increases with age, except for MSI disease.<sup>13</sup> In addition, acquired *RAS* mutations have been clearly demonstrated by our group and others to arise under the therapeutic pressure of EGFR blockade as secondary mechanisms of resistance.<sup>14,15</sup>

To assess the most efficient way of targeting the *RAS* oncogene, a comprehensive understanding of its biological role is needed. *RAS* proteins are members of a family of small guanosine triphosphate (GTP) phosphatases (GTPases) regulating many intracellular networks, which are fundamental in cell proliferation, migration, differentiation, senescence, and apoptosis.<sup>16</sup> *RAS* is activated by ligand binding to membrane receptor tyrosine kinases (RTKs), including members of the human EGFR (HER) family. *RAS* proteins are turned off if guanosine diphosphate (GDP)-bound and turned on when GTP-bound. Despite *RAS* intrinsic capability of GTP hydrolysis and nucleotide exchange, this process is mainly regulated by extrinsic guanine nucleotide exchange factors (GEF) such as son of sevenless homologue 1 (SOS1) for GDP-to-GTP transition, and GTPase-activating proteins (GAP) such as neurofibromin for GTP

hydrolysis.<sup>16,17</sup> In its active GTP-bound state, *RAS* changes its conformation and activates several downstream effector pathways, including the MAPK pathway (*RAS*-*RAF*-*MEK*-*MAPK* or namely *ERK*) and phosphatidylinositol 3-kinase (*PI3K*) pathway (*PI3K*-*AKT* or protein kinase B-*mTOR* or mammalian target of rapamycin).<sup>17</sup> To exert its function, *RAS* needs to be associated with the plasma membrane, through post-translational modifications of the cysteine-aliphatic-aliphatic-terminal amino acid (CAAX) motif within the hypervariable region (HVR) at the carboxyl terminus of the protein, mainly mediated by farnesyltransferase (FTase); phosphodiesterase- $\delta$  (*PDE $\delta$* ) chaperone protein then facilitates *RAS* localization to the plasma membrane.<sup>17</sup> Oncogenic *RAS* mutations, altering its regulation and functioning, can lead to persistent MAPK pathway activation and unbalanced proliferative signaling.<sup>16</sup>

In this review, we discuss different therapeutic strategies tackling *RAS* mutations specifically in mCRC, focusing on those approaches which have already been tested in clinical trials (clinical trials with available results are reported in [Table 1](#), whereas ongoing studies are listed in [Table 2](#)). Five main strategies were identified in this regard: direct *RAS* targeting, targeting the MAPK pathway, harnessing *RAS* through immunotherapy combinations, *RAS* targeting through metabolic pathways (all included in the visual summary [Figure 2](#)), and other miscellaneous approaches.

**Table 1. Clinical trials tackling RAS-mutant metastatic colorectal cancer with results available in the scientific literature**

Strategy	Drugs	Phase	RAS MT mCRC pts	ORR (%)	DCR (%)
<b>Direct RAS targeting</b>					
FTIs	Tipifarnib (R115777)	III	235 <sup>a</sup>	1 <sup>a</sup> (0.4)	58 <sup>a</sup> (24.7)
	Lonafarnib (SCH 66336)	II	21 <sup>a</sup>	0 <sup>a</sup> (0.0)	3 <sup>a</sup> (14.3)
	BMS-214662	I	22 <sup>a</sup>	0 <sup>a</sup> (0.0)	0 <sup>a</sup> (0.0)
Statins	Simvastatin + irinotecan + cetuximab	II	52	1 (1.9)	34 (65.4)
	KRAS <sup>G12C</sup> inhibitors				
	Sotorasib (AMG 510)	I	42	3 (7.1)	31 (73.8)
	Adagrasib (MRTX849)	II	18	3 (16.7)	17 (94.4)
Multikinase inhibitors	Rigosertib	I	10	0 (0.0)	0 (0.0)
<b>Targeting the MAPK pathway</b>					
MEKi	Trametinib	I	13	0 (0.0)	4 (30.8)
	Cobimetinib	I	28	0 (0.0)	NA
	RO5126766 <sup>b</sup>	I	2	0 (0.0)	NA
CDK4/6i	Palbociclib	II	15	0 (0.0)	5 (33.3)
mTORi	Temsirolimus	II	64	0 (0.0)	24 (37.5)
MEKi + anti-HER2	Trametinib + lapatinib	I	12	0 (0.0)	10 (83.3)
MEKi + anti-EGFR	Cobimetinib + duligotuzumab <sup>c</sup>	Ib	15	0 (0.0)	5 (33.3)
	Selumetinib + cetuximab	I	14	0 (0.0)	5 (35.7)
MEKi + PI3K	Refametinib + copanlisib	Ib	12	0 (0.0)	5 (41.7)
	Binimetinib + alpelisib	Ib	NA	0 (0.0)	NA
	Cobimetinib + pictilisib	Ib	47 <sup>a</sup>	0 <sup>a</sup> (0.0)	NA
	Trametinib + buparlisib	Ib	33	0 (0.0)	NA
	PD-0325901 + gedatolisib <sup>e</sup>	Ib	21 <sup>a</sup>	0 <sup>a</sup> (0.0)	NA
	Trametinib + omipalisib <sup>e</sup>	I	NA	0 (0.0)	NA
MEKi + AKT	Pimasertib + voxalisib <sup>e</sup>	I	11	0 (0.0)	NA
	Selumetinib + MK-2206	I	11	0 (0.0)	1 (9.09)
MEKi + ChT	Trametinib + afuresertib (GSK2110183)	I	3 <sup>a</sup>	0 (0.0)	NA
	Selumetinib + irinotecan	I/II	31	3 (9.7)	19 (61.2)
Pimasertib + FOLFIRI	I	16	2 (12.5)	11 (68.6)	
mTORi + ChT	Temsirolimus + irinotecan <sup>d</sup>	II	35	1 (2.9)	30 (85.7)
MEKi + BCL-XLi	Trametinib + navitoclax	I/II	9	0 (0.0)	2 (22.2)
MEKi + cyclosporin A	Selumetinib + cyclosporin A	I/Ib	14	1 (7.1)	11 (78.6)
<b>Harnessing RAS through immunotherapy combinations</b>					
MEKi + anti-PD-L1	Cobimetinib + atezolizumab	III	183 <sup>a</sup>	1 (1.0)	48 <sup>a</sup> (26.2)
Anti-PD-L1 + anti-CTLA-4 + ChT	Durvalumab + tremelimumab + FOLFOX	I/II	16	10 (62.5)	14 (87.5)
Immunomodulator + anti-EGFR	Lenalidomide + cetuximab	II	43	0 (0.0)	9 (20.9)
	Imprime PGG + cetuximab	II	18	1 (5.6)	10 (55.6)
	Magrolimab + cetuximab	Ib/II	40	0 (0.0)	18 (45.0)
RAS MT vaccine	RAS MT vaccine + IL-2 and/or GM-CSF	II	38	0 (0.0)	NA
<b>RAS targeting through metabolic pathways</b>					
High-dose AA + ChT	AA + FOLFOX6 or FOLFIRI	I	10	3 (30.0)	8 (80.0)
<b>Other miscellaneous approaches</b>					
Anti-PLK1 + ChT + anti-VEGF	Onvansertib + FOLFIRI + bevacizumab	I/II	9	4 (44.4)	8 (88.8)
Anti-DR5 + ChT	Conatumumab + FOLFIRI	II	51	7 (13.7)	35 (68.6)
Anti-EGFR	Cetuximab	II	12 <sup>f</sup>	0 (0.0)	3 (25.0)
	Imgatuzumab	I/II	25	0 (0.0)	6 (24.0)
Pan-HER inhibitors	Afatinib	II	41	0 (0.0)	5 (12.2)
Anti-EGFR + ChT	Imgatuzumab + FOLFIRI	II	NA	NA	NA

