

# Evolving role of cetuximab in the treatment of colorectal cancer

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**Abstract:** In recent years, the monoclonal epidermal growth factor receptor (EGFR)-targeting antibody cetuximab was introduced into systemic therapy of colorectal cancer and gained an established role in the treatment of this disease. Cetuximab was shown to be active as a single agent in chemorefractory metastatic disease as well as in combination with varying chemotherapies. Recently, randomized trials demonstrated the activity of cetuximab combinations in the first-line setting of metastatic colorectal cancer. Interestingly, the activity of cetuximab was restricted to patients with *KRAS* wildtype tumors, as was seen with panitumumab, another EGFR antibody. While 60%–70% of tumors harbor *KRAS* wildtype genes, 30%–40% of tumors express oncogenic *KRAS* with mutations in codons 12 and 13 causing constitutive activation of signaling cascades downstream of EGFR and resistance to EGFR blockade. Since proof of *KRAS* wildtype status became a prerequisite for cetuximab treatment, *KRAS* testing is being established throughout the world. Future trials will address the question which part of the *KRAS* wildtype cohort will benefit from EGFR inhibition and how to identify those patients. Additionally, new strategies for treatment of *KRAS* mutated tumors are strongly needed. Recent developments and future strategies will be summarized.

**Keywords:** cetuximab, colorectal cancer, *KRAS*

## Initial clinical development in refractory colorectal cancer

Colorectal cancer is one of the leading tumor types worldwide with about 25% of patients having metastatic disease at diagnosis.<sup>1</sup> Additionally, patients with stage II or III disease are at considerable risk to develop recurrence and metastases after curative resection. The median survival time of patients with stage IV disease receiving best supportive care only is limited to around 3–6 months. Treatment with 5FU in combination with folinic acid in metastatic disease prolonged survival up to 12 months.<sup>2</sup> The introduction of irinotecan and oxaliplatin has significantly improved median survival times of colorectal cancer patients to around 20–24 months when all treatment options are given sequentially.<sup>3,4</sup>

In the last decade new targets have been identified for the treatment of colon cancer and specific drugs have been introduced into patient care. Especially the receptor for epidermal growth factor (EGFR) gained much interest. EGFR is expressed on more than 80% of colorectal cancer cells and preclinical data demonstrated its central role in tumor-specific functions such as proliferation, invasion, metastasis, and angiogenesis.<sup>5–8</sup> Cetuximab was initially developed by ImClone Systems (New York, NY, USA) as a specific monoclonal antibody to bind and block EGFR signaling. Cetuximab is an

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immunoglobulin G1 (IgG1)-specific human mouse chimeric antibody that demonstrated activity in preclinical models of colorectal cancer.<sup>7-9</sup>

A phase II trial of cetuximab monotherapy in 57 patients with chemorefractory colorectal cancer demonstrated modest activity with 9% response rate and a median survival of 6.4 months.<sup>10</sup> The so-called BOND trial investigated cetuximab either as monotherapy or in combination with irinotecan in patients refractory to irinotecan. Cetuximab monotherapy was associated with a response rate of 10.8% and a median survival of 6.9 months in these heavily pretreated patients. The combination of cetuximab with irinotecan improved the response rate to 22.9% and median survival was estimated with 8.6 months.<sup>11</sup> Based on these data cetuximab was approved for the treatment of irinotecan-refractory colon cancer by the US Food and Drug Administration (FDA) in February 2004 and by the European Medicines Agency (EMA) in June 2004. Data of the cetuximab–irinotecan combination in refractory patients were confirmed in a single center phase II trial verifying a response rate of 25% and a median survival of 9.8 months.<sup>12</sup> Similar results were reported in a multicenter phase II trial from Japan confirming almost identical efficacy data in an Asian population.<sup>13</sup>

### Further development as second- and third-line therapy

A large multinational trial was designed to confirm the results of the BOND trial in a heavily pretreated population with patients progressing on irinotecan-containing regimens. 1147 patients with EGFR-positive colorectal cancer received cetuximab in combination with various irinotecan schedules, either weekly, every two weeks, or every three weeks. Response rates ranged between 17.3% and 21.4% depending on the irinotecan schedule. A mean overall survival (OS) time of 9.2 months was reached.<sup>14</sup> This so-called MABEL trial confirmed the activity of cetuximab added to irinotecan in irinotecan-pretreated patients in a large population in the community setting. Infusion-related reactions of grades 3 and 4 were observed in less than 1% of patients. Severity of acne-like rash was associated with improved efficacy parameters such as progression-free survival (PFS).

