Review Article

Impact of KRAS Mutations on Management of Colorectal Carcinoma

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The epidermal growth factor receptor (EGFR) pathway is a therapeutic target in the management of colorectal cancer (CRC). EGFR antagonists are active in this disease; however, only a subset of patients respond to such therapy. A Kirsten ras sarcoma viral oncogene (KRAS) wild-type (WT) status of the tumor is necessary, but possibly not sufficient, for a response to anti-EGFR monoclonal antibody therapy. Mechanisms of primary resistance to such therapy in patients harboring KRAS WT tumors are discussed. Strategies to overcome resistance to anti-EGFR monoclonal antibody therapy, including novel agents and combinations of novel therapies, are explored. Also, the use of anti-EGFR monoclonal antibodies in the adjuvant and neoadjuvant setting is reviewed.

1. Introduction

Tumor growth and progression depends in part on the activity of cell surface membrane receptors which control signal transduction pathways. These growth factor receptors can have aberrations in their expression and regulation and activation of growth factor pathways is common in many malignancies [1]. The EGFR, a transmembrane glycoprotein also called ERBB-1 or HER1, is a member of a family of receptor tyrosine kinases (TKs). The EGFR is involved in signaling pathways controlling cell growth, differentiation, and proliferation and is expressed in many different types of normal tissues as well as several tumor types, including CRC [2, 3]. Figure 1 illustrates the main EGFR signaling pathways described [4]. When a ligand binds to the EGFR, the receptor forms a dimer resulting in a signaling cascade within the cell via tyrosine kinase activity [5]. This signaling cascade occurs by the activation of receptor autophosphorylation which triggers a number of intracellular pathways regulating cell proliferation, prevention of apoptosis, and promotion of invasion, metastasis, and neovascularization [6]. The proto-oncogene c-erb-B encodes the EGFR, and activation of the proto-oncogene results in EGFR expression in many tumors [7, 8]. There was therefore interest in investigating this pathway as a potential anticancer therapy target.

Pharmacologically, there are two classes of EGFR antagonists currently in clinical use: anti-EGFR monoclonal antibodies directed against the extracellular domain of the receptor and oral small-molecule EGFR TK inhibitors which block the receptor TK activity competitively [10]. The anti-EGFR monoclonal antibodies, cetuximab and panitumumab, act by binding to the extracellular region of the EGFR and therefore block the ligand-binding region which prevents ligand-induced TK activation [11]. These monoclonal antibodies solely recognize the EGFR, making them very selective for their target [5]. The small-molecule EGFR TK inhibitors, erlotinib and gefitinib, inhibit the catalytic activity of the TK by competing with adenosine triphosphate (ATP) to bind to the intracellular domain [10]. These small-molecule inhibitors are not exclusive to the EGFR pathway and can block different receptor tyrosine kinases, such as the vascular endothelial growth factor (VEGF) receptor and other members of the EGFR family.
Anti-EGFR monoclonal antibodies have been evaluated in both untreated metastatic CRC and chemotherapy refractory disease. Figure 2 summarizes the current treatment paradigm for metastatic colorectal cancer including the appropriate incorporation of anti-EGFR monoclonal antibody therapy which improves survival for appropriately selected patients [9]. Table 1 summarizes selected clinical trials of anti-EGFR monoclonal antibodies in metastatic CRC. Response rates with single-agent anti-EGFR monoclonal antibodies range from 9–12%, with much higher response rates observed when cetuximab is used in combination with chemotherapy [12–22]. When administered to unselected metastatic CRC patients, only a minority responded to EGFR inhibitor therapy. Therefore, a method to identify and predict sensitivity to these drugs was needed.

