Novel modalities in the treatment of patients with KRAS-mutated colorectal cancer

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Mutations in the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene are a well-described mechanism of resistance to monoclonal antibodies that target the epidermal growth factor receptor in patients with metastatic and nonoperable colorectal cancer. Treatment options in this population are limited to conventional chemotherapy regimens and antiangiogenesis compounds. Numerous strategies have been proposed in preclinical models as being effective in the presence of KRAS mutations. As basic and translational research further unravels the complex interactions and regulation points in the pathways downstream of epidermal growth factor receptor, more drugs become available for clinical testing. Indeed, there are many ongoing clinical trials that focus on the safety and efficacy of novel compounds in patients with KRAS-mutated colorectal cancer. This is a review of the literature with regard to the rationale of various approaches on this topic and also a summary of the current active clinical trials limited to patients with KRAS-mutated colorectal cancer. *Anti-Cancer Drugs* 22:384–391 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Colorectal cancer is the third most frequent and the third most lethal cancer in the United States [1]. Current treatment options for patients with metastatic disease and nonresectable metastases include chemotherapy with fluorouracil, irinotecan, and oxaliplatin in various combinations with bevacizumab, an antiangiogenesis compound [2]. In addition, because of the common expression and pivotal role of the epidermal growth factor receptor (EGFR) in colorectal cancer, monoclonal antibodies that target the receptor have been designed and tested in clinical trials.

EGFR is a transmembrane receptor of the human epidermal growth factor receptor (HER) family. When activated from extracellular signals, a cascade of phosphorylations in the tyrosine kinase domain of the molecule recruit growth-bound receptor protein-2 and son of sevenless, which then cause guanosine triphosphate (GTP) to bind to rat sarcoma (RAS) protein. There are three human *RAS* genes: *HRAS*, *NRAS*, and Kirsten rat sarcoma viral oncogene homolog (*KRAS*). KRAS protein transmits the signal to a series of serine–threonine kinases, the mitogen-activated protein kinases (MAPKs) [3]. Rapidly accelerated fibrosarcoma (RAF), the first in the MAPK cascade, activates MAPK extracellular receptor kinase (MEK) and MEK activates the extracellular receptor kinase (ERK) [3]. In contrast, EGFR can activate phosphoinositide-3 kinase (PI3K) through insulin receptor substrate-1. PI3K then activates v-akt murine thymoma viral oncogene homolog (AKT), which phosphorylates tuberous sclerosis complex II (TSCII) and inhibits TSCII-mediated hydrolysis of GTP bound to Ras homolog enriched in brain. When bound to GTP, Ras homolog enriched in brain triggers the mammalian target of rapamycin (mTOR) to activate the regulators of translation 4EBP1 and pS6K [4]. There is considerable interaction between RAS and PI3K pathways. Both ERK and PI3K upregulate cyclin D1 levels and promote cell proliferation [5] and cell survival. The presence of a PI3K-mediated activation of MAPK [6] and a RAS-mediated activation of PI3K [7–9] underscores the fact that EGFR downstream pathways form a complicated network. In addition to AKT, ERK can phosphorylate TSCII as well [10–12] and in contrast AKT can interact directly with RAF and inhibit RAF/MEK signaling [13]. In addition, there are well-characterized negative feedback loops between ERK and KRAS and RAF [14–16] and between pS6K and PI3K [6]. Furthermore, MAPK phosphatases/dual-specificity phosphatases can negatively regulate MAPKs [17]. Figure 1 shows the KRAS and PI3K pathways and what is known so far about pathway interaction and existing feedback loops.

