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 KRASG12C 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 KRASG12C 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 KRASG12C. 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.1 Five-year relative overall survival (OS) is around 14% for those patients diagnosed with metastatic CRC (mCRC).1 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.5 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).2-6 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.7 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 BRAFV600E mutations, and checkpoint inhibitor immunotherapy in microsatellite unstable (MSI) mCRC, KRAS/NRAS mutations still represent the main clinical unmet need in this disease.7-10

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.11 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.4,11,12 G12 

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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, available at https://doi.org/10.1016/j.esmoop.2021.100156). Prevalence of KRAS mutations increases with age, except for MSI disease. 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.

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. 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. 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). 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-Ô (PDEÔ) chaperone protein then facilitates RAS localization to the plasma membrane. Oncogenic RAS mutations, altering its regulation and functioning, can lead to persistent MAPK pathway activation and unbalanced proliferative signaling.

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 (%) |
|-----------------------------------------|------------------------------|-------|-----------------|---------|---------|
| **Direct RAS targeting**                |                              |       |                 |         |         |
| FTIs                                    | Tipifarnib (R115777)         | III   | 235             | 1 (0.4) | 58 (24.7) |
|                                        | Lonafarnib (SCH 66336)       | II    | 21              | 0 (0.0) | 3 (14.3)  |
|                                        | BMS-214662                   | I     | 22              | 0 (0.0) | 0 (0.0)  |
| Statins                                 | Simvastatin + irinotecan + cetuximab | II  | 52              | 1 (1.9) | 34 (65.4) |
| KRASG12C 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)  |
| **Targeting the MAPK pathway**          |                              |       |                 |         |         |
| MEKi                                    | Trametinib                   | I     | 13              | 0 (0.0) | 4 (30.8)  |
|                                        | Cobimetinib                  | I     | 28              | 0 (0.0) | NA      |
|                                        | ROS126766<sup>a</sup>        | I     | 2               | 0 (0.0) | NA      |
| mTORi                                   | Palbociclib                  | II    | 15              | 0 (0.0) | 5 (33.3)  |
| MEKi + anti-HER2                        | Temsirolimus                 | II    | 64              | 0 (0.0) | 24 (37.5) |
| MEKi + anti-EGFR                        | Trametinib + lapatinib       | I     | 12              | 0 (0.0) | 10 (83.3) |
|                                        | Selumetinib + cetuximab      | I     | 14              | 0 (0.0) | 5 (35.7)  |
| MEKi + PI3K                             | Rigosertib + copanisib       | Ib    | 12              | 0 (0.0) | 5 (41.7)  |
|                                        | Binimetinib + alpelisib      | Ib    | NA              | 0 (0.0) | NA      |
|                                        | Cobimetinib + pictilisib     | Ib    | 47<sup>b</sup> | 0 (0.0) | NA      |
|                                        | Trametinib + buparlisib      | Ib    | 33              | 0 (0.0) | NA      |
| PD-0325901 + gedatolisib<sup>c</sup>   | Trametinib + omalizumib      | I     | 21<sup>c</sup>  | 0 (0.0) | NA      |
|                                        | Pimasertib + voxtalisib      | I     | 11              | 0 (0.0) | NA      |
| MEKi + AKT                              | Selumetinib + MK-2206        | I     | 11              | 0 (0.0) | 1 (9.09) |
|                                        | Trametinib + afuresertib (GSK2110183) | I  | 3<sup>c</sup>  | 0 (0.0) | NA      |
| MEKi + ChT                              | Selumetinib + irinotecan     | I/I   | 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-XXI                          | Trametinib + navitoclax      | I/I   | 9               | 0 (0.0) | 2 (22.2) |
| MEKi + cyclosporin A                    | Selumetinib + cyclosporin A  | I/b   | 14              | 1 (7.1) | 11 (78.6) |
| **Harnessing RAS through immunomodulator combinations** |                              |       |                 |         |         |
| MEKi + anti-PD-1L                       | Cobimetinib + atezolizumab   | III   | 183             | 1 (1.0) | 48<sup>c</sup> (26.2) |
| Anti-PD-L1 + anti-CTLA-4 + ChT          | Durvalumab + tremelimumab + FOLFOX | I/I   | 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       | I/b   | 40              | 0 (0.0) | 18 (45.0) |
| RAS MT vaccine                          | Immutazumab + FOLFOX         | II    | 38              | 0 (0.0) | NA      |
| **RAS targeting through metabolic pathways** |                              |       |                 |         |         |
| High-dose AA + ChT                      | AA + FOLFIRI FOLFIRI         | I     | 10              | 3 (30.0)| 8 (80.0) |
| **Other miscellaneous approaches**      |                              |       |                 |         |         |
| Anti-PIK1 + ChT + anti-VEGF             | Ovnansertib + FOLFIRI + bevacizumab | I/I   | 9               | 4 (44.4)| 8 (88.8) |
| Anti-DR5 + ChT                          | Conatumumab + FOLFIRI        | II    | 51              | 2 (13.7)| 35 (68.6) |
| Anti-EGFR                               | Cetuximab                    | I     | 12<sup>c</sup>  | 0 (0.0) | 3 (25.0) |
|                                        | Imgatuzumab                  | I/I   | 25              | 0 (0.0) | 6 (24.0) |
| Pan-HER inhibitors                      | Afatinib                     | II    | 41              | 0 (0.0) | 5 (12.2) |
| Anti-EGFR + ChT                         | Imgatuzumab + FOLFIRI        | NA    | NA              | NA      | NA      |

