Genetic and immune factors underlying the efficacy of cetuximab and panitumumab in the treatment of patients with metastatic colorectal cancer

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Efficacy of monoclonal anti-EGFR antibodies (cetuximab, panitumumab) used in combination with chemotherapy or alone has been demonstrated in clinical trials of patients with mCRC. Both drugs block signaling EGFR pathway in malignant cells (blocking ligand binding and EGFR dimerization). Obtaining treatment responses with anti-EGFR agents is possible only in a selected subgroup of patients with mCRC. Successful treatment with cetuximab and panitumab is possible almost exclusively in patients without RAS mutations. Research on predictive value of *EGFR* gene copy number, *PI3KCA* gene mutations, *P53* and *PTEN*, and *EGFR* their ligands concentrations is ongoing. Cetuximab, as IgG1 class antibody, can cause antibody dependent cellular cytotoxicity against neoplasm cells, while panitumumab, as IgG2 class antibody, does not induce such effect. Therefore a potential predictor cetuximab therapy may be the presence of different polymorphic forms of the genes for receptor immunoglobulin Fc fragments: FcγRIIa and FcγRIII subclasses.

Key words: cetuximab, panitumumab, metastatic colorectal cancer, mCRC, EGFR, ADCC.

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Introduction

Colorectal cancer (CRC) is the second (after lung cancer) most common cause of death from malignancy in Poland. In 2010, it led to the death of 3,944 men and 3,435 women [1]. Similarly to many other cancer types, high mortality is a consequence of delayed diagnosis. It often happens that by the time the disease is detected, it has already disseminated and developed distant metastases. A major therapeutic modality for colorectal cancer is systemic treatment including chemotherapy and molecularly targeted therapies. Three molecularly targeted drugs: bevacizumab, cetuximab and panitumumab have been used in the therapy of advanced colorectal cancer. Bevacizumab is an anti-VEGF (vascular endothelial growth factor) monoclonal antibody which acts by inhibiting the process of neoangiogenesis and normalizing the formation of blood vessels within the tumour. Cetuximab and panitumumab are anti-EGFR (epidermal growth factor receptor) monoclonal antibodies. Their mechanism of action involves blocking cancer cell proliferation signal through the inhibition of the signalling pathways EGFR/Pi3K/AKT/mTOR or EGFR/Ras/Raf/MAPK/ERK. Signal blocking leads to the inhibition of cell divisions in the G1 phase due to the lack of required transcription factors, followed by cell elimination by apoptosis (Fig. 1).

Direct inhibition of the binding of a ligand to EGFR through the blocking of the extracellular domain of the receptor by monoclonal antibodies is also accompanied by the process of EGFR homo- or heterodimerization with another member of the HER family, which is a prerequisite for the activation of a signal cascade inside cancer cells. This also leads to the internalization of the EGFR receptor. The therapeutic effect of cetuximab (and, to a limited extent, also panitumumab) also seems to be dependent on the cytotoxic response of the immune system induced against cancer cells coated with EGFR-bound antibodies (antibody-dependent cell-mediated cytotoxicity – ADCC), and the activation of the complement system [2–6].

According to some studies, panitumumab has a higher potential for binding to EGFR, however it is currently believed that both drugs demonstrate similar capacity for receptor binding. Moreover, both drugs achieve comparable therapeutic concentrations in blood plasma. There are, nevertheless, certain differences which may have an impact on the efficacy of therapy and on the potential for adverse reactions of both drugs. The differences result from the molecular structures of both antibodies. Cetuximab belongs to the class of IgG1 antibodies. It is a chimeric molecule containing a murine antigen-binding region. The remaining parts of heavy and light chains are of