Beside the original schedule of weekly cetuximab several trials attempted to investigate whether a more convenient schedule with infusions every other week might influence pharmacokinetics and efficacy of cetuximab. Similar efficacy results and toxicity data were observed in a schedule of cetuximab in a dose of 500 mg/m<sup>2</sup> and irinotecan (180 mg/m<sup>2</sup>)

given every two weeks as compared to previous data with weekly cetuximab.<sup>15,16</sup> However, the two-weekly applications have not been approved yet.

Further attempts to improve the efficacy of chemotherapy in combination with cetuximab were performed in the second-line setting. Souglakos and colleagues tested the combination of cetuximab with oxaliplatin and capecitabine in patients refractory to first-line oxaliplatin-containing regimen in a phase II trial. The combination of cetuximab with capecitabine and oxaliplatin was safe. The overall response rate in this population was 18.7%. Time to tumor progression however, was short at three months.<sup>17</sup> In the EPIC trial, patients with oxaliplatin-refractory EGFR-expressing disease were treated with irinotecan either alone or in combination with cetuximab. In this large phase III trial the addition of cetuximab to irinotecan improved the response rate from 4.2% to 16.4% and PFS from 2.6 to 4.0 months. The median OS was not different in both groups with 10.0 and 10.7 months. The authors noted that 87% of patients in the irinotecan arm received cetuximab-containing treatments after disease progression.<sup>18</sup> This post-study crossover might have washed out survival benefits in the combination group.

### Clinical trials in the first-line treatment of metastatic colorectal cancer

Initial data from first-line treatment with cetuximab were reported from a small cohort of 21 patients with EGFR-positive metastatic colon cancer receiving cetuximab in combination with weekly irinotecan and 5FU/FA with two dose groups of 5FU (1500 and 2000 mg/m<sup>2</sup>, respectively). The higher 5FU dose was associated with diarrhoea and the dose of 1500 mg/m<sup>2</sup> was recommended for further trials. In this small patient group the overall response rate (ORR) was 67% and PFS reached 9.9 months.<sup>19</sup> Early data from combinations with oxaliplatin were reported from a phase II trial with 43 patients using FOLFOX-4 as chemotherapy backbone. The confirmed ORR was 72%, median PFS was 10.8, and median OS was high with 30 months. Secondary resectability rate was 23% with resection of liver metastases in curative intention in 10 patients.<sup>20</sup> A small randomized phase II trial investigated the addition of cetuximab to oxaliplatin plus capecitabine in the first-line setting. While an improvement regarding response rate (41%) and PFS (7.2 months) was observed in the cetuximab arm, the low response rate of 14% and a PFS of 5.8 months in the control group were of some concern in this trial.<sup>21</sup>

Data from a larger population were reported within the OPUS study, a large randomized phase II trial studying FOLFOX-4 with or without cetuximab in 338 untreated patients with positive EGFR staining. Response rate was improved from 36% to 46% in the cetuximab group. The difference in response rate was not significant ( $p = 0.064$ ) for the whole patient population and PFS was identical with 7.2 months in both arms. With increasing data on KRAS mutations in colorectal cancer being associated with lack of response in EGFR-inhibiting therapies, KRAS mutation status was evaluated in 233 patients. In patients with KRAS wildtype tumors the difference in response was highly significant with 37% vs 61% ( $p = 0.011$ ) and a lower risk of disease progression was observed. Patients with KRAS mutant tumors did not benefit from the addition of cetuximab.<sup>22</sup>

The CRYSTAL study investigated the combination of cetuximab with FOLFIRI in 1217 untreated patients with colorectal cancer in a randomized fashion. Similar to the

OPUS trial the addition of cetuximab to chemotherapy resulted in an 8% increase of the ORR ( $p = 0.004$ ) and PFS was prolonged from 8.0 to 8.9 months ( $p = 0.0479$ ). Interestingly, PFS curves separated late with one-year PFS rates of 23% and 34%, respectively. When tumor tissue was analyzed for KRAS, mutations were detected in 36% of patients. In those the addition of cetuximab did not improve response or PFS. In wildtype patients, PFS was prolonged from 8.7 to 9.9 months ( $p = 0.017$ ).<sup>23</sup> See Table 1 for an overview of clinical trials.