Cetuximab, a humanized immunoglobulin (Ig) G1 anti-EGFR antibody, and panitumumab, a fully human IgG2 anti-EGFR antibody, have both proven to be effective in the clinical setting [18,19]. However, KRAS mutations, present in approximately 40% of colorectal cancer [20],

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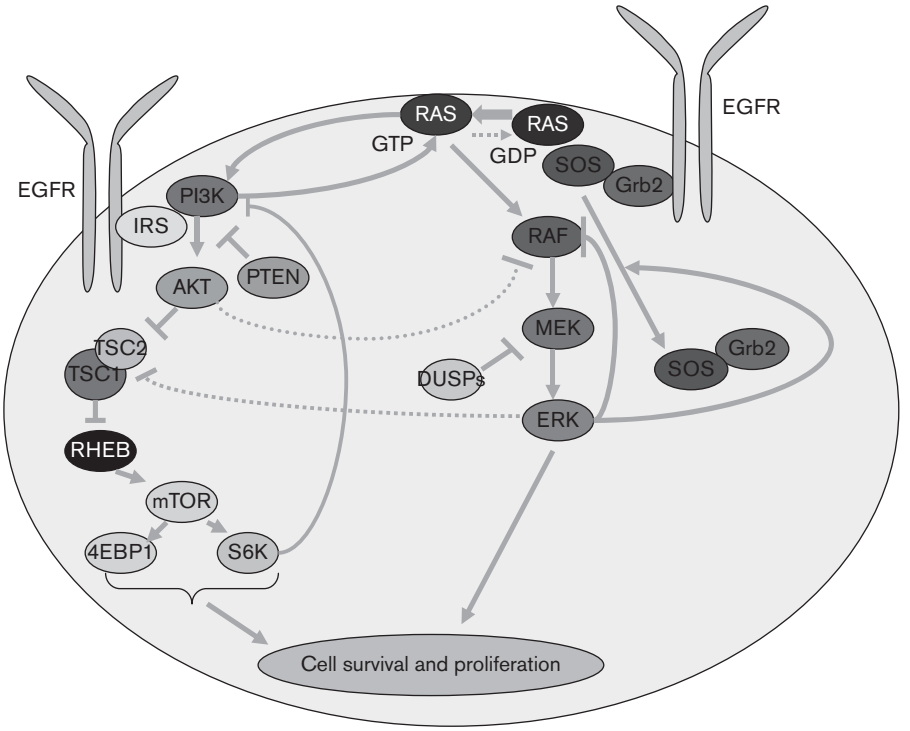
Fig. 1



Rat sarcoma (RAS) and PI3K pathways and their complex interactions and regulation feedback loops. DUSPs, dual-specificity phosphatases; EGFR, epidermal growth factor receptor; ERK, extracellular receptor kinase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; IRS, insulin receptor substrate; MEK, mitogen-activated protein kinase extracellular receptor kinase; mTOR, mammalian target of rapamycin; SOS, son of sevenless; TSC, tuberous sclerosis complex.

are causing tumors to become resistant to EGFR inhibition; KRAS is a downstream protein in the EGFR pathway. When the *KRAS* gene is mutated most commonly in codon 12 or 13 [20], the pathway is activated and is independent of EGFR. A number of clinical trials have shown that both cetuximab and panitumumab can offer clinical benefit only in patients with wild-type tumors, limiting the indications of these two drugs in colorectal cancer [21,22]. Furthermore, there is evidence to support that colorectal cancer that harbors v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) mutations is resistant to EGFR inhibition as well [23]. This notion is reasonable as BRAF protein is downstream of KRAS and EGFR; however, it is difficult to prove because the BRAF mutation rate in colorectal cancer is low. EGFR inhibition with small tyrosine kinase inhibitors is also ineffective in non-small cell lung cancer that harbors activating mutations in KRAS [24].

As almost half of the patients with colorectal cancer harbor KRAS or BRAF mutations, it is important to design effective drugs and treatment strategies that provide clinical benefit in the presence of these genetic alterations. This is a review of preclinical and clinical data on recent therapeutic advances in this subset of patients with colorectal cancer.

