

Review

Ras Mutational Status Is a Biomarker for Resistance to EGFR Inhibitors in Colorectal Carcinoma

WOLFRAM C.M. DEMPKE¹ and VOLKER HEINEMANN²

¹Elbländ Clinic Riesa, Medical Oncology, D-01589 Riesa, Germany;

²University of Munich, University Hospital of Grosshadern (Hematology and Oncology), D-81377 Munich, Germany

Abstract. The epidermal growth factor receptor (EGFR) has been validated as a therapeutic target in several human tumours, including colorectal cancer (CRC). Although EGFR expression is used for patient selection, clinical experience shows that levels of EGFR expression (measured by immunohistochemistry) do not predict clinical benefit. Ras mutations in codons 12, 13 and 61 (found in 40-45% of CRC cases) result in inhibition of GTPase activity, thus leading to the constitutive activation of the ras proteins, which may render tumour cells independent of EGFR signalling and thereby, resistant to cetuximab, panitumumab and EGFR TKIs. Data from several recently published studies, as reviewed in this article, in patients with metastatic CRC (OPUS, CRYSTAL) clearly indicated that benefit from cetuximab, when added to chemotherapy, was only restricted to patients with wild-type K-ras tumours. These results showed that K-ras mutations predict the lack of clinical benefit from cetuximab and panitumumab therapies in CRC and indicate that K-ras status should be considered when selecting CRC patients as candidates for these antibodies. Moreover, the results from these studies should also trigger retrospective analyses of K-ras mutations from all available trials in CRC (as well as non-small cell lung cancer and pancreatic cancer). These studies may enable further establishment of the correlation between K-ras mutations and resistance to cetuximab and panitumumab in CRC patients.

Anti-epidermal growth factor receptor (anti-EGFR)-targeted therapies have improved the efficacy of conventional chemotherapy in both preclinical and clinical studies (Table I). Although such therapies may lead to partial response or disease stabilisation in some patients, many patients do not benefit from anti-EGFR therapy, and those who do, eventually develop resistance to it. Great interest, therefore, exists in elucidating resistance mechanisms for anti-EGFR therapies, as well as those for chemotherapy agents. The molecular mechanisms of resistance to anti-EGFR agents may be attributed to several general processes: (i) resistance due to activation of alternative tyrosine kinase receptors that bypass the EGFR pathway (e.g. c-Met, IGF-1R), (ii) resistance due to increased angiogenesis, (iii) resistance, based on constitutive activation of downstream mediators (e.g. PTEN, K-ras, and others), and (iv) the existence of specific EGFR mutations.

Although a large body of preclinical and clinical studies has shed light into the underlying molecular mechanisms for the observed resistance (reviewed in (1)), the lack of validated predictive markers of benefit from anti-EGFR agents may be the result of the complex biology of the EGFR system itself. This complexity arises from the existence of multiple EGFR ligands, a variety of receptor dimerisation partners and the frequent occurrence of receptor cross-talk with members of other receptor families, among other things. Furthermore, it is likely that the biological consequences of EGFR activation vary as a consequence of other mutations present in the tumour. Taking all these factors into consideration, although it has been possible to identify some predictors of clinical benefit (e.g. EGFR overexpression), it may be more fruitful to identify negative predictive factors of benefit to anti-EGFR agents. These factors may be markers that, when present, would render tumours EGFR-independent and therefore not sensitive to EGFR inhibition.

Since emerging data from recently published studies have now suggested that a hyperactive mutant K-ras is likely to

Correspondence to: Wolfram C.M. Dempke, MD, Ph.D., Elbländ Clinic Riesa, Medical Oncology, Weinbergstrasse 8, D-01589 Riesa, Germany. Tel: +49 3525753552, Fax: +49 3525753578, e-mail: wolfram.dempke@elbländkliniken.de

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Table I. EGFR inhibitors currently approved for cancer treatment.

Drug (commercial name)	Category (target)	Status
Erlotinib (Tarceva®)	Tyrosine kinase inhibitor (EGF-R, erbB-1)	Approved for NSCLC, pancreatic cancer
Gefitinib (Iressa®)	Tyrosine kinase inhibitor (EGF-R, erbB-1)	Approved for NSCLC (Asian countries)
Lapatinib (Tyverb® and Tykerb®)	Tyrosine kinase inhibitor (erbB-1, erbB-2)	Approved for metastatic breast cancer (preliminary approval)
Cetuximab (Erbitux®)	Human-mouse chimeric monoclonal antibody (IgG1 subtype) (EGF-R)	Approved for CRC (<i>K-ras</i> wild-type patients only), head and neck tumours
Panitumumab (Vectibix®)	Fully human monoclonal antibody (IgG2k subtype) (EGF-R)	Approved for CRC
Trastuzumab (Herceptin®)	Humanised monoclonal antibody (IgG1 subtype) (erbB-2)	(<i>K-ras</i> wild-type patients only) Approved for breast cancer (adjuvant and metastatic)

be a powerful negative predictive factor of EGFR inhibitor response (reviewed in (1)), it was the aim of this review to summarize current clinical data in terms of whether or not the *K-ras* mutational status (Table II) may serve as a biomarker for anti-EGFR therapies.