Based on these data cetuximab has been approved for first-line therapy in metastatic colorectal cancer for patients harboring KRAS wildtype tumors. More details on the role of KRAS will be discussed later.

## Cetuximab monotherapy in refractory disease

Based on a phase II-study and the mono-arm of the BOND trial cetuximab was introduced as monotherapy option in

**Table 1** Selected cetuximab trials. Landmark trials in the development of cetuximab according to clinical treatment lines with main characteristics and results such as progression-free survival (PFS) and response rate (RR). In several trials time to progression (TTP) was used as clinical endpoint (in parentheses). Recent studies analyzed efficacy results for KRAS wildtype patients separately. Data of wildtype cohorts are noted (*italic*) below results of the entire populations

Author	Phase	Patients (n)	Treatment line	Therapy	PFS (mo)	RR (%)
Saltz et al 2004	II	57	≥2nd (irinotecan refr.)	Cet mono	1.4	9
Cunningham et al 2004	III	329	≥2nd (irinotecan refr.)	Cet + irinotecan vs Cet mono	4.1 vs 1.5	22.9 vs 10.8
Lenz et al 2006	II	346	≥2nd (iri + oxali refr.)	Cet mono	1.4	11.6
Wilke et al 2008	III	1147	≥2nd (irinotecan refr.)	Cet + irinotecan (three schedules)	3.2	20.1
Souglakos et al 2007	II	40	≥2nd (previous oxaliplatin)	Cet + CapOx	2.9 (TTP)	20
Sobrero et al 2008	III	1298	≥2nd (previous oxaliplatin)	Cet + irinotecan vs irinotecan	4.0 vs 2.6	16.4 vs 4.2
Folprecht et al 2006	II	21	First line	Cet + irinotecan/5FU/FA	9.9 (TTP)	67
Taberero et al 2007	II	43	First line	Cet + FOLFOX	12.3	72
Borner et al 2008	II	74	First line	XELOX ± Cet	7.2 vs 5.8 (TTP)	41 vs 14
Bokemeyer et al 2009	II	337	First line	FOLFOX-4 ± Cet	7.2 vs 7.2 (7.9 vs 7.2)	46 vs 36 (61 vs 37)
Van Cutsem et al 2009	III	1198	First line	FOLFIRI ± Cet	8.9 vs 8.0 (9.9 vs 8.7)	46 vs 38 (59 vs 43)
Saltz et al 2007	II	83	≥2nd (previous irinotecan)	Cet + beva ± irinotecan	7.3 vs 4.9 (TTP)	37 vs 20
Tol et al 2009	III	755	First line	CapOx/beva ± Cet	9.6 vs 10.7 (10.5 vs 10.6)	52.7 vs 50 (61.4 vs 50)
Jonker et al 2007	III	572	≥2nd	Cet vs BSC	HR 0.68 (3.7 vs 1.9)	8 vs 0 (13 vs 0)

irinotecan-refractory colorectal cancer.<sup>10,11</sup> In both trials, a mean response of 10% was observed. Another phase II trial by Lenz and colleagues demonstrated a similar response rate of 11.6% and a median OS of 6.6 months in a cohort of 346 patients in chemorefractory patients.<sup>24</sup> Adverse events included hypersensitivity reactions, acne-like rash, asthenia, diarrhea, and others. Skin toxicities started within 1–3 weeks after initiation of cetuximab treatment. In a phase III setting in heavily pretreated patients, cetuximab was superior to best supportive care in KRAS wildtype patients with a median PFS of 14.8 weeks (mutant 7.2) and meaningful difference in OS of 9.5 months vs 4.5 months in KRAS mutants.<sup>25</sup>

Pessino and colleagues addressed the question whether cetuximab could be used as monotherapy in the first-line setting. Interestingly, the response rate of 10% in cetuximab monotherapy in chemo-naïve patients was similar to that observed in pretreated patients.<sup>26</sup> Time to progression was only two months. Therefore, cetuximab monotherapy can not be considered as effective first-line treatment.