Inhibition of the rat sarcoma/RAF/mitogen-activated protein kinase/MAPK extracellular receptor kinase/extracellular receptor kinase pathway

KRAS-mutated colorectal cancer does not respond to upstream inhibition as the pathway is constitutively active and independent of EGFR. A reasonable approach would be to target downstream molecules such as RAF or MEK. Direct blockade of KRAS is difficult; inhibition requires design of compounds that disrupt protein–protein interaction, which proves difficult to achieve in the absence of well-defined pockets and because of the large, flat interface area [25]. However, strategies of KRAS inhibition have been developed and tested in preclinical in-vitro models. An interesting approach among them is to inhibit the exchange of guanosine diphosphate with GTP by guanine exchange factor inhibitors [26] as KRAS bound to guanosine diphosphate is inactive. In addition, farnesyl transferase inhibitors were suggested as possible KRAS inhibitors [27] as KRAS is functional after posttranslational isoprenylation by farnesyl transferases or geranylgeranyl transferases [28]. Nevertheless, randomized, placebo-controlled clinical trials have failed to show any benefit from farnesyl transferase inhibitor monotherapy in colorectal cancers [29,30]. In the same context, statins can reduce

isoprenylation by inhibiting hydroxyl-methylglutaryl-coenzyme A [31]. Statins in combination with FOLFIRI showed promising efficacy in a phase II study in patients with metastatic colorectal cancer [32]. Additional phase II trials testing simvastatin with panitumumab or cetuximab in patients with mutated KRAS are underway (Table 1).

Sorafenib is an inhibitor of the vascular endothelial growth factor receptor, the platelet-derived growth factor receptor and also of RAF [33], and has been approved for the treatment of renal cell [34] and hepatocellular carcinomas [35]. Although RAF does not seem to be the main target of sorafenib, the drug has caused restriction of growth and cell-cycle arrest in colorectal cancer cell lines that harbor a KRAS mutation [33]. Sorafenib has been tested in three phase I studies in patients with colorectal cancer in combination with irinotecan [36], oxaliplatin [37], or tanespimycin [38]. Interestingly, tanespimycin, which is a heat shock protein inhibitor, affects the RAF pathway and has the potential of synergistic action with other RAF inhibitors [39]. All three studies showed that sorafenib is safe in patients with colorectal cancer. More phase I and II studies are under way to test the combination of FOLFOX or FOLFIRI and bevacizumab and cetuximab with sorafenib. In addition, there is an ongoing study to test sorafenib in combination with irinotecan in patients with KRAS mutations (Table 1). PLX-4032 is a more potent RAF inhibitor than sorafenib and is active in tumors that harbor activating BRAF mutations [40,41]. A phase I trial on patients with melanoma has already shown that this compound is safe and promising when mutated RAF is present [42]. In addition, a phase I trial in colorectal carcinoma and melanoma with activated BRAF is under way.

MEK1/2 transmits mitogenic signals downstream of RAS/RAF (Fig. 1) and is therefore an attractive target in tumors in which the RAS axis is activated. Several MEK1/2 inhibitors have been designed and shown to be active in various preclinical settings in KRAS-mutated or BRAF-mutated cell lines and xenografts [43–45]. MEK1/2 inhibition is sufficient to disrupt anchorage-independent growth in colorectal cancer cell lines that harbor either KRAS or BRAF mutations [46]. However, this effect is not linked to reduction of ERK1/2 activity, indicating that other pathways of ERK activation may be present [46–48].

Table 1 Ongoing clinical trials in patients with colorectal cancer with KRAS mutations (source: *www.clinicaltrials.gov*)