AA, ascorbic acid; BCL-XL, B-cell lymphoma-extra large; CDK, cyclin-dependent kinase; ChT, cytotoxic chemotherapy; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DCR, disease control rate; EGFR, epidermal growth factor receptor; FTI, farnesyl transferase inhibitor; GM-CSF, granulocyte-monocyte colony-stimulating factor; HER2, human epidermal growth factor receptor 2; IL-2, interleukin-2; i, inhibitor; MAPK, mitogen-activated protein kinase; mCRC, metastatic colorectal cancer; MT, mutant; mTOR, mammalian target of rapamycin; ORR, overall response rate; NA, not available; PD-L1, programmed death-ligand 1; PI3K, phosphatidylinositol 3-kinase; PLK1, polo-like kinase 1; pts, patients; VEGF, vascular endothelial growth factor.

<sup>a</sup> Data are reported on overall mCRC population as this trial did not provide adequate information regarding RAS mutational status.

<sup>b</sup> RO5126766 is a RAF/MEK dual inhibitor.

<sup>c</sup> Duligotuzumab is an EGFR/HER3 dual inhibitor.

<sup>d</sup> Drug rechallenged in previously refractory patients.

<sup>e</sup> PI3K/mTORi.

<sup>f</sup> KRAS G13 MT mCRC.

## DIRECT RAS TARGETING

### Post-translational inhibitors: the ancestry of RAS-targeting

Targeting post-translational RAS modifications was one of the first attempted strategies to reduce the expression of the MAPK pathway, with the aim of preventing the RAS protein interaction with the plasma membrane, and thus

the subsequent activation of its downstream signaling. Owing to the central role of farnesylation in this process and to the accessibility of HVR and CAAX motifs, FTase became a promising target.<sup>17</sup> Despite inhibiting tumor growth in preclinical models of *HRAS*-driven cancers, however, FTase inhibitors (FTI) tipifarnib, lonafarnib, and BMS-214662 showed no clinical efficacy in patients with