2. Prediction of Response to Anti-EGFR Monoclonal Antibodies

The RAS family of proto-oncogenes include HRAS, KRAS, and NRAS [23]. KRAS (Kirsten ras sarcoma viral oncogene) is a guanosine triphosphate-(GTP-) binding protein downstream of the EGFR and is a central component of the mitogen-activated protein kinase (MAPK) pathway, a component of the EGFR signaling cascade [23]. Roughly 40% of colorectal cancers are characterized by a mutation in the KRAS gene [24]. About 90% of these mutations occur in codons 12 and 13 in exon 2 of the KRAS gene, with the remaining mutations occurring in codons 61 and 146 (roughly 5% each) [25, 26]. Such KRAS mutations lead to EGFR-independent constitutive activation of the signaling pathway and predict for a lack of response and benefit from anti-EGFR monoclonal antibodies cetuximab and panitumumab [27–35]. De Roock et al. showed that codon 61 mutations predicted for lack of response to cetuximab similar to codon 12 and 13 mutations; however, codon 146 mutations did not affect cetuximab efficacy [26]. Failure to test for codon 61 mutations may miss a significant mutation which would confer resistance to anti-EGFR monoclonal antibody therapy. There is a very high concordance of KRAS mutational status between the primary tumor and metastasis, ranging from 92–100% [36–38]. However, KRAS mutation status heterogeneity between primary tumors, lymph nodes and distant metastases in 5–10% of patients has been reported, with mixed responses to anti-EGFR monoclonal antibody therapy in those with metastatic CRC [37, 39, 40]. Because of this, some clinicians have called for a reassessment of KRAS mutation status on metastatic foci in situations where only the primary tumor was assessed for KRAS status [41].

Table 2 summarizes clinical trials of anti-EGFR monoclonal antibodies which included analysis of treatment effect and KRAS mutation status. Amado et al. assessed the predictive role of KRAS mutational status in a randomized phase III trial comparing panitumumab monotherapy with best supportive care (BSC) in patients with chemotherapy refractory metastatic CRC [24]. This trial showed that the clinical benefit associated with panitumumab was restricted to the KRAS WT population. KRAS mutations predicted for lack of clinical benefit to panitumumab [24]. Similarly, Karapetis et al. showed that treatment with cetuximab significantly improved OS and PFS in patients with KRAS WT tumors; however, in this chemotherapy-resistant patient population, those with mutated KRAS tumors did not benefit [38]. Use of cetuximab as first-line treatment for metastatic disease was investigated by Van Cutsem et al.; patients were randomly assigned to receive FOLFIRI with or without cetuximab [36]. A statistically significant benefit in PFS for patients with KRAS WT tumors receiving cetuximab and chemotherapy was confirmed in a final presentation of this trial [42]. Bokemeyer et al. investigated the use of cetuximab in combination with FOLFOX chemotherapy as initial treatment for metastatic disease [34]. A retrospective analysis of this data revealed that cetuximab and chemotherapy had a statistically significant increased response rate and lower risk of disease progression compared with chemotherapy alone in patients with KRAS WT tumors [43]. Prospectively, panitumumab has been investigated with either FOLFOX or FOLFIRI chemotherapy in the first-line metastatic setting [44, 45]. The addition of panitumumab to FOLFOX chemotherapy was associated with a statistically significant improvement in PFS [44]. Taken together, the data in Table 2 supports that in metastatic CRC, KRAS WT and mutation status predict for potential sensitivity to, and definite resistance to, respectively, both anti-EGFR monoclonal antibodies, regardless of prior treatment and irrespective of use as monotherapy or in combination. Notably, while KRAS status is an established predictor of response to anti-EGFR monoclonal antibody therapy, it has been disproven as a prognostic marker. In contrast to KRAS mutational status, evaluation of EGFR expression of CRC cells has failed to demonstrate predictive value for anti-EGFR monoclonal antibody therapy. Cunningham et al. reported that the intensity of EGFR staining by immunohistochemical analysis did not correlate with response rate to cetuximab [13]. Similar data has also been reported with panitumumab [46]. KRAS mutated CRC absent of anti-EGFR monoclonal
antibody therapy is not inferior to patients with KRAS WT disease. The evaluation of KRAS mutational status is a mandatory aspect of management of patients at the time of diagnosis of metastatic CRC.