Identifier	Regimen	Phase	Setting	Remarks
NCT01085331	MSC1936369B and FOLFIRI versus FOLFIRI and placebo	I/II	2nd line	MSC1936369B is a MEK inhibitor Randomized, placebo-controlled
	(single arm) pemetrexed and gemcitabine	II	Metastatic	Refractory to fluoropyrimidines, oxaliplatin and irinotecan
NCT00827684	Temsirolimus versus temsirolimus and irinotecan	II	Metastatic	Refractory to fluoropyrimidines, oxaliplatin and irinotecan Randomized, no placebo
NCT00813605	FOLFIRI and either AMG479 or AMG655, or placebo	II	2nd line	AMG479 and AMG655 are IGF1R inhibitors Refractory to fluoropyrimidines and oxaliplatin, 1 : 1 : 1 randomization, placebo controlled
NCT01032291	Lenalidomide and cetuximab versus lenalidomide alone	II	Metastatic	Randomized, no placebo Refractory to fluoropyrimidines, oxaliplatin, irinotecan and bevacizumab
NCT00989469	(single arm) sorafenib and irinotecan	I/II	>1st line	Refractory to irinotecan
NCT01116271	Selumetinib (AZD6244) and irinotecan	II	2nd line	Refractory to oxaliplatin and bevacizumab KRAS or BRAF mutation positive
NCT01086267	BMS-908662 versus BMS-908662 and cetuximab	I/II	Metastatic	BMS-908662 is a RAF inhibitor Randomized, no placebo Refractory or intolerant to existing therapies
NCT00856375	NKTR-102 versus irinotecan	II/III	Metastatic	NKTR-102 is a topoisomerase I inhibitor-polymer conjugate Randomized, no placebo arm Refractory to at least one prior regimen for metastatic disease
NCT01110785	(single arm) panitumumab and simvastatin	II	Metastatic	Refractory to fluoropyrimidines, oxaliplatin and irinotecan
NCT01190462	(single arm) cetuximab and simvastatin	II	Metastatic	Refractory to fluoropyrimidines, oxaliplatin and irinotecan
NCT00959127	(single arm) ARRY-438162 (MEK162)	I	Metastatic	ARRY-438162 is a MEK inhibitor Refractory to fluoropyrimidines, oxaliplatin, irinotecan and irinotecan if available Patients with KRAS or BRAF mutated colorectal or biliary cancer are recruited
NCT00869570	Radiotherapy and capecitabine and sorafenib	I/II	Neoadjuvant	Locally advanced rectal cancer
NCT01149434	Pharmacokinetic study arm: JI-101 and everolimus Pharmacodynamic arm: JI-101	I/II	Advanced/ metastatic	JI-101 is an inhibitor of VEGFR2, PDGFRβ, EphB4 Patients with ovarian, neuroendocrine, or KRAS mutant colon cancer, refractory to standard treatment Drug interaction (pharmacokinetic arm) and PFS (pharmacodynamic arm) are the study objectives
NCT00721266	RO5083945	II	Advanced/ metastatic	RO5083945 is an anti-EGFR antibody EGFR-positive tumors only
NCT00912327	Imprime PGG and cetuximab	II	Metastatic	Imprime PGG is an immunomodulator that activates body's neutrophils Refractory to pxaliplatin and irinotecan containing regimens

EGFR, epidermal growth factor receptor; EphB4, Ephrin B4; PDGFRβ, platelet-derived growth factor receptor β; PGG, poly-[1-6]-D-glucopyranosyl-[1-3]-D-glucopyranose; VEGFR2, vascular endothelial growth factor receptor 2.

Activation of ERK1/2 independently of KRAS/BRAF status has been shown in endometrial cancer cell lines as well [49]. A mode for secondary resistance after initial growth arrest is based on p38 activation [50]. p38 is part of an MAPK cascade interacting with the RAS/RAF/MEK/ERK pathway. Inhibition of p38 along with MEK1/2 is able to inhibit growth in KRAS-mutated colorectal cancer cell lines that had acquired secondary resistance to MEK inhibition alone. MEK1/2 inhibitors were tested in two phase I studies in a variety of tumors, including colorectal cancer, and proven to be safe [51,52]. A phase II study was conducted in an unselected population of patients with advanced non-small cell lung, breast, colon, or pancreatic cancers and showed no efficacy of MEK inhibition with CI-1040 [53]. Several other phase I and II trials are under way. In some of them, MEK inhibition is combined with chemotherapy and the study populations are limited to colorectal cancers with KRAS or BRAF mutations (Table 1).