**Table 2. Ongoing clinical trials tackling RAS-mutant metastatic colorectal cancer**

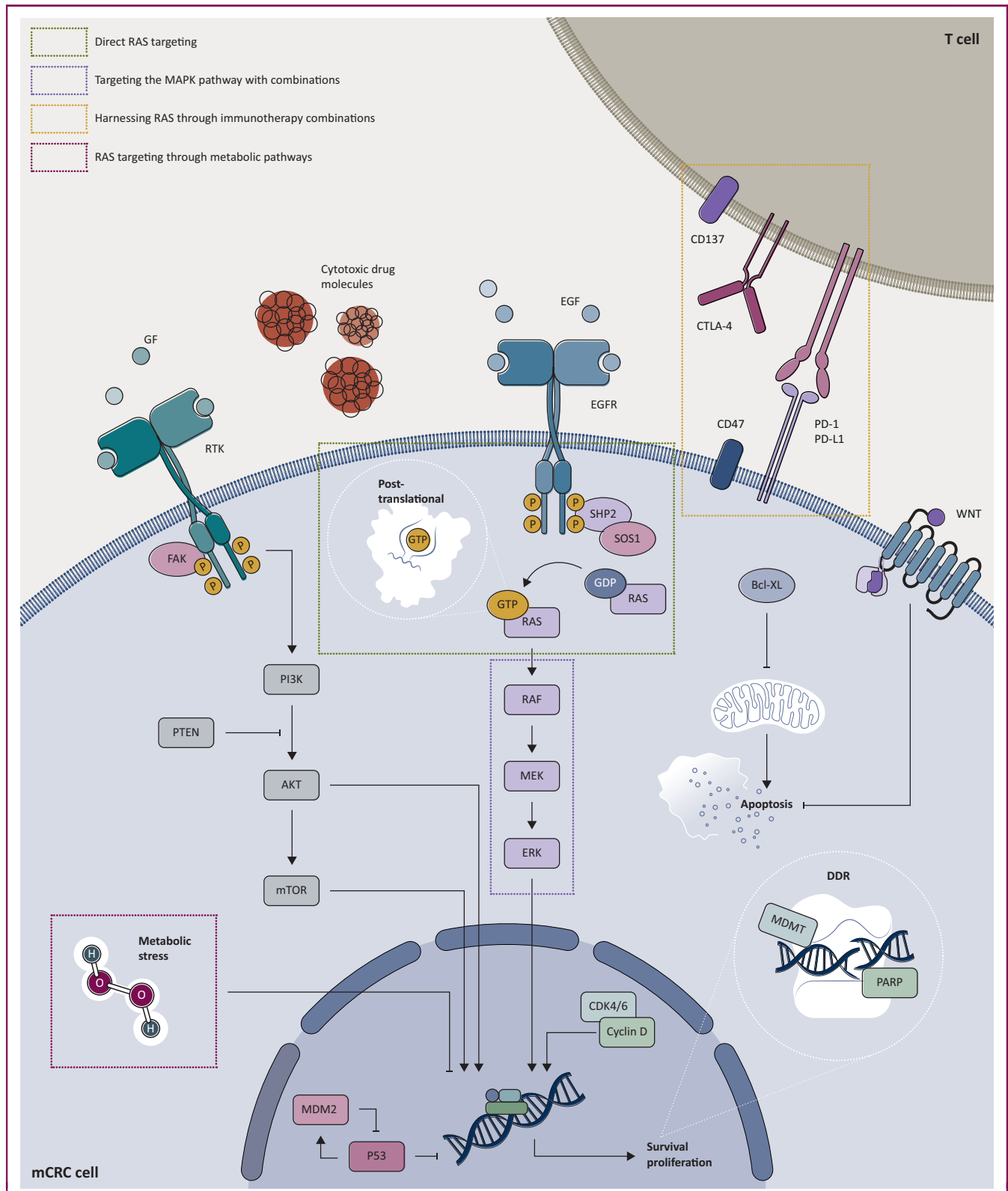
Strategy	NCT/trial name	Drugs	Phase
<b>Direct RAS targeting</b>			
KRAS <sup>G12C</sup> inhibitors	NCT03600883/CodeBreaK100	Sotorasib (AMG 510)	II
	NCT03785249/KRYSTAL-1	Adagrasib (MRTX849)	I/II
	NCT04006301	JNJ-74699157	I
	NCT04165031 <sup>b</sup>	LY3499446	I/II
KRAS <sup>G12C</sup> inhibitor-based combinations	NCT04185883/CodeBreaK101	AMG 510 with anti-PD-1, MEKi, SHP2 allosteric inhibitor, pan-ErbB inhibitor, anti-PD-L1, anti-EGFR, ChT, mTORi, or CDK4/6i	I
	NCT03785249/KRYSTAL-1	MRTX849 with pembrolizumab, cetuximab or afatinib	I/II
	NCT04330664/KRYSTAL-2	MRTX849 + TNO155 (SHP2 inhibitor)	I/II
	NCT04793958/KRYSTAL-10	MRTX849 + cetuximab versus ChT	III
KRAS-derived mRNA binder	NCT03101839	AZD4785	I
SOS1 inhibitor	NCT04111458	BI 1701963 ± trametinib (MEKi)	I
SHP2 inhibitors	NCT03634982	RMC-4630	I
	NCT03518554	JAB-3068	I
	NCT03565003	JAB-3068	I/II
SHP2 inhibitor-based combinations	NCT03989115	RMC-4630 with osimertinib (anti-EGFR) or cobimetinib (MEKi)	I/II
<b>Targeting the MAPK pathway</b>			
RAF/MEKi ± mTORi	NCT02407509	RO5126766 ± everolimus	I
ERK inhibitors	NCT02857270	LY3214996 ± midazolam, abemaciclib (CDK4/6i), nab-paclitaxel (ChT), gemcitabine (ChT), encorafenib (MEKi), or cetuximab (anti-EGFR)	I
	NCT02313012	CC-90003	I
	NCT03065387	Neratinib with everolimus (mTORi), palbociclib (CDK4/6i), or trametinib (MEKi)	I
Pan-ErbB inhibitor-based combinations	NCT02510001	Crizotinib with PD-0325901 or binimetinib	I
cMET inhibitor + MEKi	NCT03087071	Panitumumab ± trametinib	II
EGFR inhibitor + MEKi	NCT01927341	Panitumumab + binimetinib	I/II
	NCT02065063	Trametinib + palbociclib	I
MEKi + CDK4/6i	NCT03981614	Binimetinib + palbociclib	II
FAK inhibitor + RAF/MEKi	NCT03875820/FRAME trial	VS-6063 + RO5126766	I
PI3K + MEKi	NCT01337765	BEZ235 <sup>c</sup> + binimetinib	I
	NCT01859351	WX-037 ± WX-554	I
MEKi + ChT	NCT02613650	Binimetinib + mFOLFIRI	I
	NCT03317119	Trametinib + TAS-102	I
MEKi + MDM2 inhibitor	NCT03714958	Trametinib + HDM201	I
<b>Harnessing RAS through immunotherapy combinations</b>			
Anti PD-1 + MEKi ± anti-CTLA-4	NCT03271047	Nivolumab + binimetinib ± ipilimumab	I/II
Anti-PD-L1 + MEKi + PARPi	NCT03637491	Avelumab + binimetinib + talazoparib	I/II
Anti-CTLA-4 + anti-PD-L1 + ChT	NCT03202758/MEDETREME	Tremelimumab + durvalumab + FOLFOX	I/II
Anti PD-1 + ChT + anti-VEGF	NCT04194359	Sintilimab + XELOX + bevacizumab	III
CD137 agonist + ChT + anti-EGFR	NCT03290937	Utomilumab + irinotecan + cetuximab	I
Adoptive cell transfer	NCT03745326	Anti-KRAS <sup>G12D/G12V</sup> mTCR PBL	I/II
Sequential ChT and anti-PD-1	NCT03519412/ARETHUSA	Temozolomide followed by pembrolizumab	II
<b>RAS targeting through metabolic pathways</b>			
Glutaminase inhibitor + CDK4/6i	NCT03965845	Telaglenastat (CB-839) + palbociclib	I/II
Fatty acid synthase inhibitor	NCT02980029	TVB-2640	I
Metabolic damaging	NCT03146962	High-dose i.v. vitamin C	I
	NCT02969681	High-dose i.v. vitamin C + FOLFOX ± bevacizumab	III
<b>Other miscellaneous approaches</b>			
Selective WEE1 inhibitor + ChT	NCT02906059	Adavosertib (AZD1775) + irinotecan	I
TRAIL receptor agonist	NCT03082209	Eftozanermin (ABBV-621) ± FOLFIRI and bevacizumab	I
Anti-EGFR + ChT	ACTRN12612000901808 <sup>a</sup> /ICECREAM	Cetuximab ± irinotecan	II
Pan-ErbB inhibitor + anticonvulsant	NCT03919292	Neratinib + valproate	I/II

CDK, cyclin-dependent kinase; ChT, cytotoxic chemotherapy; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; FAK, focal adhesion kinase; I, inhibitor; i.v., intravenous; MEK, mTOR, mammalian target of rapamycin; NCT, unique identification code given to each clinical study upon registration at [ClinicalTrials.gov](https://clinicaltrials.gov); PARP, poly (ADP-ribose) polymerase; PBL, peripheral blood lymphocyte; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PI3K, phosphatidylinositol 3-kinase; SHP2, Src homology region 2 domain-containing phosphatase-2; SOS1, sevenless homologue 1; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor.

<sup>a</sup> Referring to Australian New Zealand Clinical Trials Registry (ANZCTR).

<sup>b</sup> Early termination due to unexpected toxicity.

<sup>c</sup> BEZ235 is MEK/mTORi.