3. Mechanisms of Resistance

While KRAS mutations are a major mechanism of primary resistance to anti-EGFR monoclonal antibody therapies, resistance mechanisms in KRAS WT patients are also being defined. Forty-sixty percent of patients with KRAS WT tumors fail to respond to treatment with anti-EGFR monoclonal antibodies [51]. Therefore, other possible molecular determinants of response are being identified in those patients with EGFR monoclonal antibody-resistant WT KRAS disease.

The importance and frequency of NRAS (a ras oncogene variant) mutations in CRC remains under-investigated [52, 53]. Lambrechts et al. found that NRAS, KRAS, and BRAF mutations were all mutually exclusive events, with combined WT status of these genes associated with higher response rates and longer progression-free survival times [54]. Lambrechts et al. also reported that an NRAS mutation was associated with a lack of response to cetuximab. Irahara et al. investigated the relationship between NRAS mutations and clinical outcome in a collection of 225 colorectal cancers from two prospective cohort studies [55]. NRAS mutations were detected in 2.2% of the CRCs. There was no apparent association between the NRAS mutations and any clinical or pathologic features, including patient survival. However, the low frequency of NRAS mutations may obscure a significant relation. De Roock et al. conducted a retrospective analysis of over 700 tumor samples from patients treated with cetuximab plus chemotherapy and found a NRAS mutation frequency of 2.6%. Additionally, in KRAS wild types, carriers of NRAS mutations had a significantly lower response rate to cetuximab than NRAS wild types (7.7% versus 38.1%, \( P = .013 \)) [26]. There was, however, no significant difference in median PFS (14 versus 26 weeks, \( P = .055 \)) and median OS (38 versus 50 weeks, \( P = .051 \)) between NRAS wild types and mutants [26].

B-type Raf kinase (BRAF) is a component of the RAS-RAF-MEK signaling cascade of the EGFR (see Figure 1) [56]. A specific mutation in the BRAF gene (V600E) is present in approximately 5–8% of CRCs and is thought to be limited to those tumors without mutations in exon 2 of KRAS [42, 57]. BRAF, which is located directly downstream from RAS, can have activating mutations leading to stimulation of the MEK pathway [56, 58]. BRAF mutations appear to confer a poor prognosis, and it appears that BRAF mutations also predict for a lack of response to anti-EGFR monoclonal antibodies [42, 57, 59, 60]. Loupakis et al. analyzed 87 patients with KRAS WT tumors for the BRAF V600E mutation who were receiving irinotecan and cetuximab for refractory metastatic.
Table 1: Clinical trials of antiEGFR monoclonal antibodies in metastatic CRC.