AA, ascorbic acid; BCL-XXI, 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; FII, 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; mTORi, 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> ROS126766 is a RAS/MK/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. 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
| Strategy | NCT/trial name | Drugs | Phase |
|----------|----------------|-------|-------|
| Direct RAS targeting | | | |
| KRAS<sup>G12C</sup> inhibitors | NCT03600883/CodeBreak100 | Sotorasib (AMG 510) | II |
| | NCT03785249/KRYSTAL-1 | Adagrasib (MRTX849) | I/II |
| | NCT04069301 | JNJ-74699446 | I |
| | NCT04165031 | LY3499446 | I/II |
| KRAS<sup>G12C</sup> inhibitor-based combinations | NCT04185883/CodeBreak101 | AMG 510 with anti-PD-1, MEKi, SHP2 allosteric inhibitor, pan-PI3K inhibitor, anti-PD-L1, anti-EGFR, ChT, mTORi, or CDK4/6i | I |
| | NCT03785249/KRYSTAL-1 | MRTX849 with pembrolizumab, cetuximab or afatinib | I/II |
| | NCT03518554 | JAB-3068 | I |
| | NCT03565003 | JAB-3068 | I/II |
| KRAS-derived mRNA binder | NCT03101839 | AZD4785 | I |
| SOS1 inhibitor | NCT04114458 | BI 1701963/C6 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 |
| Targeting the MAPK pathway | | | |
| 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 |
| Pan-ErbB inhibitor-based combinations | NCT02313012 | CC-90003 | I |
| | NCT03065387 | Neratinib with everolimus (mTORi), palbociclib (CDK4/6i), or trametinib (MEKi) | I |
| cMET inhibitor + MEKi | NCT02510001 | Crizotinib with PD-025901 or binimetinib | I |
| EGFR inhibitor + MEKi | NCT03080781 | Panitumumab ± trametinib | II |
| | NCT01927341 | Panitumumab ± binimetinib | I/II |
| MEKi + CDK4/6i | NCT02065063 | Trametinib + palbociclib | I |
| | NCT03981614 | Binimetinib + palbociclib | II |
| FAK inhibitor + RAF/MEKi | NCT03785240/FRAME trial | V5-6063 + ROS126766 | I |
| PI3K + MEKi | NCT0137765 | BEZ235 ± binimetinib | I |
| | NCT01859351 | WX-037 ± WX-534 | I |
| MEKi + ChT | NCT02613650 | Binimetinib + mFOLFIRI | I |
| MEKi + MDM2 inhibitor | NCT03714958 | Trametinib + HDI201 | I |
| Harnessing RAS through immunotherapy combinations | | | |
| Anti PD-1 + MEKi ± anti-CTLA-4 | NCT03271047 | Nivolumab + binimetinib ± ipilimumab | I/II |
| Anti-PD-L1 + MEKi + PARP | 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 | II |
| 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 |
| RAS targeting through metabolic pathways | | | |
| Glutaminase inhibitor + CDK4/6i | NCT03658545 | Telaglenastat (CB-839) + palbociclib | I/II |
| Fatty acid synthase inhibitor | NCT02980029 | TVB-2640 | I |
| Metabolic damaging | NCT03146962 | High-dose i.v. vitamin C | II |
| | NCT02969681 | High-dose i.v. vitamin C + FOLFIRI + bevacizumab | III |
| Other miscellaneous approaches | | | |
| Selective WEE1 inhibitor + ChT | NCT02966059 | Adavosertib (AZD1775) + irinotecan | I |
| TRAIL receptor agonist | NCT03082209 | Etofizarin (ABBV-621) ± FOLFIRI and bevacizumab | I |
| Anti-EGFR + ChT | ACTRN12612000901808/ICECREAM | Cetuximab ± irinotecan | I |
| 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; MEKi, murine T-cell receptor; mTOR, mammalian target of rapamycin; NCT, unique identification code given to each clinical study upon registration at 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 BioRendered.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, O6-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. 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. Dual targeting of FTase and GGTase has been proposed as a circumventing strategy, as well as targeting downstream RAS processing proteins like PDEδ with deltarasin or novel PDEδ inhibitor deltzainone 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. 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. 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 β-hydroxy-β-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. 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). 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.