Findings of anti-EGFR monotherapy have been reviewed recently.<sup>27</sup>

## Combination of cetuximab with bevacizumab

A similar design to the BOND trial, but with the addition of bevacizumab, was tested in irinotecan-refractory patients in a small phase II study. The combination of bevacizumab plus cetuximab resulted in a response rate of 20% and an OS of 11.4 months in this heavily pretreated cohort. The combination of both antibodies plus irinotecan further improved the response with an ORR of 37% and OS of 14.5 months.<sup>28</sup> The results of this BOND-2 study formed the basis for the randomized CAIRO2 trial investigating the addition of cetuximab to a combination of capecitabine, oxaliplatin, and bevacizumab. Although no unexpected toxicities were observed,<sup>29</sup> efficacy data were surprisingly inferior for the cetuximab-treated patients. In the intention-to-treat (ITT) population PFS was 10.7 months in the control group and 9.4 months in the cetuximab arm. In the subanalysis of the KRAS wildtype population there was no difference in PFS and OS between treatment arms. In KRAS mutant patients PFS was significantly lower in the cetuximab arm with 8.6 months. Interestingly, the control group had a higher PFS (12.5 months) compared to all other groups.<sup>30</sup>

A similar attempt using the EGFR antibody panitumumab was studied in the PACCE trial. In this randomized first-line trial panitumumab was added to either oxaliplatin- or irinotecan-based chemotherapy plus bevacizumab.

The combination was associated with inferior outcome as compared to the control arm with PFS times of 10 vs 11.4 months and OS of 19.4 vs 24.5 months for panitumumab and the control arm, respectively. Additionally, toxicities were significantly increased in the panitumumab arm. KRAS analyses resulted in inconsistent data between groups. Oxaliplatin-based chemotherapy plus both antibodies was associated with inferior response compared to the control group in KRAS wildtype patients.<sup>31</sup>

Based on these two negative randomized trials, the idea of EGFR and vascular endothelial growth factor (VEGF) double-targeting with monoclonal antibodies has failed. The underlying mechanism is not understood so far. One explanation could be the downregulation of VEGF production under EGFR inhibition. This may cause activation of alternative proangiogenic factors and lack of efficiency of VEGF blockade.

## The biology of KRAS in EGFR-targeted treatment of colorectal cancer

Since the discovery of the epidermal growth factor (EGF) receptor in the early 1980's,<sup>32</sup> growing knowledge about ligands, activation, and signaling helped to understand its biological function.<sup>33</sup> Upon activation, the transmembrane EGF receptor forms a dimer leading to receptor autophosphorylation through its tyrosine kinase activity.<sup>34</sup> EGFR activates at least five different signaling pathways: the RAS/RAF/MAPK, the phospholipase C, the PI3K/AKT, the STAT, and the SRC pathways.<sup>35</sup> Of those, RAS/RAF/MAPK and PI3K/PTEN/AKT pathways are considered as central effectors of EGFR activation. Each of these finally triggers intracellular signals that may support the malignant phenotype. In recent years, increasing evidence suggested that self-activating or loss of function mutations could occur in nearly all signaling pathways and steps.

Besides alterations of p53, FAP, and other oncogenes activating mutations of KRAS have been described as part of the oncogenic transformation during development of colorectal cancer.<sup>36</sup> Indeed, KRAS mutations have a prevalence of 30%–40% in larger series of colorectal cancer trials.<sup>22,23,37</sup> While the prognostic role of mutated KRAS in colorectal cancer remains controversial,<sup>38,39</sup> initial data from small cohorts had suggested, that in patients treated with cetuximab response was only observed in wildtype tumors.<sup>40</sup> This finding was confirmed by *in vitro* experiments showing lack of response to cetuximab in colon cancer cells

expressing mutant KRAS as compared to wildtype cells.<sup>41</sup> In a larger series of 89 patients among which 27% had KRAS mutant tumors, wildtype patients had a response rate of 40% while none of the patients with mutant tumors responded to cetuximab treatment.<sup>42</sup> These findings were confirmed by another group analyzing 113 patients treated with cetuximab. Early tumor shrinkage was identified as additional predictive marker.<sup>43</sup>

In a randomized phase III trial comparing EGFR inhibition with panitumumab monotherapy to best supportive care in patients refractory to chemotherapy, the objective response for all patients treated with panitumumab was 10%.<sup>44</sup> In wildtype patients treated with panitumumab, the response rate was 17% compared to 0% in the mutant group.<sup>45</sup> Based on these data, panitumumab was approved as single agent only for patients with KRAS wildtype tumors.