| Study                  | Setting                                    | Treatment                          | No. of patients | ORR (%) | mTTP (mos) | mPFS (mos) | mOS (mos) |
|------------------------|--------------------------------------------|------------------------------------|-----------------|---------|------------|------------|-----------|
| Single Arm phase II [12]| Irinotecan-refractory                      | Cetuximab monotherapy               | 57              | 9       | 1.4        | N.R.       | 6.4       |
| Randomized phase II [13]| Refractory disease to 5-FU and Irinotecan  | Cetuximab monotherapy vs. Cetuximab + Irinotecan | 111 vs. 218     | 10.8* vs. 22.9 | 1.5* vs. 4.1 | N.R.       | 6.9 vs. 8.6 |
| Single Arm phase II [14]| Refractory disease to 5-FU, Irinotecan, and Oxaliplatin | Cetuximab monotherapy               | 346             | 12.4    | 1.4        | N.R.       | 6.6       |
| Single Arm phase II [15]| First-line treatment                       | Cetuximab + Irinotecan + 5-FU/FA    | 21              | 67      | 9.9        | N.R.       | 33.0      |
| Randomized phase III [16]| Refractory disease to 5-FU, Irinotecan and Oxaliplatin | Cetuximab monotherapy vs. BSC      | 287 vs. 285     | 8* vs. 0 | N.R.       | 1.9* vs. 1.8  | 6.1* vs. 4.6 |
| Single Arm phase II [17]| First-line treatment                       | Cetuximab + FOLFOX-4               | 43              | 72      | N.R.       | 12.3       | 30        |
| Randomized phase III [18]| Refractory to Oxaliplatin                  | Cetuximab + Irinotecan vs. Irinotecan | 648 vs. 650     | 16.4* vs. 4.2 | N.R.       | 4.0* vs. 2.6 | 10.7 vs. 10.0 |
| Randomized phase III [19]| First-line treatment                       | Cetuximab + FOLFIRI vs. FOLFIRI    | 602 vs. 600     | 46.9* vs. 38.7 | N.R.       | 8.9* vs. 8.0 | N.R.      |
| Randomized phase III [20]| Refractory disease to 5-FU, Irinotecan and Oxaliplatin | Panitumumab monotherapy versus BSC | 231 vs. 232     | 10.0* vs. 0 | N.R.       | 8 weeks* vs. 7.3 weeks | 6.5 vs. 6.5 |
| Randomized phase III [20]| Refractory to Oxaliplatin                  | Panitumumab monotherapy versus BSC | 231 vs. 232     | 10.0* vs. 0 | N.R.       | 8 weeks* vs. 7.3 weeks | 6.5 vs. 6.5 |
| Randomized phase II [21]| Refractory to Irinotecan                   | Irinotecan + Cetuximab + Bevacizumab | 43 vs. 40       | 37 vs. 20 | 7.3 vs. 4.9 | N.R.       | 14.5 vs. 11.4 |
| Single Arm phase II [22]| Refractory to Irinotecan + Bevacizumab    | Cetuximab + Bevacizumab + Irinotecan | 33              | 9       | 3.9        | N.R.       | 10.6      |

* Statistically significant improvement.

ORR: overall response rate; mTTP: median time to progression; mPFS: median progression-free survival; mOS: median overall survival; N.R.: not reported; 5-FU: 5-fluorouracil; BSC: best supportive care; FA: folinic acid; NS: not significant.