Inhibition of the PI3K pathway

As KRAS along with EGFR can activate the PI3K pathway, inhibition of PI3K and its downstream molecules might be meaningful in KRAS-mutated colorectal tumors. PI3K activates AKT, which activates mTOR. MTOR consists of two distinct complexes, mTORC1 and mTORC2. Although it is well described that mTORC1 promotes cell growth through phosphorylation of S6K and 4EBP1, little is known about mTORC2. PI3K and mTOR inhibitors are efficient against cell lines with high-frequency microsatellite instability because of hypermethylation of MLH1 irrespectiveof its KRAS status [54]. Rapamycin, an mTORC1 inhibitor, as well as mTORC1 knockdown, is able to inhibit the growth of some, but not all, colorectal cancer cell lines that harbor KRAS mutations. In contrast, mTORC2 inhibition is able to inhibit the growth of rapamycin-resistant and KRAS-mutant cell lines and xenografts [55]. Taken together, mTOR or mTORC2, but not mTORC1 knockdown, results in growth inhibition *in vitro* and *in vivo* in the presence of KRAS or BRAF mutations as well [56]. A phase II study testing the combination of temsirolimus, an mTOR inhibitor, and irinotecan in patients with KRAS-mutant colorectal cancer is under way (Table 1). A phase I/II study of the combination of everolimus with a multikinase inhibitor, JI-101, is about to start recruiting patients with ovarian neuroendocrine, or KRAS-mutated colorectal cancer (Table 1).

Combined inhibition of KRAS and PI3K pathways

A possible mechanism of resistance to MEK inhibition is activation of the PI3K pathway. The PI3K pathway can be activated by mutations in PI3K, loss of phosphatase and tensin homolog (PTEN), or by loss of functional PTEN mutations. Either of these can coexist in patients with colorectal cancer or in colorectal cancer cell lines with KRAS mutations [57–59]. Wee *et al.* [59] reported that loss of PTEN causes complete resistance to MEK inhibition, whereas activating PI3K mutations cause partial resistance in KRAS-mutated colorectal cancer cell lines. The researchers conclude that a possible explanation for this effect might be that PTEN has targets other than PI3K or that loss of PTEN can activate the PI3K pathway more effectively than activating PI3K mutations. Another study showed that activation of the PI3K pathway is a cause of primary resistance of colorectal cancer cell lines with KRAS mutations to MEK1/2 inhibition [60]. Cell lines and xenografts that are positive for KRAS mutations are less sensitive to MEK1/2 inhibition than cell lines and xenografts that harbor BRAF mutations [61]. This reflects the ability of activated KRAS to trigger pathways other than the RAF/MEK pathway. Indeed, RAS can activate PI3K and further cross-talk between the PI3K and RAS pathways has been shown. Negative feedback loops are another reason that might make MEK inhibition ineffective as single agents. Recently, Cirit *et al.* [62] reported that the negative feedback loop between ERK and RAF accounts for most of the negative feedback regulation after using a quantitative assessment. Taken together, these data indicate that dual inhibition of the MEK and PI3K pathways is a reasonable approach in patients with colorectal cancer with KRAS mutations. There is one phase I/II study that is currently recruiting patients with KRAS-mutant or BRAF-mutant colorectal cancers, and tests the safety and efficacy of the combination of BMS-908662, a BRAF inhibitor with cetuximab (Table 1). In the phase II part of the study, patients will be assigned randomly to either single treatment with the BRAF inhibitor or to a combination of EGFR and BRAF inhibition.