**Figure 2. Main therapeutic strategies targeting RAS-mutant metastatic colorectal cancer.**

Five therapeutic strategies targeting RAS-mutant metastatic colorectal cancer were identified and categorized by different pharmacodynamic interferences with the RAS signal: direct RAS targeting, targeting the MAPK pathway, harnessing RAS through immunotherapy combinations, RAS targeting through metabolic pathways, and other miscellaneous approaches. The main molecular targets are shown and grouped according to the most suitable anti-RAS therapeutic strategy. Created with BioRender.com.

Bcl-XL, B-cell lymphoma-extra large; CDK, cyclin-dependent kinase; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DDR, DNA damage response and repair; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; GF, growth factor; GDP, guanosine diphosphate; GTP, guanosine triphosphate; MAPK, mitogen-activated protein kinase; mCRC, metastatic colorectal cancer; MDM2, murine double minute 2; MGMT, O<sup>6</sup>-methylguanine-DNA methyl-transferase; mTOR, mammalian target of rapamycin; PARP, poly (ADP-ribose) polymerase; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; RTK, receptor tyrosine kinase; SHP2, Src homology region 2 domain-containing phosphatase-2; SOS1, sevenless homologue 1.



advanced RAS MT mCRC.<sup>18-20</sup> These results have been attributed to RAS isoform-specific differences in the post-translational processing, as FTase can be superseded by alternative prenylation by geranylgeranyltransferase (GGTase) in *NRAS*- and *KRAS*-driven tumors, but not *HRAS*-driven ones.<sup>17</sup> Dual targeting of FTase and GGTase has been proposed as a circumventing strategy, as well as targeting downstream RAS processing proteins like PDE $\delta$  (with deltarasin or novel PDE $\delta$  inhibitor deltazinone 1). However, these strategies have never been tested in clinical trials, since increased toxicity is expected from shared activity of these enzymes on both normal and transformed cells.<sup>21</sup> Novel lipophilic bisphosphonate BPH1222 also showed preclinical inhibition of the post-translational processing of RAS prenylation and may be considered for future clinical trials in mCRC.<sup>22</sup> Besides, lipid-lowering statins were proven to interfere with the post-translational modification of the RAS protein, through blockade of the mevalonate pathway by means of  $\beta$ -hydroxy- $\beta$ -methylglutaryl-CoA reductase inhibition. The mevalonate pathway is a metabolic cascade resulting in cholesterol synthesis, together with various end-products including farnesyl and geranylgeranyl moieties, which are both critical for the post-translational prenylation of RAS. By impeding farnesylation and geranylgeranylation, statins interfere with RAS binding to the plasma membrane, and thereby its activation. In spite of this, the addition of simvastatin to anti-EGFR drugs in order to restore sensitivity reported lack of efficacy in clinical trials.<sup>23,24</sup> Conflicting data emerged from the addition of statins to chemotherapy regimens, with negative results from a retrospective analysis of the CAIRO2 trial (with chemotherapy + bevacizumab + cetuximab).<sup>25</sup> However, a phase II trial showed an encouraging 65.4% disease control rate (DCR) and 7.6 months progression-free survival (PFS) for irinotecan-refractory *KRAS* MT mCRC patients treated with irinotecan, cetuximab, and simvastatin.<sup>26</sup>

### **RAS direct inhibitors: new perspectives limited to the *KRAS*<sup>G12C</sup> mutation**

Differently from FTI, direct inhibition of specific RAS isoforms and codon mutations accounts for a more encouraging approach. Among the most common RAS mutations, *KRAS*<sup>G12C</sup> has been recently demonstrated druggable.<sup>27,28</sup> *KRAS*<sup>G12C</sup> is identified in approximately 4% of CRCs.<sup>12</sup> Its oncogenic activity is linked to impaired GAP-mediated hydrolysis, resulting in marked predominance of the GTP-bound active state. However, *KRAS*<sup>G12C</sup> preserves peculiar near-wild-type (WT) intrinsic GTPase activity and thus slight GTP-to-GDP cycling ability, differently from other *KRAS* codon mutations.<sup>29</sup> This diverseness enables direct inhibitors to halt the *KRAS*<sup>G12C</sup> protein in its inactive GDP-bound conformation, by means of covalent binding to a reactive thiol group in the cysteine 12 residue.<sup>30</sup> The discovery of this allosteric nucleotide-binding pocket (called 'switch-II pocket') was pioneered by Shokat and colleagues,<sup>31</sup> and led to the development of chemical compounds irreversibly targeting *KRAS*<sup>G12C</sup>, and translating into

decreased viability and increased apoptosis in cell lines. Thenceforward, several novel inhibitors were developed. Sotorasib (AMG 510 by Amgen, Inc., Thousand Oaks, CA) has been the first small molecule to be tested in clinical trials. A phase I study with sotorasib monotherapy evaluated 42 heavily pretreated patients (median of three prior lines) with refractory *KRAS*<sup>G12C</sup> mCRC. Overall response rate (ORR) and DCR were 7.1% (3/42) and 73.8% (31/42), respectively. Of interest, these increased to 12.0% (3/25) and 80.0% (20/25), respectively, in patients receiving the expansion phase dosage (960 mg daily). Among all dose levels, median duration of stable disease (SD) was 5.4 months and median PFS was 4.0 months. Sotorasib was well tolerated with no dose-limiting toxicities or adverse events causing treatment discontinuation.<sup>27,32</sup> The phase II monotherapy trial is ongoing (CodeBreaK100/NCT03600883). Adagrasib (MRTX849 by Mirati Therapeutics, Inc., San Diego, CA) is another *KRAS*<sup>G12C</sup> inhibitor which is currently being tested in a phase Ib/II clinical trial (KRYSTAL-1/NCT03785249). Of 24 patients treated with the recommended phase II dose (600 mg twice), results are available for 18 with 16.7% ORR (3/18) and 94.4% DCR (17/18). Treatment duration was  $\geq 4$  months for 55% of patients (10/18).<sup>28,33</sup> Further phase I/II trials exploiting different *KRAS*<sup>G12C</sup> inhibitors such as JNJ-74699157 (NCT04006301) and LY3499446 (NCT04165031) are ongoing. Given its good toxicity profile and in order to increase its efficacy, sotorasib has been combined with MEK inhibitors (MEKi), immune checkpoint inhibitors (ICIs), cytotoxic agents and anti-EGFR drugs, significantly improving inhibition of tumor growth *in vivo*.<sup>34-36</sup> Several phase I/II trials combining an anti-*KRAS*<sup>G12C</sup> together with ICIs (extensive topic addressed in the following chapter *Harnessing RAS through immunotherapy combinations*), MEKi, anti-AKT drugs, Src homology region 2 domain-containing phosphatase-2 (SHP2) allosteric inhibitors (TNO155), pan-HER RTK inhibitors, EGFR inhibitors, and cytotoxic agents are ongoing (CodeBreaK101/NCT04185883, KRYSTAL-1/NCT03785249, KRYSTAL 2/NCT04330664).<sup>34,37</sup> Besides, a phase III study is now randomizing patients to receive adagrasib and cetuximab versus chemotherapy as the second-line treatment of *KRAS*<sup>G12C</sup> MT mCRC (KRYSTAL-10/NCT04793958).