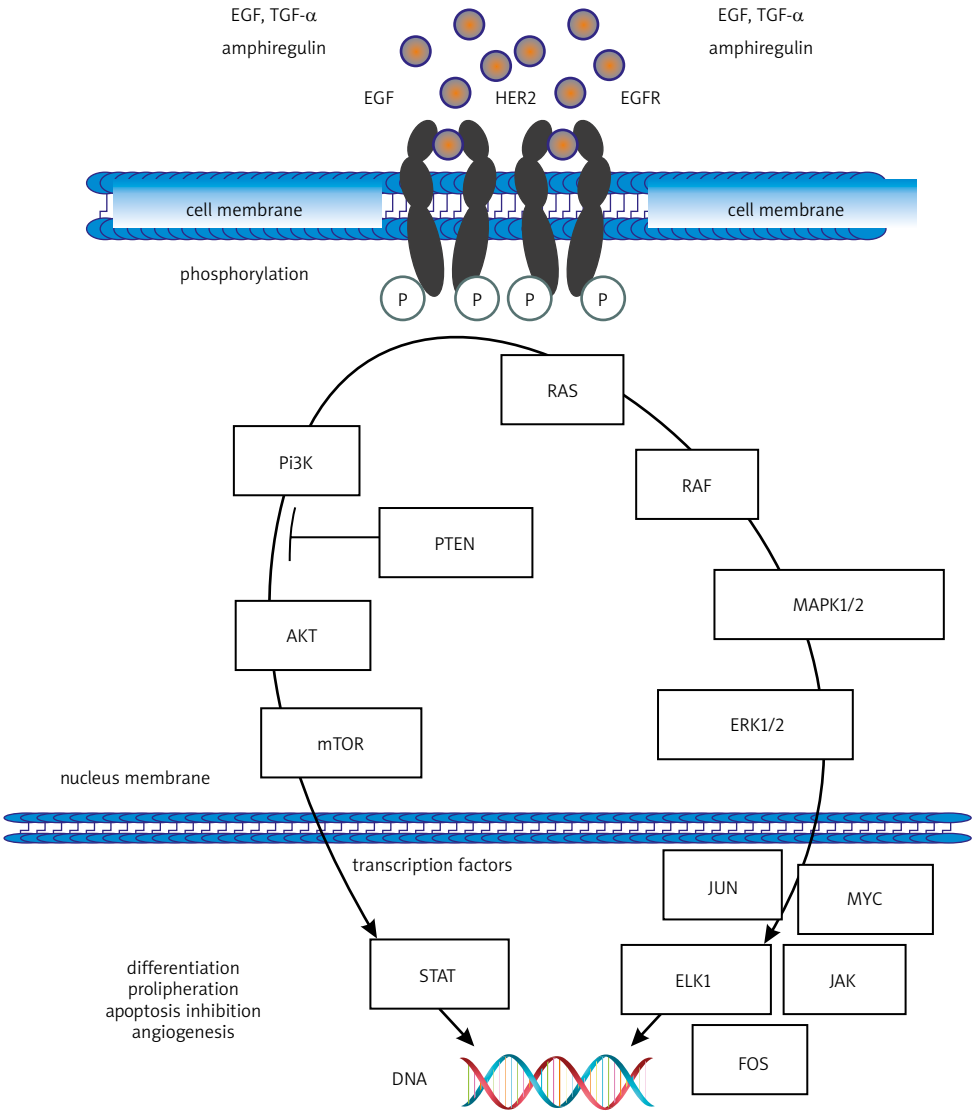

Fig. 1. Intracellular signalling pathway originating at EGFR and inducing the activation of proliferation, inhibition of apoptosis, and differentiation of epithelial and cancer cells

human origin (allergic reactions occur in 2–4% of treated patients, and corticosteroid and antihistamine premedication is required). Measurable concentrations of human anti-chimeric antibodies (HACA) have been detected in 3.4% of patients treated with cetuximab. The formation of HACA, however, is not associated with the development of hypersensitivity reactions to cetuximab, and no HACA-induced neutralizing effect on cetuximab is observed. Panitumumab is a fully human IgG2 antibody which induces allergic reactions in less than 1% of treated patients. It should be noted, though, that contrary to IgG1 antibodies (cetuximab), IgG2 antibodies have no ability to induce ADCC immune response. Importantly, the blood plasma half-life of cetuximab is up to one week, and for panitumumab it reaches two weeks, which is why cetuximab is administered every seven days and panitumumab – every 14 days. As there are no clinical trials directly comparing the efficacy of both agents, they have been approved for use in Poland and in the EU for similar indications in the treatment of colorectal cancer [4, 5].