EGFR and Ras Oncogenes: Molecular Biology

Receptor tyrosine kinases (RTKs), such as EGFR and vascular endothelial growth factor receptor (VEGFR), are transmembrane proteins with an extracellular ligand-binding domain and an intracellular tyrosine kinase catalytic domain. On binding to their cognate ligands, most RTKs dimerise and become activated through autophosphorylation of intracellular tyrosine residues. Activation of RTKs results in up-regulation of multiple cellular signalling pathways that promote cell growth, survival and angiogenesis or environmental stimuli. Inappropriate activation of RTKs via mutation, overexpression or ectopic ligand production is a frequent feature of human tumour development and progression and is thought to be a major mechanism by which cancer cells subvert normal growth control (2-4). Consequently, in recent years, modulation of RTK signal transduction has been an active area in oncology drug discovery.

EGFR (also called erbB1) and other erbB family RTKs (erbB-2/HER-2/neu, erbB-3/HER-3 and erbB-4/HER-4) encoded by the *c-erbB* proto-oncogenes have been strongly implicated in cancer development and progression ((3) and reviewed in (5)). Several mechanisms cause aberrant receptor activation, resulting in tyrosine kinase activity, which is observed in cancer. Such mechanisms include receptor overexpression, mutation, ligand-dependent receptor dimerisation and ligand-independent activation. For erbB-2, where a specific ligand has not been identified, activation occurs by homo- or hetero-dimerisation alone, whereas erbB-3 does not have significant kinase activity (5, 6). However, on activation, all four receptors are capable of signal transduction, causing activation of the ras/MAP kinase pathway, the

Table II. Frequency of ras mutations in various tumours. The data are from the study by Dempke (28).

Tumour	Mutation	Incidence
Pancreatic carcinoma	K-ras	90%
Colorectal carcinoma	K-ras	40-45%
Seminoma	N-ras	43%
Lung cancer	N-ras, K-ras	15-30%
MDS/AML	N-ras	30%
Ovarian carcinoma	H-ras	23%
Malignant melanoma	N-ras	20%

PI3K/Akt pathway, src family kinases and STAT proteins. Activation of these pathways promotes cell proliferation, survival and angiogenesis (7).

Several other downstream signalling mediators (e.g. Akt mTOR, src kinases, STAT proteins, *K-ras* and MEK1/2) have been reported to bypass EGF-R inhibition by constitutive activation of multiple pathways and some of them (mTOR and MEK1/2 inhibitors) are now being targeted in combination with EGFR inhibitors in early-phase clinical trials (8). Amongst them, the ras/MAP pathway is of potential clinical interest. The ras proteins are members of a large superfamily of guanosine-5'-triphosphate (GTP)-binding proteins that play a complex role in the normal transduction of growth factor receptor-induced signals (9). Stimulation of growth factor receptors, such as EGFR, causes activation of multiple regulatory molecules, including the ras protein. EGFR activates ras by stimulating its binding to GTP. Ras, in its active, GTP-bound state, binds several key target proteins, resulting in the subsequent activation of several downstream pathways, including those mediated by MAP kinase, PI3K and others (10). Engagement of these pathways leads to stimulation of cell-cycle progression, desensitisation of the cell to pro-apoptotic stimuli, changes in cytoskeletal organisation and invasion and other processes