### **Beyond *KRAS*<sup>G12C</sup>: future options for RAS inhibition**

*KRAS*<sup>non-G12C</sup> mutations translate into biologically distinct proteins from *KRAS*<sup>G12C</sup>, lacking cysteinic substrate for covalent inhibition and expressing a lower intrinsic GTP hydrolysis rate.<sup>29</sup> Thus, novel compounds are being studied in preclinical models such as the RAS(ON) platform by Revolution Medicines (Redwood City, CA).<sup>21</sup> Dealing with the diversity of RAS codon mutants also led to the conceiving of pan-RAS inhibitors (binding both WT and MT *KRAS* beyond G12C) and protein-interaction disrupters, despite concerns for their tolerability.<sup>16</sup> Besides, the RAS protein-interaction disrupter rigosertib did not show any benefit in a clinical trial.<sup>38</sup> Novel compounds demonstrated *in vitro* and *in vivo* activity against *KRAS*<sup>G12D</sup>, *KRAS*<sup>G61H</sup>, and *KRAS*<sup>G12V</sup> codon

mutations in CRC and other histologies.<sup>39-43</sup> As the *KRAS*<sup>G12D</sup> mutation is the most common in mCRC, the development of a specific effective inhibitor would be relevant in clinical practice.<sup>11</sup> A genetic depletion strategy by novel antisense oligonucleotide AZD4785 (binding *RAS*-derived mRNA) is also under clinical investigation (NCT03101839).<sup>44</sup> Finally, GEF inhibition was identified as a potential target, through binding of effectors such as SOS1 and SHP2, the latter being a scaffold protein that increases SOS1 nucleotide exchange activity by tethering SOS1 together with growth factor receptor-bound protein 2 (GRB2).<sup>45</sup> A currently ongoing phase I clinical trial is testing the SOS1 inhibitor BI-170196 (NCT04111458). Inhibitors of SHP2, like RMC-4630 and JAB-3068, are also in phase I/II clinical trials, alone or combined with MEKi (NCT03634982, NCT03518554, NCT03565003, NCT04111458, NCT03989115). Strategies impeding *RAS* oligomerization have not reached clinical trials yet.<sup>21</sup>

## TARGETING THE MAPK PATHWAY

### Single-agent MAPK blockade

The inhibition of MAPK effectors other than *RAS* represents a further strategy targeting *RAS* MT mCRC, mainly in the form of combination therapies targeting multiple downstream kinases or upstream membrane RTK.<sup>46</sup> According to current evidence, MEKi were established as the cornerstone for drug association in this setting, favored over *BRAF* inhibitors in *RAS* MT cancers. Indeed, clinically approved *BRAF*<sup>V600E</sup> inhibitors, such as vemurafenib, dabrafenib, and encorafenib, are only effective with *RAF* monomers like *BRAF*<sup>V600</sup>, and not *BRAF* and *CRAF* dimers, and can lead to paradoxical activation of the EGFR/MAPK pathway through ERK-mediated regulatory feedback.<sup>47</sup> Conversely, MEKi such as trametinib, binimetinib, and cobimetinib, prevent MEK phosphorylating ERK1/2, thus avoiding its dimerization and nuclear translocation.<sup>48</sup> However, trametinib, cobimetinib, and also RO5126766 (a potent *RAF*/MEKi; NCT02407509 still recruiting) alone were not proven active in this subset of patients.<sup>49-51</sup> Again, this can be explained by redundant signaling through upstream RTK and activation of parallel signal transduction cascades bypassing MEK inhibition and reactivating ERK signaling.<sup>52</sup> Likewise, preliminary results of ERK inhibitor monotherapy with LY3214996 or CC-90003 did not show marked activity (NCT02857270, NCT02313012).<sup>53,54</sup> Given the poor outcome with monotherapies, combinations exploiting vertical and/or horizontal (on parallel pathways) blockade have been assessed.

### MEK and RTKs blockade

Several recent early studies focused on concurrent blockade of MEK and upstream RTK. For instance, MEKi were combined with anti-EGFR drugs, with the aim of overcoming primary resistance to the latter in *RAS* MT mCRC.<sup>14,55</sup> The benefit of combining cetuximab, lapatinib (anti-HER2), or the EGFR/HER3 dual inhibitor duligotuzumab with MEKi produced, at most, disease stabilization with no objective

responses.<sup>56-59</sup> Two phase I trials are evaluating the combination of MEKi with other RTK inhibitors, neratinib or crizotinib (NCT03065387, NCT02510001). Other trials combining MEKi and panitumumab are ongoing (NCT03087071, NCT01927341).

### MEK inhibition and cell cycle regulation

MAPK pathway activation might lead to cell cycle dysregulation through the cyclin-dependent kinase (CDK) pathway, contributing to the G1-S phase progression through retinoblastoma protein phosphorylation, the latter seldom inactivated in CRC.<sup>60</sup> Palbociclib alone showed limited activity in a phase II trial (0% ORR, 33% DCR).<sup>61</sup> Given the limited toxicity of CDK4/6 inhibitors (CDK4/6i), trametinib was added to palbociclib which was demonstrated to be effective in *KRAS* MT CRC patient-derived xenograft (PDX) models. This combination has been tested in a phase Ib study (NCT02065063), the results of which have not been published yet. Up to now, the only available outcome data come from a case report of an *NRAS* MT mCRC patient achieving a prolonged partial response (PR) of 10.8 months with this combination.<sup>62,63</sup> Finally, a phase II trial will compare binimetinib plus palbociclib versus trifluridine/tipiracil (TAS-102) in refractory *RAS* MT mCRC (NCT03981614). Focal adhesion kinase (FAK) is a major focal adhesion-associated protein kinase involved in cellular proliferation. It acts through elicitation of intracellular signal transduction pathways such as PI3K-AKT-mTOR, and inhibition of apoptosis in several types of cancer, including CRC. The FAK inhibitor VS-6063 in combination with RO5126766 (dual *RAF*/MEKi) is currently under investigation (NCT03875820).<sup>64</sup>

### MEK and mTOR pathway inhibition

*PIK3CA* mutations or up-regulation of the PI3K-AKT-mTOR signaling pathway through *ERBB3* gene amplification can preclude responsiveness to MEKi. Precisely, *PIK3CA* mutations can restore G1-S cell cycle progression making cancer cells independent from MAPK signaling.<sup>65</sup> Available data on PI3K-AKT-mTOR pathway inhibition suggest minimal anti-tumor activity of mTOR inhibitors alone in *KRAS* MT mCRC. Indeed, temsirolimus led to 38% SD in pretreated patients.<sup>66</sup> Combinations of MEKi with PI3K inhibitors, AKT inhibitors, or mTOR inhibitors are in clinical trials, despite several combinations having been proved ineffective in refractory mCRC. Indeed, 0% ORR was observed with combinations of MEKi plus PI3K inhibitors (copanlisib, alpelisib, pictilisib, or buparlisib), PI3K/mTOR dual inhibitors (gedatolisib, voxtalisisib, or omipalisib), and AKT inhibitors (MK-2206, ipatasertib, or afuresertib).<sup>67-78</sup> Results from three similar studies are pending (NCT01337765, NCT01859351, NCT02407509).