Indications for cetuximab or panitumumab, and results of major clinical trials conducted in colorectal cancer patients

Cetuximab is indicated for the treatment of patients with epidermal growth factor receptor (EGFR)-expressing, *KRAS* wild-type metastatic colorectal cancer, in combination with irinotecan-based chemotherapy, in first-line in combination with FOLFOX, as a single agent in patients who who have failed oxaliplatin- and irinotecan-based therapy and who are intolerant to irinotecan. Cetuximab monotherapy is regulated within the framework of a drug programme. Panitumumab has similar indications to cetuximab, however according to the SPC it is approved for first-line treatment in combination with FOLFOX, for second-line treatment in combination with FOLFIRI in patients who have received first-line fluoropyrimidine-based chemotherapy (excluding irinotecan) and in monotherapy after failure of chemotherapy regimens containing fluoropyrimidine, oxaliplatin and irinotecan [7].

The efficacy of cetuximab used in monotherapy or in combination with chemotherapy has been analyzed, among others, in five large randomized clinical trials which enrolled over 3,700 patients with mCRC. Study EMR 62 202-013 conducted in patients with *KRAS* wild-type gene demonstrated superiority of first-line FOLFIRI chemotherapy combined with cetuximab over chemotherapy alone in all the analyzed characteristics. Significant increases were observed for median overall survival (OS) from 20 to 23.5 months ($p = 0.0093$) and median progression-free survival (PFS) from 8.4 to 9.9 months, accompanied by an increase in response rate (RR) from 39.7% to 57.3%. No comparable efficacy was found in patients with *KRAS* gene mutations [8–10]. Study CA225006 compared treatment with cetuximab plus irinotecan with irinotecan monotherapy in patients with progressive disease (PD) following oxaliplatin- and fluoropyrimidine-based therapy. The study showed a significant increase in median PFS (4 vs. 2.6 months, $p < 0.0001$) and an increase in objective response rate from 4.2% to 16.4% regardless of the status of the *KRAS* gene in the group of patients treated with cetuximab combined with irinotecan. No difference was noted in median overall survival (ca. 10 months) between the two study groups [11]. Study CA225025 sought to compare cetuximab used in monotherapy with *placebo* in patients with progressive disease following treatment with oxaliplatin, irinotecan and fluoropyrimidine. Cetuximab proved to be similarly effective to best supportive care (BSC) exclusively in patients with *KRAS* wild-type gene (response to treatment was seen exclusively in this group, in 12.8% of subjects). Median OS was found to have increased significantly from 4.8 to 9.5 months, and median PFS – from 1.9 to 3.7 months. In the group of patients with *KRAS* gene mutations parameters describing the efficacy of cetuximab were almost identical to the BSC-treated group [12].

The efficacy of panitumumab in patients with mCRC was comparable to the efficacy of cetuximab for the same therapeutic regimens. Four major randomized studies involved a total of 3,885 patients. In PRIME study, panitumumab was used in combination with FOLFOX for first-line treatment. In patients with no *KRAS* gene mutation the addition of panitumumab to chemotherapy induced a statistically significant increase in therapeutic response rate (48% vs. 57%), a prolongation of median PFS (8.6 vs. 10 months) and median OS (19.7 vs. 23.9 months). Panitumumab used in patients with a *KRAS* gene mutation had no effect on RR (ca. 40%). A significant reduction in median PFS and an insignificant reduction in median OS compared to chemotherapy alone were noted in this group of patients (7.4 and 9.2 months, and 15.5 and 19.2 months, respectively) [13, 14]. The effects of panitumumab plus FOLFIRI vs. FOLFIRI alone as second-line therapy was investigated in study NCT00339183. Among *KRAS* gene wild-type patients a statistically significant increase in the response rate (10% vs. 36%), an extension of median PFS (6.6 months vs. 7.6 months) and median OS (12.5 months vs. 14.5 months) were achieved. In the group of *KRAS* mutation positive patients the efficacy of the FOLFIRI regimen was similar regardless of whether it was combined with panitumumab or used alone [15]. Administered in monotherapy, panitumumab – similarly to cetuximab – induced an objective treatment response only among patients with *KRAS* wild-type gene. Median PFS in this group of patients was 16 weeks, as opposed to 8 weeks in the placebo group. A median PFS of 8 weeks was observed in the group of *KRAS* mutation positive patients receiving panitumumab or placebo [16].