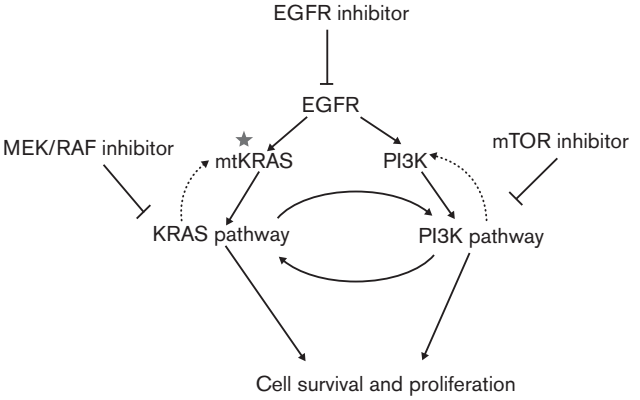
Figure 2 illustrates the combination treatment as a possible strategy for patients resistant to anti-EGFR antibodies because of activating mutations at the KRAS level.

Antibody-dependent cellular cytotoxicity following EGFR targeting

It has been described that, when cetuximab binds to EGFR, the fragment c γ portion of the antibody binds to the fragment c receptor (FcR) present on the surface of immune cells that is recruited and attacks tumor cells [63,64]. This mechanism is independent of EGFR downstream pathways and therefore the KRAS status should not matter. A similar mode of activity has been described for herceptin and rituximab [65,66]. However, as cetuximab does not have any clinical benefit over placebo in the KRAS-mutant colorectal cancer population, it seems that antibody-dependent cellular cytotoxicity (ADCC) is not clinically significant without further selection of patients.

Certain polymorphisms in the FcR were able to predict response of follicular lymphoma to rituximab [67]. FcR that did have the polymorphisms was able to bind to IgG1

Fig. 2



KRAS mutation (mtKRAS) is a well-described mode of resistance of colorectal cancer to anti-EGFR treatment: given the constant activation of mtKRAS (shown with a star), the interaction between KRAS and PI3K pathways, and the feedback loops (shown with dashed lines) between KRAS, PI3K, and their downstream molecules, a combination of drugs that act on the MAPK extracellular receptor kinase (MEK)/RAF, epidermal growth factor receptor (EGFR), or mammalian target of rapamycin (mTOR) level is a reasonable strategy to overcome resistance to anti-EGFR treatment alone.

in a tighter manner than the receptor without polymorphisms. Nevertheless, studies in colorectal cancer failed to show any clinical significance of these particular polymorphisms; the results were conflicting and nonreproducible [68–70].

Lenalidomide is a compound similar to thalidomide that has shown effectiveness in hematologic malignancies. Lenalidomide is able to enhance natural killer cell-mediated ADCC on cetuximab-coated cells *in vitro*, irrespective of their KRAS status. There is one ongoing phase II study in patients with colorectal cancer with mutated KRAS that tests the combination of cetuximab and lenalidomide versus lenalidomide alone (Table 1).

A different approach in the context of ADCC is suggested with bi-specific T-cell engagers (BiTE) technology. A BiTE antibody binds to EGFR at low concentrations and a second BiTE antibody linked to the first binds to CD3 on T lymphocytes. The complex of BiTE antibodies is therefore able to recruit Fcγ-lacking T cells, which is not possible with IgG1 antibodies such as cetuximab. Indeed, BiTE technology showed efficacy against KRAS-mutated and BRAF-mutated colorectal cancer cells *in vitro* and *in vivo* [71].