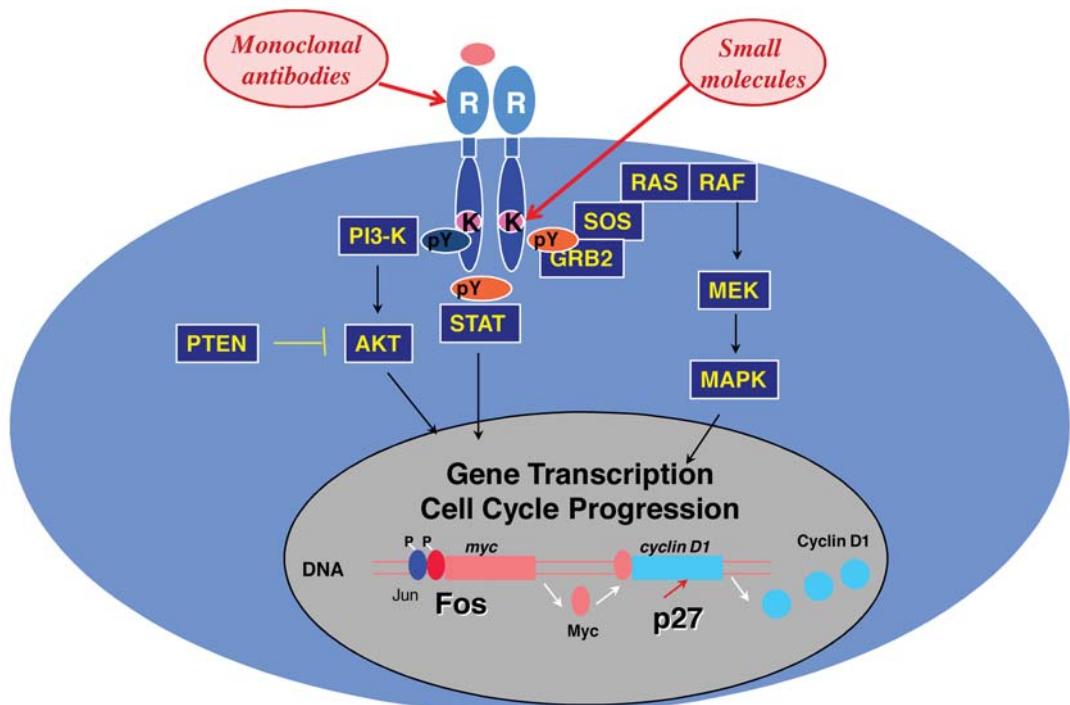


Figure 1. Cascade of EGFR- and ras-modulated signal transduction.

required for cell proliferation. Activating mutations in the *K-ras* gene, which result in EGFR-independent activation of the MAP-kinase pathway, are found in approximately 15–30% of patients with non-small cell lung cancer (NSCLC) and 40–45% of patients with colorectal cancer (CRC) (Table II), and their presence generally correlates with a worse prognosis with respect to the outcome of the cancer. In most cases, the somatic *ras* missense mutations found in cancer introduce amino acid substitutions at positions 12 (Gly->Val), 13 and 61. These mutations disable the endogenous GTPase activity of the *ras* protein and cause cancer-associated *ras* to accumulate in the active, GTP-bound conformation. This, in turn, results in activation of PI3K, MAP kinase and others, causing malignant transformation. Because *ras* is downstream from EGFR, aberrant *ras* signalling, like that occurring in cells with mutant *K-ras*, may lead to dysregulation of *ras*-dependent pathways and downstream signalling even when the upstream receptor is silenced by anti-EGFR monoclonal antibodies or RTKs (Figure 1).

In several studies, *K-ras* mutations have been significantly associated with a lack of response to EGFR tyrosine kinase inhibitors in patients with NSCLC and a lack of response to cetuximab or panitumumab in patients with advanced CRC, as detailed below. Both findings suggest that EGFR-independent, constitutive activation of the *K-ras* signalling pathway would impair the response to anti-EGFR drugs (11).

Clinical Data

Effect of *K-ras* mutation on response to anti-EGFR therapy. The clinical relevance of *K-ras* mutations has been evaluated retrospectively in several clinical trials investigating the effect of EGFR inhibitors such as cetuximab or panitumumab in first-line treatment of metastatic CRC. Van Cutsem *et al.* (12) reported the CRYSTAL trial which compared 5-fluorouracil, folinic acid and irinotecan (FOLFIRI) plus cetuximab to FOLFIRI alone. An analysis of 45% of the study population (540 out of 1198 patients) revealed a *K-ras* mutation in 35.6% of evaluable tumours. This study demonstrated that the addition of cetuximab to FOLFIRI significantly improved progression-free survival (PFS) in *K-ras*-wild-type (*K-ras*-WT) patients (hazard ratio (HR)=0.68, *p*=0.017), while no improvement was observed in patients with *K-ras*-mutant tumours (*K-ras*-Mut patients) (HR=1.07, *p*=0.47). Likewise, the overall response rate (ORR) was significantly improved in *K-ras*-WT patients (43% vs. 59%, *p*=0.0025), but not in the mutant population (40% vs. 36%, *p*=0.46).

The OPUS trial selected a combination of 5-fluorouracil, folinic acid and oxaliplatin (FOLFOX4) as a chemotherapy backbone and investigated the addition of cetuximab in a randomised trial (13). Of 337 patients included in this first-line trial, 233 patients were evaluable for their *K-ras* status

and a *K-ras* mutation was found in 42% of them. In *K-ras*-WT patients, the addition of cetuximab to FOLFOX4 caused a significant increase of ORR (61% vs. 37%, $p=0.011$) and PFS (HR=0.57, $p=0.016$). In contrast, a negative impact on treatment efficacy was noted when cetuximab was applied in *K-ras*-Mut patients with regard to PFS (HR=1.83, $p=0.0192$) and ORR (33% vs. 49%, $p=0.106$).