Role of determining EGFR expression for the eligibility of treatment with cetuximab or panitumumab

Activation of the signal transduction pathway which originates at EGFR in abnormal cells plays a central role in the development of many types of cancer including the two most common, i.e. non-small-cell lung carcinoma (NSCLC) and colorectal cancer. These cancers are usually associated with elevated blood plasma levels of EGFR ligands including EGF, amphiregulin (AREG), epiregulin (EREG) and TGF-α (transforming growth factor α), and high expression of HER family membrane receptors: HER1 (EGFR), HER2, HER3 and HER4 on the surface of cancer cells. EGFR expression on CRC cells is identified in over 80% of patients. Data on correlations existing between the degree of EGFR expression on cancer cells and the degree of clinical advancement of CRC, survival time, and rate and extent of metastatic spread, are controversial. Some studies have demonstrated that EGFR expression is the greatest in the most invasive tumour areas, in locations where cancer infiltrates peri-intestinal tissues, and in lymph node and distant metastases. It appears, then, that high expression of EGFR (and its ligands such as amphiregulin) may be a poor prognostic factor in CRC patients. Several other studies [17–21], however, have found no evidence to support the above hypothesis.

Considering that cetuximab and panitumumab act by blocking the extracellular domain of EGFR, it appeared that the efficacy of both drugs would be conditional on the presence of EGFR on the surface of cancer cells. The requirement for immunohistochemical (IHC) detection of EGFR expression in tumour material preserved in paraffin blocks to determine eligibility of mCRC patients for cetuximab therapy is included in the SPC of the drug [20]. In Poland, the opinion issued by the Consultative Council of the Agency for Health Technology Assessment (AOTM) has been used as a basis for the development of drug programmes under which eligibility for cetuximab or panitumumab treatment is limited to patients with positive EGFR expression and lack of *KRAS* gene mutations in cancer cells.

Most early clinical trials required an assessment of EGFR expression for determining eligibility for cetuximab or panitumumab therapy. The predictive value of the degree of EGFR expression, however, was not confirmed in two randomized studies (CRYSTAL and OPUS) [8, 9]. In the randomized phase III COIN trial (Continuous Chemotherapy plus Cetuximab or Intermittent Chemotherapy) EGFR expression was no longer listed among inclusion criteria for cetuximab treatment. The study showed no significant benefits of adding cetuximab to chemother-