Almost identical data have been reported from a randomized phase III trial with cetuximab monotherapy versus best supportive care in chemorefractory patients. In this trial enrolling 572 patients, the response rate was 8% vs 0% in the cetuximab vs control groups, respectively.<sup>46</sup> Post-hoc KRAS analyses of 69% of tumors detected KRAS mutant status in 42% of patients. In those, there was no difference in PFS and OS when treatment and control groups were compared. In wildtype patients, median OS significantly improved from 4.8 to 9.5 months when cetuximab therapy was given.<sup>25</sup>

The KRAS analyses from the CRYSTAL and OPUS trials confirmed the importance of KRAS mutation status for EGFR-targeted therapy in the first-line treatment of metastatic colorectal cancer. First-line cetuximab in combination with FOLFOX-4 significantly improved the response rate from 37% to 61% in KRAS wildtype tumors when cetuximab was added to chemotherapy. PFS was significantly improved from 7.2 to 7.7 months.<sup>22</sup> A similar effect was observed in the CRYSTAL study using FOLFIRI as backbone with an increase in RR from 43% to 59% in wildtype patients and improvement of PFS from 8.7 to 9.9 months.<sup>23</sup> In the smaller OPUS trial KRAS mutant patients seemed to do worse under cetuximab treatment with lower response rates (49% vs 33%) and PFS (8.6 vs 5.8 months) when compared to chemotherapy only. In the CRYSTAL trial there was no significantly inferior outcome in the mutant group. Whether this finding represents a true effect of inferior outcome caused by EGFR inhibition in KRAS mutant tumors in particular in combination with FOLFOX remains unclear.

Based on the presented data, the EMEA approved cetuximab treatment exclusively for patients with KRAS wildtype metastatic colorectal cancer.<sup>47</sup> The American Society of

Clinical Oncology published a provisional clinical opinion stating that all patients who are candidates for anti-EGFR therapy should have their tumors tested for KRAS mutation status. Patients with KRAS mutations should not receive anti-EGFR antibodies.<sup>48</sup> This development reflected an exciting step towards personalized therapy in solid tumors.

Appropriate and standardized KRAS mutation detection tests are subjects of practical considerations.<sup>49</sup> Another important question is whether primary and metastases have identical KRAS mutation status. Santini and colleagues analyzed 38 patients with KRAS mutant tumors and found a high concordance of 96%. Only one patient had a wildtype primary and mutant metastases and three patients had mutant primary tumors and wildtype KRAS in their metastases.<sup>50</sup> Based on this data there is no need to analyze both primary and metastases.

## Biomarkers in cetuximab therapy

In early trials, proof of positive EGFR staining on the tumor tissue was mandatory in order to treat only patients expressing the appropriate target for cetuximab. Further data suggested, that patients with absence of immunohistological EGFR staining might also respond to cetuximab treatment.<sup>51,52</sup> A larger translational study analyzing 346 patients found no correlation of EGFR-staining score and treatment response.<sup>24</sup> Although evidence from randomized trials is not available, EGFR immunohistochemical (IHC) staining is no longer required for cetuximab treatment according to current expert opinion.<sup>53</sup> The lack of EGFR IHC to predict response may be related to the short presentation of receptors on the surface due to receptor turnover.

Further attempts to evaluate meaningful predictive markers for EGFR-blocking agents in colorectal cancer focused primarily on gene amplifications and polymorphisms of the EGFR gene. Increased gene copy numbers of EGFR as detected by fluorescent *in situ* hybridization (FISH) have been linked to an increased response rate and prolonged OS in cetuximab-treated patients.<sup>54</sup> Similar results have reported from a panitumumab cohort.<sup>55</sup> In patients with rectal cancer receiving neoadjuvant radiochemotherapy plus cetuximab, elevated EGFR gene copy numbers were significantly associated with tumor regression.<sup>56</sup> In contrary, data suggesting no evident association with EGFR gene copy number or mutations have been reported earlier.<sup>24</sup> Due to the heterogeneity of data and problems in methodological standardization FISH analysis has not entered clinical routine, so far.