A comparable effect was also noted in the CAIRO II trial which compared capecitabine/oxaliplatin (CapOx) plus bevacizumab to the same regimen plus cetuximab (14). The addition of cetuximab did not affect ORR or PFS in *K-ras*-WT patients. However, in *K-ras*-Mut patients, it induced a markedly shorter duration of PFS (8.6 months vs. 12.5 months, $p=0.043$) and OS (19.2 months vs. 24.9 months). An explanation for this apparently negative interaction of cetuximab with oxaliplatin-based chemotherapy in *K-ras*-Mut patients is far from clear.

The PACCE trial was designed to investigate double-targeting of VEGF and EGFR. Patients with metastatic CRC received first-line treatment with irinotecan- or oxaliplatin-based chemotherapy and were randomised to additional treatment with either bevacizumab plus panitumumab or bevacizumab alone (15). A subgroup analysis was performed for patients with irinotecan-based chemotherapy ($n=200$). In *K-ras*-WT patients ($n=115$), the addition of panitumumab to irinotecan/bevacizumab-based therapy induced an ORR of 54% compared to 47% without the EGFR inhibitor. Also in this study, no improvement of ORR was observed when panitumumab was given to *K-ras* mutant patients (30% vs. 38%). Taken together, the PACCE study and the CAIRO II study indicate that in the presence of VEGF-inhibition by bevacizumab, additional inhibition of EGFR does not provide further clinical benefit.

All four trials described above uniformly demonstrate that *K-ras* mutation confers resistance to anti-EGFR-directed antibodies. Furthermore, these data are supported by a large body of evidence from phase II and case-control studies showing the lack of efficacy of anti-EGFR antibodies in pre-treated patients (16-21). As a consequence, registration of cetuximab and panitumumab limits their use to patients with *K-ras* wild-type tumours. Determination of the *K-ras* mutational status is, therefore, required before the clinical application of anti-EGFR-directed antibodies. At present, multiple methods are available for the detection of *K-ras* mutations. Given that cross-validation of sensitivity, specificity and reliability is presently being evaluated, a single best method has not yet been defined.

Skin toxicity developing during the first weeks of treatment is an important predictor of response to anti-EGFR therapy (22), but is independent of *K-ras* mutational status. The greatest benefit from anti-EGFR therapy may, therefore, be expected in *K-ras* wild-type patients reacting to treatment with marked skin toxicity (23).

The results from these studies should therefore trigger retrospective analyses of *K-ras* mutations from all available trials in CRC (as well as NSCLC and pancreatic cancer). Analysis of *K-ras* mutations may also be important in the cetuximab-containing N0147 and the PETACC-8 adjuvant studies in CRC. These studies would enable further establishment of the correlation between *K-ras* mutation and resistance to cetuximab and panitumumab in CRC patients (22).

Effect of *K-ras* mutation status on response to anti-VEGF therapy. Given that VEGF is regulated downstream of EGFR and that inhibition of EGFR may cause a down-regulation of VEGF expression, it is of interest to investigate the effect of *K-ras* mutations on anti-VEGF-directed therapy (24, 25). Ince *et al.* (26) performed a retrospective analysis of the pivotal phase III trial which tested the addition of bevacizumab to first-line therapy with irinotecan, 5-fluorouracil and leucovorin (IFL) (26, 27). Micro-dissected tumours from 295 patients were available for determination of mutations in *K-ras* (35%), *B-raf* (5.6%), and p53 (68%). With regard to overall survival, *K-ras*- and *B-raf* wild-type patients had a better prognosis than patients with mutant tumours, but all subgroups showed a benefit from the treatment with bevacizumab. In patients who were wild-type for both *K-ras* and *B-raf*, HR in favour of bevacizumab treatment was 0.57 (95% confidence interval (95%CI)=0.31-1.06), while it was 0.67 (95% CI=0.37-1.20) in patients with mutant tumours. Considering the limitations of such a retrospective analysis, it was suggested that the survival benefit induced by bevacizumab is independent of *K-ras*-, *B-raf*- and TP53-mutation status (26).

Studies over the last few years have identified several anti-EGFR and anti-VEGFR resistance mechanisms. These findings have led to clinical trials using newly designed targeted therapies that can overcome these resistance mechanisms and have shown promise in laboratory studies. Ongoing research efforts will likely continue to identify additional resistance mechanisms and these findings will hopefully translate into effective therapies for different cancers.

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