### MEKi and cytotoxic agents

MEKi can alter the expression of the B-cell lymphoma 2 (Bcl-2) family protein favoring cell apoptosis. Thus, synergistic activity of MEKi combination with cytotoxic agents was investigated.<sup>79</sup> In a phase I/II study with selumetinib and irinotecan as second-line therapy, 9.7% ORR and 61.3%

DCR were achieved.<sup>80</sup> Likewise, temsirolimus achieved 63% SD when associated with irinotecan.<sup>66</sup> Since chemotherapy doublets became the standard of care for RAS MT mCRC in the second-line setting, FOLFIRI plus pimasertib was evaluated in a phase I study, but early stopped due to toxicity concerns.<sup>81</sup> However, further phase I trials with binimetinib and FOLFIRI, or trametinib plus TAS-102, are ongoing (NCT02613650, NCT03317119).

### RAS MT TP53 WT CRC: prospect of further MEK-based therapy

The protein p53 is the main determinant of cell cycle arrest and a pivotal tumor suppressor encoded by the *TP53* gene, even if other regulatory proteins can interfere with cell cycle functioning such as murine double minute 2 protein (MDM2). Cell cycle regulation in *TP53* WT cells may be harnessed by *MDM2* gene amplification or, likewise, by *CDKN2A* loss, which encodes the MDM2 antagonist p14<sup>ARF</sup>. Disruption of the interaction between p53 and MDM2, with subsequent reactivation of p53, represents a potential strategy in *TP53* WT cancer cells.<sup>82</sup> In RAS MT *TP53* WT mCRC patients, a phase I study is evaluating the combination of trametinib and HDM201 (MDM2 inhibitor) (NCT03714958). Besides, further novel molecules are being proposed to restore *TP53* activity (i.e. inhibitors of the oncogenic *KRAS*-induced p53-binding 'Snail') and may soon enter clinical trials.<sup>83</sup>

### Other combinations exploiting MEK inhibition

Bcl-XL (Bcl-2-like protein 1 or Bcl-extra large) is an anti-apoptotic Bcl-2 family protein and a key suppressor of the apoptotic response to MEKi, since it binds and inhibits pro-apoptotic proteins induced by MEKi such as Bim (Bcl-2 interacting mediator of cell death). After proof of tumor regression in mouse models of RAS MT cancers, combined Bcl-XL/MEK inhibition entered clinical trials.<sup>84</sup> In a phase Ib/II trial, navitoclax (Bcl-XL inhibitor) was given together with trametinib in subjects with RAS MT advanced solid tumors. Differently from other histologies, no sign of activity was noted in mCRC.<sup>85</sup> Likewise, preclinical studies attributed MEKi resistance to Wnt pathway overexpression in *KRAS* MT cells. Thus, selumetinib combined with cyclosporin A (a non-canonical Wnt pathway modulator) achieved 5% ORR (2/38) and 47% DCR (18/38).<sup>86</sup>

### HARNESSING RAS THROUGH IMMUNOTHERAPY COMBINATIONS

Immunotherapy is emerging as a new standard treatment in the upfront setting for MSI mCRC patients. However, in the RAS MT subgroup pembrolizumab was not superior to cytotoxic regimens.<sup>10</sup> Indeed, *KRAS* mutations might facilitate cancer immunoescape mechanisms. In preclinical models, *KRAS* mutations modulate tumor microenvironment (TME), inducing immunosuppressive chemokines like interleukin (IL) 6 and IL-10, transforming growth factor- $\beta$ , and granulocyte-macrophage colony-stimulating factor. This leads to M2 macrophages, myeloid-derived suppressor cells,

and CD4+ regulatory T cell (Treg) recruitment, and CD8+ T cell depletion.<sup>87</sup> Moreover, *KRAS* mutations were associated with programmed cell death protein 1 (PD-1) and reduction of expression of programmed death-ligand 1 (PD-L1) in MSI CRC.<sup>88</sup>

### Sensitizing TME to immunotherapy by blocking the RAS pathway

MAPK pathway inhibition may revert immunosuppressive TME, thus enhancing the activity of ICIs in *KRAS* MT mCRC. In preclinical models, the combination of sotorasib (AMG 510) with ICIs increased intratumoral CD8+ T cells, interferon- $\gamma$  (IFN- $\gamma$ ) pathway activation, and boosted secretion of chemokines and cytokines. *In vivo*, this led to sustained complete tumor regression in 9 out of 10 PDX (90%) derived from human *KRAS*<sup>G12C</sup> MT CRC cells.<sup>34</sup> Similar results were obtained when combining adagrasib (MRTX849) with anti-PD-1 agents.<sup>89</sup> Thus, two clinical trials are evaluating sotorasib and adagrasib combined with anti-PD-1 (CodeBreak101/NCT04185883, KRYSTAL-1/NCT03785249). Based on similar preclinical evidence, a MEKi and anti-PD-L1 combination has been investigated. However, in a phase III trial (IMblaze 370) atezolizumab and cobimetinib demonstrated no OS and PFS improvement compared with regorafenib in a molecular-unselected population of mCRC.<sup>90</sup> A phase Ib/II trial is currently evaluating nivolumab and binimetinib with or without ipilimumab (anti-cytotoxic T-lymphocyte-associated protein 4, namely anti-CTLA-4) in patients with *KRAS* MT MSS mCRC (NCT03271047). *In vitro*, poly (ADP-ribose) polymerase inhibitors (PARPi) increased the formation of neoantigens triggering IFN release, potentially sensitizing immunotherapy, even if the role of PARPi in CRC remains to be established.<sup>91</sup> In addition, MEKi decreased the expression of multiple homologous recombination (HR) components, thus sensitizing cancer cells to PARPi.<sup>92</sup> Accordingly, a phase Ib/II study is currently investigating a triple combination of avelumab (anti-PD-L1), binimetinib, and talazoparib (PARPi) in patients with *KRAS* MT solid tumors (NCT03637491).