Several polymorphisms in the EGFR gene have been identified. Of those, EGFR intron-1 S/S, EGFR 497 G > A

and EGFR R521K seem to play considerable roles.<sup>57–59</sup> In a cohort of 110 patients, increased skin toxicities and treatment response was associated with EGFR intron-1 S/S carriers.<sup>57</sup>

Pharmacogenetic analyzes of peripheral blood allows to examine germ-line variants of relevant genes such as EGFR ligands. Variants in the ligand EGF, namely EGF 61A > G seemed to be associated with an improved OS compared to the A/A genotype.<sup>57</sup> In another trial, analyses of EGF polymorphisms in KRAS wildtype tumors suggested that EGF 61A > G was significantly associated with decreased response rate and OS.<sup>60</sup> These contradictory findings need to be re-evaluated in larger cohorts and should be linked to levels of EGF in the serum of patients.

Further potential predictive markers have been identified. Increased expression of EGFR ligands epiregulin and amphiregulin was linked to better disease control rates and longer PFS according to gene array studies analyzing tissue from 110 patients.<sup>61</sup> Indeed, treatment with cetuximab induces up-regulation of epiregulin, amphiregulin as well as transforming growth factor- $\alpha$  (TGF- $\alpha$ ), another EGFR ligand, in preclinical models and patients treated with cetuximab.<sup>62</sup>

Skin toxicity was reported to correlate with efficacy of cetuximab.<sup>14</sup> While in patients without skin rash no objective response occurred, patients with increasing rash grades had responses ranging from 7.2% in mild rash up to 20% in severe skin toxicity.<sup>24</sup> The EVEREST trial reported data from dose-escalation of cetuximab in patients lacking skin toxicities. Patients receiving escalating doses up to 500 mg/m<sup>2</sup> experienced responses in 30% (13% in controls) and a median PFS of 4.8 months as compared to 3.9 months in controls.<sup>63</sup> The lack of response in KRAS mutated tumors demonstrate that skin toxicity and KRAS status are independent predictive markers of cetuximab.<sup>64</sup>

## Additional targets in the EGFR signaling cascade

The role of KRAS mutations has been discovered recently and was discussed before. Aside from KRAS further genes of signaling proteins might be affected by oncogenic mutation and associated with resistance to EGFR inhibition.

Principal effector downstream of KRAS is the serine–threonine–kinase BRAF. A mutation of BRAF with replacement of valine in codon 600 by glutamic acid resulting in an enhanced kinase activity independent from upstream signaling has been described.<sup>65</sup> This V600E allele mutation occurs in approximately 10% of colorectal cancer patients. It is associated with microsatellite instability and poor survival of colon cancer patients.<sup>66,67</sup> BRAF mutations in

colorectal cancer cause resistance to EGFR-targeted therapy with shorter PFS and OS compared to nonmutated patients.<sup>68</sup> Remarkably, treatment with the BRAF-inhibitor sorafenib in *in vitro* experiments restored sensitivity to cetuximab in colon cancer cells.<sup>68</sup>

PI3K-activating mutations occur in around 13% of colorectal cancer patients.<sup>69</sup> The hot spots are located in exon 9 and 20; they correlate with resistance to cetuximab in metastatic colorectal cancer *in vivo* and *in vitro*.<sup>70,71</sup> On the other hand, Prenen and colleagues reported a series of 200 patients with 12% PI3K mutants without any correlation of PI3K mutations to cetuximab response.<sup>72</sup> Therefore, further data on this topic are urgently needed.