RAS direct inhibitors: new perspectives limited to the KRASG12C 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, KRASG12C has been recently demonstrated druggable. KRASG12C is identified in approximately 4% of CRCs. Its oncogenic activity is linked to impaired GAP-mediated hydrolysis, resulting in marked predominance of the GDP-bound active state. However, KRASG12C preserves peculiar near-wild-type (WT) intrinsic GTPase activity and thus slight GTP-to-GDP cycling ability, differently from other KRAS codon mutations. This diverseness enables direct inhibitors to halt the KRASG12C protein in its inactive GDP-bound conformation, by means of covalent binding to a reactive thiol group in the cysteine 12 residue. The discovery of this allosteric nucleotide-binding pocket (called ‘switch-II pocket’) was pioneered by Shokat and colleagues, and led to the development of chemical compounds irreversibly targeting KRASG12C, 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 KRASG12C 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. The phase II monotherapy trial is ongoing (CodeBreaK100/NCT03600883). Adagrasib (MRTX849 by Mirati Therapeutics, Inc., San Diego, CA) is another KRASG12C inhibitor which is currently being tested in a phase Ib/II clinical trial (KRYS1TAL-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 ≥4 months for 55% of patients (10/18). Further phase I/II trials exploiting different KRASG12C inhibitors such as NJI-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), cytokotoxic agents and anti-EGFR drugs, significantly improving inhibition of tumor growth in vivo. Several phase I/II trials combining an anti-KRASG12C together with ICIs (extensive topic addressed in the following chapter Harnessing RAS through immuno-therapy combinations), MEKi, anti-AKT drugs, Src homology region 2 domain-containing phosphatase-2 (SHIP2) allostERIC inhibitors (TNO155), pan-HER RTK inhibitors, EGFR inhibitors, and cytokotoxic agents are ongoing (CodeBreaK101/ NCT04185883, KRYSTAL-1/NCT03785249, KRYS1TAL 2/ NCT04330664). Besides, a phase III study is now randomizing patients to receive adagrasib and cetuximab versus chemotherapy as the second-line treatment of KRASG12C MT mCRC (KRYS1TAL-10/NCT04793958).