### Immunotherapy and cytotoxic agents

In preclinical studies, several chemotherapy regimens showed the capability to stimulate antitumor immunity through different pathways, especially by increasing PD-L1 expression and CD8+ T-cell recruitment. In addition, PD-1/PD-L1 blockade enhanced cancer vulnerability to oxaliplatin by reducing the expression of ERK or p38 MAPK, a potential mechanism involved in secondary resistance to platinum.<sup>93</sup> The phase I/II MEDETREME trial (NCT03202758) is evaluating tremelimumab (anti-CTLA-4) and durvalumab (anti-PD-L1) on top of FOLFOX in the upfront setting for *KRAS* MT mCRC. An interim analysis supported its efficacy with a 6-month PFS of 62.5% (10 of 16 patients), of which 5 were complete response (CR) and 5 PR.<sup>94</sup> Similarly, a phase III trial is assessing the efficacy of sintilimab (anti-PD-1) in association with XELOX and bevacizumab in the same setting (NCT04194359).



### Other approaches to elicit immune response

Lenalidomide is an immunomodulatory agent enhancing inflammatory response through T-cell proliferation, IL-2, IL-12, and IFN- $\gamma$  up-regulation and Treg inhibition. A lenalidomide and cetuximab combination failed to achieve meaningful activity in a clinical trial of *KRAS* MT mCRC patients.<sup>95</sup> Similarly, Imprime PGG (a novel innate immune cell modulator) was adopted in combination with cetuximab with poor results in *RAS* MT CRC.<sup>96</sup> Based on the inhibition of phagocytosis by tumor protein CD47, magrolimab (anti-CD47 antibody, Hu5F9-G4) proved antitumoral activity in a phase I basket trial, thus a phase Ib/II trial combined with cetuximab demonstrated at most 45% SD in *KRAS* MT mCRC patients.<sup>97</sup> Cancer vaccines with mutant *RAS* peptides were also investigated in CRC patients, demonstrating the induction of specific immune responses in 54% of patients. The adjuvant use of granulocyte-macrophage colony-stimulating factor increased the immune response up to 92%, despite no patient showing a clinical response, likely due to Treg up-regulation.<sup>98</sup> Moreover, triggering CD137 (4-1BB), a costimulatory receptor expressed on T lymphocytes and natural killer (NK) cells, increased antibody-dependent cellular toxicity (ADCC) by NK cells *in vivo*. Its potential synergistic activity with cetuximab led to an ongoing phase I trial of utomilumab (CD137 agonist) plus cetuximab and irinotecan in mCRC patients including those with *KRAS* MT disease (NCT03290937).<sup>99</sup> In recent times, adoptive cell transfer (ACT) entered clinical research in solid tumors. ACT exploits patients' peripheral blood lymphocytes, which are transfected with a retroviral vector encoding a murine T-cell receptor (mTCR) directed against a specific cancer antigen. After *in vitro* expansion, the lymphocytes are reinfused in the same patient to boost the immune response. A phase I/II trial is evaluating the administration of lymphocytes loaded with anti-*KRAS*<sup>G12D</sup> or anti-*KRAS*<sup>G12V</sup> mTCR in patients with *KRAS*<sup>G12D/G12V</sup> MT cancers (NCT03745326). Finally, the usefulness of alkylating drugs has also been assessed as a bridge loophole taking advantage of tumor resilience, with the aim of providing immunotherapy to previously insensitive tumors. Some 55% of *KRAS* MT mCRC cells present O<sup>6</sup>-methylguanine-DNA methyltransferase methylation (dMGMT), thus implying an increased vulnerability to temozolomide and dacarbazine.<sup>100</sup> Besides, in proficient mismatch repair (pMMR)/MSS CRC cells, temozolomide increased tumor mutational burden (TMB) and infiltrating lymphocytes with IFN- $\gamma$  release.<sup>101</sup> The phase II ARETHUSA trial (NCT03519412) is currently evaluating the effect of temozolomide priming in chemorefractory *KRAS* MT dMGMT pMMR/MSS mCRC patients, followed by pembrolizumab in case of TMB elevation.<sup>102</sup>

### RAS TARGETING THROUGH METABOLIC PATHWAYS

*KRAS* mutations can induce metabolic reprogramming through enhanced glucose uptake and increased expression of glutamine metabolic proteins.<sup>103</sup> In this regard, a phase Ib/II trial is evaluating the association of the glutaminase inhibitor telaglenastat (CB-839) plus palbociclib in pretreated

*KRAS* MT mCRC patients (NCT03965845). Fatty acid synthase (FASN) is an enzyme involved in lipid synthesis and frequently up-regulated in *KRAS* MT cells. A phase I trial is testing the effect of preoperative doses of TVB-2640 (FASN inhibitor) in resectable solid tumors, including *KRAS* MT CRC (NCT02980029).<sup>104</sup> In addition, it has been reported that, when exposed to high-dose ascorbic acid (AA, vitamin C), *KRAS* MT cells are driven to energetic crisis and cell death. Indeed, increased dehydroascorbate uptake (oxidized form of AA) requires glutathione to reduce dehydroascorbate to AA, and its depletion induces oxidative stress. Oxygen radicals are then responsible for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) inactivation, which is pivotal for the high glycolytic metabolic profile of *KRAS* MT mCRC cells (to a greater extent than *RAS* WT cells).<sup>105</sup> Besides, MAPK pathway is selectively inhibited by AA in *KRAS* MT CRC cells.<sup>106</sup> Phase I clinical trials found that high dose (1.5 g/kg or 90 g/m<sup>2</sup>) intravenous AA can be safely given with chemotherapy regimens FOLFOX and FOLFIRI.<sup>107,108</sup> A phase II trial is studying high-dose AA monotherapy in *KRAS* MT mCRC in a cohort of pretreated patients and in the perioperative treatment before and after Y90 radioembolization in a cohort of patients with resectable hepatic metastases (NCT03146962). Finally, a phase III randomized trial is combining high-dose AA and FOLFOX plus bevacizumab in a first-line setting (NCT02969681).