CRC. This mutation was found in 15% of the patients and was associated with a lack of response to therapy (0% versus 32%, \( P = .016 \)) and a shorter overall survival (4.1 months versus 13.9 months, \( P = .037 \)) [61]. An additional retrospective analysis of 113 patients treated with antiEGFR monoclonal antibodies found the V600E BRAF mutation in 14% of the KRAS WT patients and was associated with no response to therapy and a statistically significant shorter progression-free survival and overall survival compared with BRAF WT patients [59]. In De Roock’s retrospective analysis of tumor samples from patients treated with cetuximab plus chemotherapy, a BRAF mutation was discovered in 4.7% of tumors [26]. In KRAS wild types, carriers of BRAF mutations had a significantly lower response rate to cetuximab than in BRAF wild types (8.3% versus 38.0%, \( P = .0012 \)), a significantly shorter PFS (8 versus 26 weeks, \( P < .0001 \)), and a significantly shorter OS (26 versus 54 weeks, \( P < .0001 \)) [26]. KRAS and BRAF mutation status do not, however, appear to affect the clinical benefit of oxaliplatin or irinotecan on PFS or OS [62]. Several compounds (PLX4032, PLX4720, and GDC-0879) which selectively inhibit the kinase enzyme BRAF containing the V600E mutation are in clinical development [63]. In BRAF mutant cancer cell lines, these selective BRAF inhibitors potently block RAF-MEK-ERK signaling. However, in those tumors that are BRAF WT, but possess a KRAS mutation, these BRAF inhibitors activate this same pathway and therefore should be avoided in those cancers with RAS mutations [64–66].
| Study                          | Setting                      | Treatment                                      | KRAS genotype | No. of patients | ORR (%) | mPFS (mos) | mOS (mos) |
|-------------------------------|------------------------------|-----------------------------------------------|---------------|-----------------|---------|------------|-----------|
|                               |                              |                                               |               |                 |         | Single arm studies |           |
| Lièvre et al. [34]            | Second-line treatment        | Cetuximab                                     | WT            | 65              | 40*     | 31.4 wk*   | 14.3*     |
|                               |                              |                                               | Mut           | 24              | 0       | 10.1       | 10.1      |
| De Roock et al. [29]          | Irinotecan refractory        | Cetuximab or cetuximab + irinotecan           | WT            | 57              | 41†     | 34 wk† (combo) | 27 wk† (combo) |
|                               |                              |                                               | Mut           | 46              | 0       | 12 (cetux) | 12        |
| Khambata-Ford et al. [28]     | Second or third-line treatment | Cetuximab                                     | WT            | 50              | 10      | N.R.       | N.R.      |
|                               |                              |                                               | Mut           | 30              | 0       | N.R.       | N.R.      |
| Di Fiore et al. [47]          | Refractory disease           | Cetuximab + chemotherapy                      | WT            | 43              | 20.3    | 44.7 wk   | 25.3–27.3 |
|                               |                              | Cetuximab or panitumumab + cetuximab +       | Mut           | 16              | 0       | 12 (cetux) |           |
|                               |                              | chemotherapy                                   |               |                 |         |            |           |
| Benvenuti et al. [48]         | Various lines of treatment   | Cetuximab or panitumumab + cetuximab +       | WT            | 32              | 31      | N.R.       | N.R.      |
|                               |                              | chemotherapy                                    | Mut           | 16              | 6       | N.R.       | N.R.      |
|                               |                              |                                               |               |                 |         | Randomized studies |           |
| Amado et al. [24]             | Refractory disease           | Panitumumab + BSC vs. BSC                     | WT            | 124             | 17      | 12.3 wk*   | 8.1       |
|                               |                              |                                               | Mut vs.       | 84              | 0       | 7.4 wk     | 4.9       |
|                               |                              |                                               | WT            | 119             | 0       | 7.3 wk     | 7.6       |
|                               |                              |                                               | Mut           | 100             | 0       | 7.3 wk     | 4.4       |
| Van Cutsem et al. [33]        | First-line treatment         | FOLFIRI + cetuximab vs. FOLFIRI               | WT            | 172             | 59.3    | 9.9*       | 24.9      |
|                               |                              |                                               | Mut vs.       | 105             | 36.2    | 7.6        | 17.5      |
|                               |                              |                                               | WT            | 176             | 43.2    | 8.7        | 21.0      |
|                               |                              |                                               | Mut           | 87              | 40.2    | 8.1        | 17.7      |
| Van Cutsem et al. [42]        | First-line treatment         | FOLFIRI + cetuximab vs. FOLFIRI               | WT            | 316             | 57.3*   | 9.9*       | 23.5*     |
|                               |                              |                                               | Mut vs.       | 214             | 31.3    | 7.4        | 16.2      |
|                               |                              |                                               | WT            | 350             | 39.7    | 8.4        | 20.0      |
|                               |                              |                                               | Mut           | 183             | 36.1    | 7.7        | 16.7      |
| Bokemeyer et al. [31]         | First-line treatment         | FOLFOX + cetuximab vs. FOLFOX                 | WT            | 61              | 61*     | 7.7*       | N.R.      |
|                               |                              |                                               | Mut vs.       | 52              | 33      | 5.5        | 18.5      |
|                               |                              |                                               | WT            | 73              | 37      | 7.2        | 13.4      |
|                               |                              |                                               | Mut           | 47              | 49      | 8.6        |           |
| Bokemeyer et al. [43]         | First-line treatment         | FOLFOX + cetuximab vs. FOLFOX                 | WT            | 82              | 57*     | 8.3*       | 22.8      |
|                               |                              |                                               | Mut vs.       | 77              | 34      | 5.5        | 18.5      |
|                               |                              |                                               | WT            | 97              | 34      | 7.2        | 13.4      |
|                               |                              |                                               | Mut           | 59              | 53      | 8.6        | 17.5      |
| Karapetis et al. [35]         | Refractory disease           | Cetuximab + BSC vs. BSC                       | WT            | 115             | 12.8    | 3.7*       | 9.5*      |
|                               |                              |                                               | Mut vs.       | 81              | 1.2     | 1.8        | 4.5       |
|                               |                              |                                               | WT            | 113             | 0       | 1.9        | 4.8       |
|                               |                              |                                               | Mut           | 83              | 0       | 1.8        | 4.6       |
| Siena et al. [44]             | First-line treatment         | FOLFOX + panitumumab vs. FOLFOX               | WT = 656      | 55              | 9.6 (wt)* | 7.3 (mut) | N.R.      |
|                               |                              |                                               | Mut = 440     | 48              | 8.0 (wt) | 8.8 (mut) |           |
| Kohne et al. [45]             | First-line treatment         | FOLFIRI + panitumumab                         | WT            | 85              | 48      | N.R.       | N.R.      |
The mitogen-activated protein kinase kinase (MEK, also known as MAP2K) is downstream of BRAF in the RAS-RAF-MEK signaling cascade of the EGFR and uses extracellular signal-regulated kinase (ERK) as a substrate (see Figure 1) [67]. A number of MEK inhibitors such as AS703026, AZD6244 and RO5068760 have been or currently are being investigated in phase 1 and 2 clinical trials [68, 69]. The development of several MEK inhibitors has been halted because of either very low response rates or due to ocular toxicity [70]. These agents have however shown substantial preclinical activity in tumor cell lines harboring the BRAF V600E gene mutation [71]. It has been established that KRAS has a number of downstream effectors that are not blocked by MEK inhibition, and indeed BRAF mutant cell lines were found to be more sensitive to MEK inhibitors than KRAS mutant cells [71]. It is imperative to be able to identify which patients are likely to respond to MEK inhibitors, and it appears that those with BRAF mutations are a good start. Given that KRAS signaling operates through a number of downstream effectors, those with KRAS mutations may require a combination of targeted agents. Preclinical evidence suggests that BRAF gene amplification is a mechanism of resistance to both MEK and BRAF inhibitors and a combination of these inhibitors may be a strategy to overcome this [72].