apy mainly due to treatment delays and the necessity to reduce doses of cytostatic agents due to toxicity effects. Cetuximab significantly increased the response rate (57% vs. 64%) and prolonged median PFS in patients receiving cetuximab with a regimen containing oxaliplatin and fluorouracil in combination with folic acid (Modified de Gramont with Oxaliplatin – OxMdG) [10]. Moreover, study results have been published indicating that positive EGFR expression is not a precondition for the efficacy of cetuximab, while response to treatment is possible in patients with negative receptor expression. In one of the first reports Chung *et al.* revealed a potential for achieving response to cetuximab treatment alone or in combination with irinotecan in 25% of patients with chemotherapy refractory EGFR-negative metastatic CRC [22]. Similar results were obtained by Hebbar *et al*., who even concluded that response to treatment with cetuximab combined with irinotecan was more frequently observed in those of oxaliplatin- and irinotecan-refractory subjects who were EGFR expression-negative than EGFR expression-positive [23]. Han *et al.* demonstrated that the finding could be attributed to different monoclonal antibodies used in immunohistochemical diagnostic assays for EGFR expression which gave false negative results of EGFR expression on cancer cells [24]. CE/IVD certified IHC tests which are currently commonly used in CRC patients for the detection of EGFR expression, e.g. EGFR PharmDx (Dako), are expected to detect the presence of receptor on cancer cells in over 95% of patients [20]. In view of the results of studies cited above, it is increasingly claimed that there are no grounds for IHC diagnostic tests determining EGFR expression to assess eligibility of mCRC patients for anti-EGFR antibody treatment. This is especially important in view of large differences in results of EGFR expression assays obtained in different Polish medical centres. In some of them, a considerable number of patients may, in fact, be erroneously excluded from molecularly targeted therapy on the basis of lack of EGFR expression despite the presence of wild-type KRAS gene in tumour cells. The provision included in the therapeutic programme, however, remains unchanged and in order to be considered eligible for therapy with anti-EGFR antibodies, mCRC patients must be EGFR expression-positive.

Studies investigating the predictive value of the assessment of the number of *EGFR* gene copies for therapy with anti-EGFR antibodies in mCRC cancer patients having a wild-type *KRAS* gene have failed to yield unambiguous results. With the help of suitable techniques including fluorescence *in situ* hybridization (FISH), chromogenic *in situ* hybridization (CISH) or, less commonly, silver *in situ* hybridization (SISH) it has been shown that an incorrect *EGFR* gene copy number occurs heterogeneously in different areas of the CRC tumour. The majority of studies have demonstrated correlations between polysomy or amplification of the *EGFR* gene and the potential for achieving objective response to treatment and prolongation of PFS. In addition, in many studies the OS of patients treated with anti-EGFR antibodies has been similar regardless of having a normal or increased number of copies of the *EGFR* gene [6, 25]. In the study conducted by Scartozzi *et al*. among subjects treated with irinotecan-cetuximab the PFS of patients with a high number of *EGFR* gene copies was found to be significantly longer, whereas in the studies by Laurent-Puig *et al.* and Personeni *et al.* conducted in cetuximab-treated patients there was a slight increase of OS in subjects with a high number of *EGFR* gene copies compared to patients with a low number of copies of the gene [26–28]. The most spectacular results regarding the efficacy of cetuximab or panitumumab in different lines of treatment were obtained by Algars *et al.* who observed a significant prolongation of PFS and OS in patients without *KRAS* gene mutations and with more than four copies of the *EGFR* gene compared to patients with a low number of copies of that gene. According to the authors, clinical benefit of anti-EGFR antibody therapy occurred in 82% of *KRAS* wild-type gene patients with a high number of copies of the *EGFR* gene (with remission noted in 36% of patients), whereas in subjects with a low number of *EGFR* gene copies remission and stable disease were rarely observed (6% and 13%, respectively) [29]. In 2013, Jiang *et al*. published a metaanalysis of eight studies on the effects of EGFR gene polysomy on the efficacy of cetuximab or panitumumab in different therapeutic regimens used in patients with mCRC. The authors demonstrated that a high number of copies of the *EGFR* gene causes a significant increase in OS (HR = 0.62) and PFS (HR = 0.65) in patients receiving anti-EGFR antibodies, and is associated with a higher incidence of skin rash during the therapy. On that basis, it can be assumed that an assessment of EGFR gene copy number alterations provides a good predictive factor for the eligibility of mCRC patients for anti-EGFR antibody treatment [30, 31]. It seems that the assessment may prove to be much more valuable for appropriate qualification of patients for this type of treatment than IHC-based assays for EGFR expression.