Beyond KRASG12C: future options for RAS inhibition

KRASG12C mutations translate into biologically distinct proteins from KRASG12C, lacking cysteine substrate for covalent inhibition and expressing a lower intrinsic GTP hydrolysis rate. Thus, novel compounds are being studied in preclinical models such as the RAS(ON) platform by Revolution Medicines (Redwood City, CA). 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. Besides, the RAS protein-interaction disrupter rigosertib did not show any benefit in a clinical trial. Novel compounds demonstrated in vitro and in vivo activity against KRASG12D, KRASG12H, and KRASG12V codon
mutations in CRC and other histologies.\textsuperscript{39-43} As the \textit{KRAS}\textsubscript{G12D} mutation is the most common in mCRC, the development of a specific effective inhibitor would be relevant in clinical practice.\textsuperscript{11} A genetic depletion strategy by novel antisense oligonucleotide AZD4785 (binding \textit{RAS}-derived mRNA) is also under clinical investigation (NCT03101839).\textsuperscript{44} 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).\textsuperscript{35} 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.\textsuperscript{41}

**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.\textsuperscript{46} 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 \textit{BRAF}\textsubscript{V600E} inhibitors, such as vemurafenib, dabrafenib, and encorafenib, are only effective with RAF monomers like \textit{BRAF}\textsubscript{V600}, and not BRAF and CRAF dimers, and can lead to paradoxical activation of the EGF/RTK pathway through ERK-mediated regulatory feedback.\textsuperscript{47} Conversely, MEKi such as trametinib, binimetinib, and cobimetinib, prevent MEK phosphorylating ERK1/2, thus avoiding its dimerization and nuclear translocation.\textsuperscript{48} However, trametinib, cobimetinib, and also ROS5126766 (a potent RAF/MEKi; NCT02407509 still recruiting) alone were not proven active in this subset of patients.\textsuperscript{49-51} 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.\textsuperscript{52} Likewise, preliminary results of ERK inhibitor monotherapy with LY3214996 or CC-90006 did not show marked activity (NCT02857270, NCT02313012).\textsuperscript{53,54} 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.\textsuperscript{14,55} 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.\textsuperscript{56-59} 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.\textsuperscript{60} Palbociclib alone showed limited activity in a phase II trial (0% ORR, 33% DCR).\textsuperscript{61} 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 I/II 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.\textsuperscript{62,63} Finally, a phase II trial will compare binimetinib plus palbociclib versus triluridine/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 ROS5126766 (dual RAF/MEKi) is currently under investigation (NCT03875820).\textsuperscript{54}

**MEK and mTOR pathway inhibition**

PI3KCA mutations or up-regulation of the PI3K-AKT-mTOR signaling pathway through \textit{ERBB3} gene amplification can preclude responsiveness to MEKi. Precisely, PI3KCA mutations can restore G1-S cell cycle progression making cancer cells independent from MAPK signaling.\textsuperscript{65} 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.\textsuperscript{66} 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 (gedotilisib, voxaltib, or omiplalisib), and AKT inhibitors (MK-2206, ipatasertib, or afuresertib).\textsuperscript{67-78} 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.\textsuperscript{79} In a phase I/II study with selumetinib and irinotecan as second-line therapy, 9.7% ORR and 61.3%
DCR were achieved. Likewise, temsirolimus achieved 63% SD when associated with irinotecan. 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. 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 p14ARF. Disruption of the interaction between p53 and MDM2, with subsequent reactivation of p53, represents a potential strategy in TP53 WT cancer cells. 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.

**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. 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. 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).