An additional EGFR pathway is the PTEN/PI3K/AKT pathway [phosphatase and tensin homologue gene (PTEN)]. PTEN encodes a phosphatase which uses phosphatidylinositol-3,4,5-triphosphate (PIP3) as a major substrate [73]. Loss of PTEN function leads to increased PIP-3 concentration, with resultant AKT hyperphosphorylation protecting tumor cells from apoptosis [73]. Roughly 60% of primary CRCs have a hyperphosphorylated AKT [74]. PTEN loss, activating mutations of phosphatidylinositol 3-kinase catalytic alpha polypeptide (PIK3CA) and activating mutations in KRAS/BRAF/MAPK confer resistance to apoptosis induced by cetuximab [75]. In patients with KRAS WT tumors treated with a cetuximab-based regimen, PTEN loss was associated with a significantly shorter OS [60]. Approximately one third of CRCs harbor activating somatic mutations in PIK3CA, and it has been reported that these mutations are predictive of lack of benefit from antiEGFR therapy [76]. Additional genetic alterations which could confer resistance to antiEGFR monoclonal antibodies include an inhibitor of PI3K signaling; coamplification of PAK4 (p-21-activated protein kinase) and AKT, which are downstream mediators of PI3K signaling; and amplification of IRS2 (insulin receptor substrate 2), which is an upstream activator of PI3K signaling [77, 78].