Equally debatable are the results of studies investigating correlations between various polymorphic forms of the EGFR gene and the efficacy of anti-EGFR antibodies in mCRC patients. Genetic polymorphism refers to the simultaneous occurrence of various forms of the same gene in a population (e.g. single-nucleotide polymorphism), which may lead to differences in the structure and characteristics of the protein encoded by this gene. As opposed to driver mutations (there have only been reports on isolated CRC patients with mutations in exons 20 and 21 of the *EGFR* gene), genetic polymorphism occurs in at least 1% of members of a given population, and affects not only cancer cells but all body cells. Intron 1 of the EGFR gene can be affected by a polymorphism causing variation in the number of tandem CA repeats. The longer form of intron 1 is associated with a reduced transcriptional capability of the EGFR gene and hence lower expression of the EGFR protein on the surface of epithelial cells. Analyses were also performed for other polymorphisms in the *EGFR* gene: G216T and G497A, and in the *EGF* gene: A61G. Graziano *et al.* demonstrated the presence of the short variant of the *EGFR* gene intron-1 and the G allele in codon 61 of the *EGF* gene (higher EGF production) to be a favourable predictive factor for cetuximab-irinotec-

an therapy. In addition, a smaller number of CA repeats in intron 1 is associated with more frequent adverse reactions accompanying treatment, manifested as skin rash [6, 25, 31].

Impact of mutations in *KRAS* and *BRAF* genes, and other rare mutations on the efficacy of cetuximab or panitumumab

Mutations in the *KRAS* gene and possibly also in the *BRAF* gene are the fundamental negative predictive factors in anti-EGFR antibody treatment of mCRC patients. Mutations in the *KRAS* oncogene are the most common genetic abnormalities identified in CRC cells and in the majority of human cancers in general. They are detected in 20–50% of CRC patients. High discrepancy of results defining the incidence of KRAS gene mutations stems from the diversity of diagnostic methods and types of mutations under study. The most important KRAS gene mutations occur in exons 1 and 2 in codons 12 (most commonly), 13 and 61. The mutations represent single-nucleotide substitutions resulting in the replacement of glycine in codons 12 and 13, and glutamine in codon 61, with another amino acid (G12C, G12V, G12D, G12R, G12A, G12S, G13D, G13C, Q61K, Q61R, Q61L). Since the KRAS protein plays a key role in the intracellular transduction cascade originating at EGFR, KRAS damage and excessive activity generates a signal for cell proliferation or differentiation regardless of EGFR activation or lack of it. What this means is that effectors continuously transmit signal to the cell nucleus, activating appropriate transcription factors (Fig. 2) [32–37].

Large clinical trials have shown that the majority of chemotherapy-refractory mCRC patients with mutations in the KRAS gene (regardless of mutation type) are also refractory to chemotherapy combined with anti-EGFR antibody treatment. Objective response to this treatment modality is observed in 2–15% of patients with *KRAS* gene mutations and in ca. 35–40% of patients with wild-type *KRAS* gene [11, 15]. Assessment of the influence of *KRAS* gene mutations on the efficacy of chemotherapy and cetuximab in patients who have had no previous *chemotherapy* is ambiguous. The addition of cetuximab or panitumumab to chemotherapy compared to chemotherapy alone in untreated mCRC patients with wild-type *KRAS* gene increases the response rate from over 35% to nearly 60%, prolongs PFS to over 9 months and extends OS by a mean of ca. 4 months [8, 9, 13]. The PRIME study even demonstrated that panitumumab added to chemotherapy in the treatment of patients with *KRAS*-mutated colorectal cancers reduced progression-free survival compared to patients treated by chemotherapy alone [13]. The outcomes of the studies seem to suggest that there are no benefits of adding anti-EGFR antibodies to chemotherapy in patients with a mutated *KRAS* gene, however on account of the fact that the efficacy of this type of