Conventional chemotherapy and bevacizumab

The impact of KRAS mutations on the clinical benefit from anti-EGFR antibodies is well described. As a combination of conventional chemotherapy and bevacizumab is the standard of care in the metastatic setting of colorectal cancer, the efficacy of irinotecan, oxaliplatin, or bevacizumab in the presence of KRAS mutations becomes an important question. Retrospective analyses of two trials have compared 5-fluorouracil (FU)/irinotecan or 5-FU/oxaliplatin with 5-FU alone and irinotecan, fluorouracil, and leucovorin (IFL) and bevacizumab with IFL alone in the subset of patients with KRAS or BRAF mutations, respectively. Both trials did not include any anti-EGFR treatment in their arms. The former study [72] (based on the FOCUS trial) showed that the benefit from the addition of irinotecan or oxaliplatin to 5-FU was comparable between the KRAS wild type and KRAS-mutated subpopulations. The 5-FU/oxaliplatin group achieved superior progression-free survival (PFS) over the 5-FU group, whereas for the 5-FU/irinotecan group there was a trend toward improved PFS over the 5-FU group in the KRAS-mutant or BRAF-mutant population. A trial to compare oxaliplatin-based chemotherapy with irinotecan-based chemotherapy in the KRAS-mutated or BRAF-mutated colorectal cancer population is lacking. The latter study [73] was a retrospective analysis of the AVF2107 study and compared IFL with or without bevacizumab in patients with KRAS mutations. It showed that patients with KRAS mutations who received bevacizumab achieved a better PFS compared with patients who received only IFL. There was no difference in overall survival with the addition of bevacizumab. It seems that the clinical benefit from bevacizumab is independent of KRAS status.

Other approaches

A combination of insulin growth factor (IGF) inhibition and EGFR inhibition has been suggested as a promising option against tumors that are resistant to EGFR inhibition alone. However, preclinical data about whether this strategy would be active in the case of KRAS mutations have been controversial. A study in basal-like breast cancer showed that IGF-1R inhibition was efficient in transgenic mouse models and xenografts with an activated KRAS pathway [74]. In addition, IGF-1R inhibitors are able to reduce the proliferation rate and increase the number of dead cells in KRAS-mutated colorectal cancer DLD-1 cells [75]. In contrast, a trend toward better efficiency of IGF-1R inhibitor OSI-906 in cell lines with wild-type KRAS rather than mutated KRAS was shown, indicating that mutated KRAS might be mediating resistance to inhibitors of various tyrosine kinases besides EGFR [76]. Consistent with this report, a randomized phase II clinical trial of the IGF-1R inhibitor IMC-A12 with or without cetuximab in patients with cetuximab-refractory or panitumumab-refractory metastatic colorectal cancer showed no antitumor activity of IMC-A12. Specifically, among patients with KRAS-mutated tumors, there were no documented responses, whereas among patients with wild-type KRAS there was one that responded to the combination treatment. PFS and overall survival were similar between the randomized groups [77].

A series of novel compounds has shown activity in the KRAS-mutated colorectal cancer cell line and xenograft models. Trifluorothymidine is a fluoropyrimidine that can inhibit thymidylate synthase and also cause DNA damage. The combination of trifluorothymidine with erlotinib has synergistic effects in inhibiting growth and causing cell death in colorectal cancer cell lines that harbor mutations in either BRAF or KRAS. However, synergistic action is maximized in cell lines that are wild-type BRAF and KRAS [78]. It was suggested that reduced thymidylate synthase levels because of EGFR inhibition and off-target effects of erlotinib are responsible for this synergistic effect. Insulin growth factor-binding protein 7 induces apoptosis and reduces growth rate in xenografts from colorectal cancer and melanoma cell lines that harbor KRAS or BRAF mutations [79]. This effect is probably because insulin growth factor-binding protein 7 mediates MEK inhibition. Numerous drugs are currently being tested in clinical trials in the population of patients with KRAS-mutated colorectal cancer (Table 1).

Conclusion

EGFR-targeted therapies are ineffective in patients with colorectal cancer with KRAS mutations. Despite the potential of cetuximab to affect tumor cells through ADCC and the numerous compounds that have shown some activity *in vitro*, using MEK inhibitors seems to be the most promising approach. In addition, accumulated data exist with regard to tumors being resistant to MEK inhibition through activation of the PI3K pathway. In fact, the KRAS and PI3K pathways are highly interacting and regulate each other at multiple levels. A combined inhibition of the two pathways might be necessary to overcome resistance to single-agent treatment. A large number of ongoing trials are now focusing on patients with KRAS-mutated colorectal cancer. Additional translational and biomarker studies that define the patient populations suitable for different drugs and treatment combinations are warranted.

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