### 4. Strategies to Overcome Resistance

A number of approaches to the problem of resistance to antiEGFR monoclonal antibody therapy have been studied and are ongoing. Combining antiEGFR monoclonal antibodies with cytotoxic chemotherapy has already been discussed. Erlotinib and gefitinib, two oral small molecule EGFR inhibitors, are inactive by themselves [79, 80]. The combination of erlotinib with capecitabine and oxaliplatin in previously treated patients and the combination of gefitinib with FOLFOX were investigated in small phase II studies with favorable results, however randomized trials with chemotherapy alone as a control are needed [81–83]. Dual antiEGFR therapy with antiEGFR monoclonal antibodies plus antiEGFR TK inhibitors may overcome resistance to either drug alone. A 41% response rate was reported for the combination of cetuximab and erlotinib in patients with refractory disease, however this was limited to patients with KRAS and BRAF WT tumors [84].

EGFR and vascular endothelial growth factor (VEGF) have several signal transduction pathways in common, with preclinical data revealing that antiEGFR and antiVEGF drug combinations have synergistic activity [85]. The BOND-2 study randomized patients with irinotecan- and oxaliplatin-refractory but bevacizumab naïve disease to cetuximab and bevacizumab with or without irinotecan [21]. Response rates, TTP and OS favored the triple drug regimen, however these results did not hold up in a subsequent study of this combination [21, 22]. Two subsequent randomized phase III trials have shown that combinations of antiEGFR

### Table 2: Continued.

| Study | Setting | Treatment | KRAS genotype | No. of patients | ORR (%) | mPFS (mos) | mOS (mos) |
|-------|---------|-----------|---------------|----------------|---------|------------|-----------|
| Tol et al. [49] First-line treatment | CAPOX + bevacizumab + cetuximab vs. FOLFOX + bevacizumab | WT | 158 | 50.0 | 10.5* | 21.8 |
| | | Mut | 98 | 59.2 | 8.1 | 17.2 |
| | CAPOX + bevacizumab | WT | 156 | 61.4* | 10.6 | 22.4 |
| | | Mut | 108 | 45.9 | 12.5 | 24.9 |
| Hecht et al. [50] First-line treatment | FOLFOX + bevacizumab + panitumumab vs. FOLFOX + bevacizumab | WT | 201 | 50 | 9.8 | 20.7 |
| | | Mut | 135 | 47 | 10.4 | 19.3 |

* Statistically significant improvement
† Statistically significant improvement for the combination of cetuximab and irinotecan only.

ORR: overall response rate; mPFS: median progression-free survival; mOS: median overall survival; N.R.: not reported; BSC: best supportive care.
monoclonal antibodies plus bevacizumab do not improve outcomes and can actually cause increased toxicity irrespective of KRAS mutational status. The PACCE trial evaluated panitumumab combined with oxaliplatin- or irinotecan-based chemotherapy plus bevacizumab. The dual monoclonal antibody arm was associated with increased toxicity and significantly shorter PFS in patients with both KRAS WT and mutant tumors [50]. Similar results were observed with the combination of cetuximab to a regimen containing capecitabine, oxaliplatin, and bevacizumab in the CAIRO2 trial [49].

Novel agents and combinations are being employed in an attempt to overcome anti-EGFR monoclonal antibody resistance. Motesanib, an oral inhibitor of VEGF, platelet derived growth factor (PDGF) and Kit receptors is being investigated with or without panitumumab in patients with refractory disease [86]. A number of inhibitors of the mutant BRAF kinase are in clinical development, as discussed above [87]. AMG 102 is an investigational monoclonal antibody against human hepatocyte growth factor (also known as cMET, of which overexpression correlates with cetuximab resistance) is being studied in combination with panitumumab in patients with metastatic CRC [88, 89].