**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. Indeed, KRAS mutations might facilitate cancer immunoevasive mechanisms. In preclinical models, KRAS mutations modulate tumor microenvironment (TME), inducing immunosuppressive chemokines like interleukin (IL) 6 and IL-10, transforming growth factor-β, 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. 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.

**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-γ (IFN-γ) 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 KRASG12C MT CRC cells. Similar results were obtained when combining adagrasib (MRTX849) with anti-PD-1 agents. 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 molecularly unselected population of mCRC. 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. In addition, MEKi decreased the expression of multiple homologous recombination (HR) components, thus sensitizing cancer cells to PARPi. 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. The phase I/II MEDETREME trial (NCT03202758) is evaluating tremelimumab (anti-CTLA-4) and durvalumab (anti-PD-L1) on top of FOLFIRI 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. 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-γ up-regulation and Treg inhibition. A lenalidomide and cetuximab combination failed to achieve meaningful activity in a clinical trial of KRAS MT mCRC patients.93 Similarly, Imprime PGG (a novel innate immune cell modulator) was adopted in combination with cetuximab with poor results in RAS MT CRC.90 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.97 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.98 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).99 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 reinjected in the same patient to boost the immune response. A phase I/II trial is evaluating the administration of lymphocytes loaded with anti-KRAS G12V or anti-KRAS G12V mTCR in patients with KRAS G12D/G12V 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 O6-methylguanine-DNA methyltransferase methylation (dMGMT), thus implying an increased vulnerability to temozolomide and dacarbazine.100 Besides, in proficient mismatch repair (pMMR)/MSS CRC cells, temozolomide increased tumor mutational burden (TMB) and infiltrating lymphocytes with IFN-γ release.101 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.102

**RAS TARGETING THROUGH METABOLIC PATHWAYS**

KRAS mutations can induce metabolic reprogramming through enhanced glucose uptake and increased expression of glutamine metabolic proteins.103 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).104 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).105 Besides, MAPK pathway is selectively inhibited by AA in KRAS MT CRC cells.106 Phase I clinical trials found that high dose (1.5 g/kg or 90 g/m²) intravenous AA can be safely given with chemotherapy regimens FOLFOX and FOLFIRI.107,108 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 FOLFIRI plus bevaciuzamab 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.109 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.110 As previously discussed, the therapeutic application of PARPi in CRC was recently addressed.111 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.112 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 eftozanerin (ABBV-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. A phase I trial is now recruiting RAS MT mCRC patients to receive eztozanermin 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 (contatumumab) 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.

**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. However, different RAS codon mutations have been hypothesized to underlie discrepancies in the anti-EGFR response. Confl icting results from retrospective analyses raised doubts whether patients with KRASG13D MT mCRC could benefit from anti-EGFR drugs similarly to WT ones. In a phase II trial, 0% ORR and 25% DCR were observed in 12 KRASG13D MT mCRC patients treated with cetuximab monotherapy. 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 KRASG13D MT mCRC (ACTRN12612000901808). 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%). Efficacy of neratinib in combination with divalprox 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 benefi t 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.

**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. After being considered ‘undruggable’ for decades, the RAS oncogene was fi nally proven actionable in clinical trials with the advent of several novel inhibitors directed against the KRASG12C 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). This discrepancy might be attributed to a likely higher molecular heterogeneity and thus lower KRAS oncogene addiction in mCRC rather than NSCLC. Also, KRASG12C 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. Nevertheless, given the good tolerability of KRASG12C inhibitors, further implementation through combinational strategies with other anticancer drugs has been proposed. Noteworthy, KRASG12C inhibitors combined with anti-EGFR agents might revert resistance to KRASG12C 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. These studies might underline 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. Similarly, combining the KRASG12C blockade with inhibitors of MAPK effectors, or parallel pathways such as PI3K-AKT-mTOR, was proposed in order to achieve maximal signal suppression. Promising proof of activity came from the combination of KRASG12C inhibitors with ICI in vivo, as the anti-infl ammatory 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. 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. 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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