### OTHER MISCELLANEOUS APPROACHES

#### Targeting cell cycle effectors and DNA damage response system

WEE1 is an oncogenic nuclear protein kinase that operates at the G2/M checkpoint through the inactivation of CDK1 in response to DNA damage. Selective inhibition of WEE1 (i.e. by adavosertib) favors DNA damage buildup and thus promotes mitotic catastrophe.<sup>109</sup> On this basis, a phase I trial tested adavosertib (AZD1775) and irinotecan as second-line treatment of *KRAS* or *BRAF* MT mCRC and the results are awaited (NCT02906059). Similarly, polo-like kinase 1 (PLK1) is a serine/threonine nuclear kinase regulating mitotic checkpoints and cell division, often overexpressed in CRC. PLK1 was identified as a synthetic lethal target in *KRAS* MT CRC, since its inhibition by onvansertib (PCM-075) induced apoptosis. Based on preclinical evidence, onvansertib plus FOLFIRI and bevacizumab in second-line treatment of *KRAS* MT mCRC patients achieved 44% PR and 44% SD.<sup>110</sup> As previously discussed, the therapeutic application of PARPi in CRC was recently addressed.<sup>111</sup> In a preclinical study of *RAS* or *BRAF* MT MSS CRC cells, around 13% of CRC lines (13/99) were highly sensitive to olaparib and displayed cross-sensitivity to oxaliplatin, potentially underpinning a defect in the HR repair pathway.<sup>112</sup> Another potential tumor target is the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Due to its *in vivo* capacity to induce selective apoptosis in tumor cells, TRAIL receptor agonists like eftozanermin (ABV-621) were developed and tested in clinical trials. To activate the apoptotic cascade, these drugs need to bind to TRAIL membrane-death receptors (DR) TRAIL-R1

(DR4) and TRAIL-R2 (DR5), the expression of which was found higher in *KRAS* MT tumors.<sup>113</sup> A phase I trial is now recruiting *RAS* MT mCRC patients to receive eftozanermin monotherapy or combined with FOLFIRI and bevacizumab (NCT03082209). Activation of DR leading to apoptosis was also investigated in a randomized phase II trial using an agonistic monoclonal antibody (moAb) against DR5 (conatumumab) combined with FOLFIRI versus FOLFIRI in the second-line setting and the experimental arm showed a trend toward an improved PFS with a better ORR.<sup>114</sup>

### Anti-EGFR-based treatments for *RAS* MT mCRC

It is knowledge that *RAS* mutations cause primary and secondary resistance to anti-EGFR moABs in mCRC.<sup>4,14,55</sup> However, different *RAS* codon mutations have been hypothesized to underlie discrepancies in the anti-EGFR response. Conflicting results from retrospective analyses raised doubts whether patients with *KRAS*<sup>G13D</sup> MT mCRC could benefit from anti-EGFR drugs similarly to WT ones.<sup>115</sup> In a phase II trial, 0% ORR and 25% DCR were observed in 12 *KRAS*<sup>G13D</sup> MT mCRC patients treated with cetuximab monotherapy.<sup>116</sup> A cohort of the phase II ICECREAM trial will answer this question by assessing the efficacy of cetuximab alone or in combination with irinotecan in patients with *KRAS*<sup>G13D</sup> MT mCRC (ACTRN12612000901808).<sup>117</sup> Pan-HER inhibitors like afatinib and neratinib were also tested in clinical trials for *RAS* MT mCRC patients, with the aim of expanding RTK blockade and in virtue of preclinical models indicating tumor growth inhibition in *RAS* MT mCRC. However, DCR with afatinib was modest (12%).<sup>118</sup> Efficacy of neratinib in combination with divalproex sodium (histone deacetylase inhibitor) is under evaluation in a phase I/II clinical trial (NCT03919292). Finally, a novel humanized engineered anti-EGFR moAb (imgatuzumab) designed to enhance ADCC, showed poor ORR as monotherapy in EGFR-positive *KRAS* MT mCRC, and achieved no benefit in a phase II trial when added to FOLFIRI when compared with FOLFIRI plus cetuximab in a second-line setting, in both *RAS* WT and *RAS* MT mCRC patients.<sup>119</sup>

### DISCUSSION

*RAS* mutations in mCRC are moving from being only an unfavorable prognostic and predictive biomarker into an integral part of the evolving engine of dynamic preclinical and clinical research. Despite the failure of most of the previous wide-ranging approaches, novel *RAS*-directed drugs and therapeutic strategies have been developing, driven by the high unmet clinical need.<sup>11,16</sup> After being considered 'undruggable' for decades, the *RAS* oncogene was finally proven actionable in clinical trials with the advent of several novel inhibitors directed against the *KRAS*<sup>G12C</sup> subtype. These agents are certainly the most promising therapeutic discovery in this setting, even if differences in ORR emerged for mCRC as compared with non-small-cell lung cancer (NSCLC).<sup>27,28</sup> This discrepancy might be attributed to a likely higher molecular heterogeneity and thus lower *KRAS* oncogene addiction in mCRC rather than

NSCLC. Also, *KRAS*<sup>G12C</sup> CRC cells deeply rely on RTK activation for their proliferation, as proven by higher detection of basal phosphorylated RTKs (including EGFR) in CRC as compared with NSCLC, and thus stronger residual ERK signaling despite *KRAS* inhibition in preclinical models.<sup>36,120</sup> Nevertheless, given the good tolerability of *KRAS*<sup>G12C</sup> inhibitors, further implementation through combinational strategies with other anticancer drugs has been proposed.<sup>27</sup> Noteworthy, *KRAS*<sup>G12C</sup> inhibitors combined with anti-EGFR agents might revert resistance to *KRAS*<sup>G12C</sup> blockade in mCRC patients, as this approach was proven highly effective in CRC cells, patient-derived organoids and PDX, and is now in clinical trials.<sup>36</sup> These studies might underlie the prospect of a new combinational strategy in the future of *RAS* MT mCRC, where the use of EGFR drugs has been inconceivable up to now.<sup>4</sup> Similarly, combining the *KRAS*<sup>G12C</sup> blockade with inhibitors of MAPK effectors, or parallel pathways such as PI3K-AKT-mTOR, was proposed in order to achieve maximal signal suppression.<sup>35</sup> Promising proof of activity came from the combination of *KRAS*<sup>G12C</sup> inhibitors with ICI *in vivo*, as the anti-inflammatory TME associated with *RAS* mutations was significantly reverted by these new agents in preclinical models, to a higher extent than that previously achieved by MEKi.<sup>34</sup> These combinational strategies are now in clinical trials. Finally, an unexpected contribution might come from the supplementation of high-dose vitamin C to cytotoxic agents, which is expected to induce metabolic stress with limited additional toxicity in *RAS* MT mCRC cells.<sup>105,106</sup> In conclusion, further translation research studies and clinical investigations are warranted to improve and broaden the initial promising results of *RAS* targeting in mCRC, learning from the limitations of previous therapeutic approaches and taking into account the peculiar histology-dependent bio-molecular features of *RAS* MT mCRC cells.

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