5. Neoadjuvant and Adjuvant Therapy

Given the clinical benefit of anti-EGFR monoclonal antibodies in patients with metastatic disease, evaluation of these therapies as postoperative (adjuvant) treatment was warranted. In the adjuvant setting, eradication of micrometastatic disease is associated with increased cure rates. N0147 randomized 1760 patients with resected stage III KRAS WT colon cancer to FOLFOX with or without cetuximab [90]. Interim analysis led to premature closure of this trial after it was determined that no group of patients benefited from cetuximab [90]. Initially this trial enrolled patients regardless of KRAS mutational status, and among 658 patients with mutant KRAS, the addition of cetuximab to FOLFOX resulted in impaired disease-free survival (DFS) and a trend toward impaired OS [91].

In patients with rectal cancer, EGFR is a logical target in combination with neoadjuvant radiotherapy (RT). Retrospective analyses have demonstrated lower pathologic complete response (pCR) rates and shorter DFS in patients with rectal cancer expressing EGFR who were treated with neoadjuvant RT, suggesting that radiosensitivity might be increased by targeting the EGFR [92, 93]. Several phase I/II studies have investigated the combinations of cetuximab and chemoradiotherapy in the neoadjuvant setting for patients with rectal cancer. These studies have demonstrated that cetuximab could be safely combined with preoperative chemoradiotherapy but the pCR rates have been low (5–12%) [94–100]. In two of these studies [96, 99], subsequent analyses were done to correlate KRAS mutation status with response rate. Among patients with KRAS WT tumors, Bengala et al. reported a trend toward a greater rate of tumor regression (36.7% for KRAS WT versus 11% for KRAS mutant), however it did not reach statistical significance ($P = .12$) [101]. Devecquoy et al. also did not find a correlation between KRAS WT tumors and pathologic response to therapy [102]. To our knowledge, panitumumab has not been studied in combination with RT in patients with rectal cancer. Given the failure of anti-EGFR monoclonal antibodies to demonstrate a benefit in the adjuvant setting for stage III WT KRAS colon cancer, the value of further study of these agents for rectal cancer is doubtful.

Preclinically gefitinib has demonstrated improved radiosensitization [103]. Valentini et al. investigated the combination of gefitinib, continuous infusion 5-fluorouracil (5-FU) and pelvic RT in 41 patients with locally advanced rectal cancer and reported a pCR rate of 30%, however toxicity was an issue and further studies are necessary to establish the safety of this combination [104].

The effect of combined anti-EGFR and anti-VEGF therapy in combination with preoperative chemoradiotherapy for rectal cancer remains unknown, however given the negative results reported for combined EGFR and VEGF blockade in patients with metastatic CRC in combination with chemotherapy, studies investigating this avenue are unlikely [22, 49, 50]. Blaszkowsky et al. performed a small study investigating the combination of bevacizumab, erlotinib and 5-FU with RT in patients with locally advanced rectal cancer [105]. The regimen was found to be well-tolerated and highly active with a pCR rate of 47% and may deserve further investigation. However, the value of pCR as a surrogate for DFS and OS is uncertain.

6. Conclusion

Anti-EGFR monoclonal antibodies are among the standard treatment options for patients with metastatic CRC given their established efficacy. It is now clear that the benefit of anti-EGFR monoclonal antibodies is isolated to patients with KRAS WT tumors. It appears that KRAS mutational status is just the beginning of our understanding of the EGFR as an integral component of the biology of CRC. Given that only a subset of patients respond to anti-EGFR therapy, there is a need for better predictors to guide patient selection for such therapy. Several important components of the EGFR signaling pathway have been discovered, including BRAF, PTEN, AKT and PI3K, which deserve further study as predictors of response to existing treatments, or as targets of new interventions. The unexpected detrimental outcome associated with combined EGFR and VEGF blockade is a reminder of how much there is still to learn. New combinations and novel agents will continue to shed light on how to overcome resistance to inhibitors of the EGFR pathway, and hopefully new targets will be identified. Further study of how to employ our knowledge of EGFR pathway inhibitors to improve outcomes in the adjuvant and neoadjuvant setting is also warranted.

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