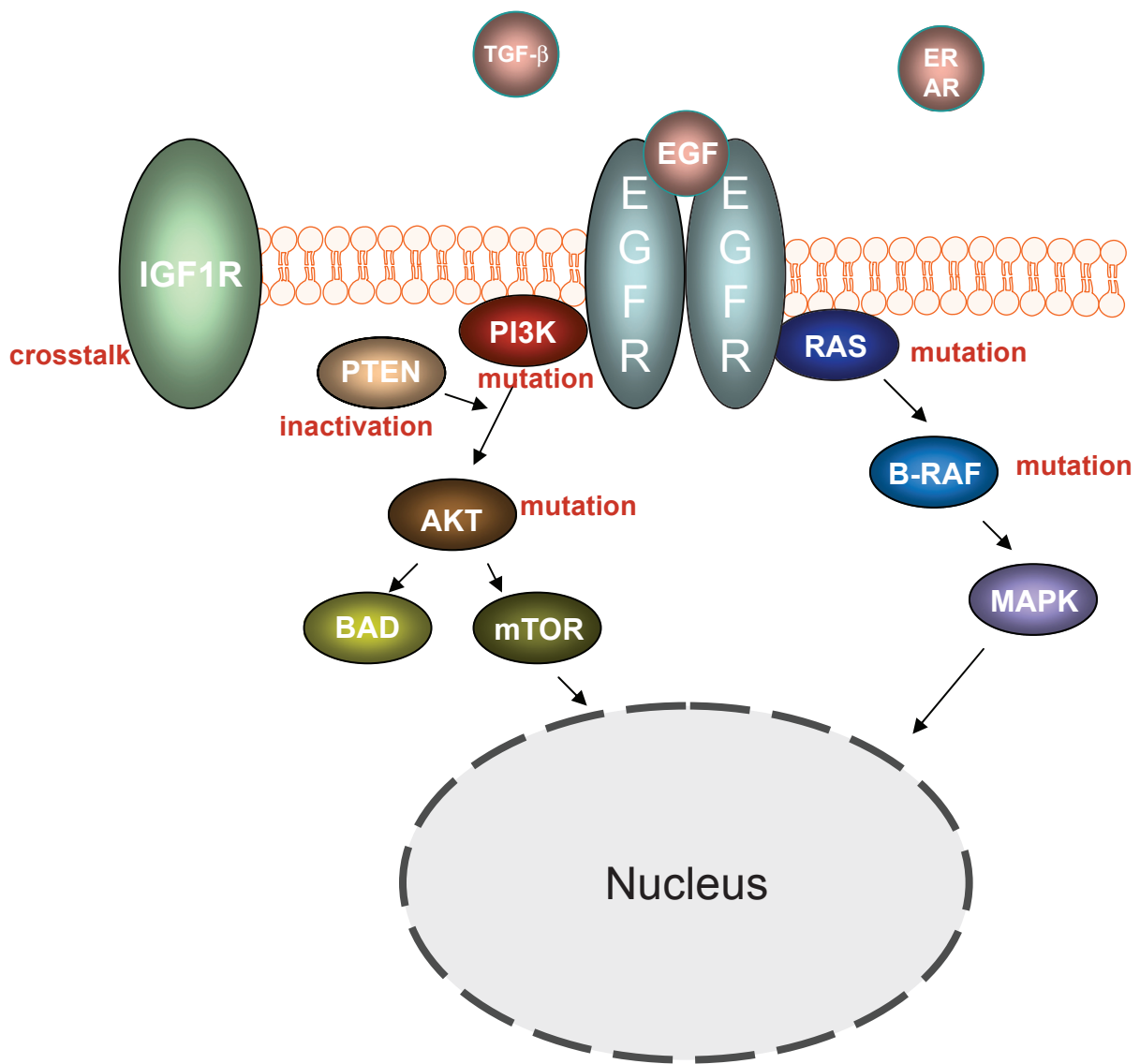
The PTEN tumor suppressor is a negative regulator of PI3K signaling. Inactivating mutations or promoter methylation cause loss of expression in 20% of microsatellite-unstable tumors and was reported to occur in up to 10% of colorectal cancer patients.<sup>73</sup> Since loss of PTEN function results in uncontrolled PI3K activation, patients do not benefit from EGFR blockade with cetuximab.<sup>74–76</sup> Interestingly, the concordance between primary tumor and metastases is low with 60%. Only PTEN loss on metastases predicted resistance to cetuximab plus chemotherapy in this patient cohort.<sup>76</sup> In *in vitro* experiments the effect of a PTEN-activating drug was shown, but it remains unclear if it would be able to induce re-expression of the protein.<sup>77</sup>

AKT is a serine–threonine–kinase known as a main effector of PI3K signaling. The E17K mutation induces an upstream-signal independent constitutive activation of AKT and resistance to cetuximab.<sup>35</sup> This signaling pathway can be selectively targeted by mTOR inhibitors.<sup>78</sup> This approach has already been successfully investigated in metastatic melanoma with constitutively activated PI3K.<sup>79</sup>

No mutations have been described in the STAT pathway, nor was STAT associated with resistance to EGFR targeting,<sup>35</sup> although, only a small number of samples have been screened.

In the SRC/FAK pathway, activation may be caused by upregulation.<sup>35</sup> Src mutations have not been confirmed in colorectal cancer.<sup>80</sup> *In vitro*, SRC upregulation has been associated with cetuximab resistance in non-small cell lung cancer cell line.<sup>81</sup> Remarkably, treatment with the SRC-inhibitor dasatinib restored cetuximab sensitivity.<sup>81</sup> In addition SRC-inhibitors were found to be effective in colorectal cancer *in vitro*.<sup>82</sup>

Another interesting target is the IGF1 receptor that can dimerize with EGFR and could directly interact with EGFR downstream signaling and bypass cetuximab blockade.<sup>83</sup>



**Figure 1** Simplified EGFR-signaling pathway. Ligands are EGF, TGF- $\beta$ , ER, and AR. The two main pathways are the PI3K/AKT/mTOR and RAS/RAF/MAPK cascades. The IGF1 receptor can dimerize with EGFR and activate EGFR signaling. Alternatively, downstream crosstalk between signaling cascades may occur. Alterations of downstream effectors known to interfere with EGFR signaling are marked in red.

**Abbreviations:** AR, amphiregulin; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, epiregulin; IGF1, insulin-like growth factor-1; TGF- $\beta$ , transforming growth factor- $\beta$ .

Resistance to EGFR-blocking therapeutics could evolve through alternate mechanisms causing independence of EGFR activation. Beside alterations in the EGFR signal transduction pathway tumors may escape EGFR blockade by increased angiogenesis, activation of alternative tyrosine kinases or receptor mutations (Figure 1).<sup>84,85</sup>

### Future directions: immunological biomarkers

Antibody-dependent cell-mediated cytotoxicity (ADCC) is one of the main modes of therapeutic antibodies such as trastuzumab or rituximab.<sup>86,87</sup> Polymorphisms in the IgG

fragment C receptor affecting ADCC have been shown to be associated with efficacy of those antibodies.<sup>86,87</sup> Zhang and colleagues reported two FCGR polymorphisms associated with efficacy of cetuximab monotherapy in 39 patients.<sup>88</sup> This could be confirmed in another cohort of 69 patients treated with cetuximab plus irinotecan with longer PFS for patients with FCGR2a-131 H/H and/or FCGR3a-158V/V genotypes. The predictive effect of Fc polymorphisms remained independent of the KRAS status.<sup>89</sup>

Recently, expression of human leukocyte antigen-E (HLA-E) antigen was studied on colorectal cancer tissue. This nonclassical major histocompatibility complex (MHC)

molecule is overexpressed in human colon cancer and associated with inhibition of natural killer (NK)-mediated cell lyses and might explain the escape to immunological control in this cancer type. Upregulation of HLA-E was also associated with shorter survival of Dukes' C patients.<sup>90</sup> *In vitro*, cetuximab-mediated cytotoxicity was hampered in HLA-E overexpressing cells.<sup>91</sup> While direct antiproliferative effects were seen in cell lines only at high concentrations, ADCC was observed already in low cetuximab concentrations in HLA-E normal-expressors suggesting ADCC as the main effect of cetuximab. The role of HLA-E overexpression needs to be further explored. Probably, it might serve as a new biomarker and future target.

## Summary

EGFR-targeted therapy with cetuximab in colorectal cancer has made significant progress in the recent years. However, the detection of KRAS mutations and their potential impact for treatment guidance opened several new questions. For the future we need to identify patients who will benefit most from EGFR inhibition. This may be available by establishment of a reliable panel of biomarkers that can predict the response to cetuximab. Besides KRAS, this approach involves genetic analyses of ligands and downstream effectors. Additionally, polymorphisms of Fc receptors and HLA-E expression in tumor tissue should be analyzed and correlated to clinical outcome. Based on these evolving data new targets may be identified and could lead to new combinations of targeted drugs. Translational research in medical oncology remains a major challenge in the next years in order to provide the background for personalized treatment of cancer patients.

## Disclosure

The authors report no conflicts of interest in this work.

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