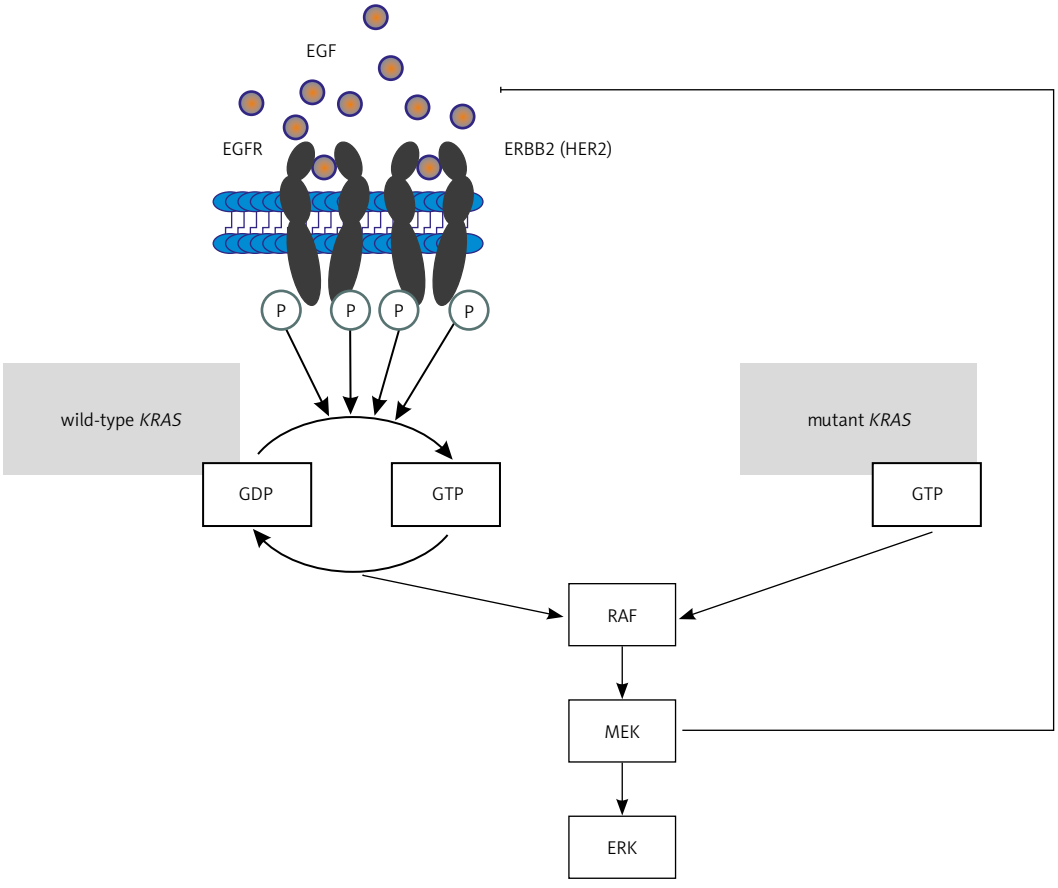

Fig. 2. Role of normal and mutated KRAS protein in the regulation of the signalling pathway associated with EGFR activation

treatment depends on multiple factors (e.g. crossover to alternative treatment after disease progression, and use of subsequent lines of therapy), it is extremely difficult to evaluate the effect of anti-EGFR monoclonal antibodies in this patient group. The role of *KRAS* gene mutations in the development of refractoriness to cetuximab and panitumumab monotherapy is discussed above [12, 16]. In other studies, Karapetis *et al.* report objective response to cetuximab monotherapy in just one patient with a *KRAS* gene mutation (1.8% of mutation-bearing patients) and in 12.8% of mutation-free patients. In the group of cetuximab-treated patients with wild-type *KRAS* gene the authors report longer PFS (3.7 months) and OS (9.5 months) compared to patients with mutated *KRAS* gene receiving this antibody (1.8 and 4.5 months, respectively). Moreover, in the latter group of patients there were no differences in median PFS and OS depending on the type of treatment [36]. Amado *et al.* identified similar differences in response rates in patients treated with panitumumab monotherapy, achieving response in 17% of patients with wild-type *KRAS* gene and no response in patients with mutated *KRAS* gene. Panitumumab-treated patients with wild-type *KRAS* gene had longer PFS and OS than patients with KRAS mutations who received the same antibody therapy [38].

In view of the study results presented above and the indisputable role of *KRAS* gene mutations as a negative predictive factor both for cetuximab and panitumumab therapy, subsequent clinical studies always incorporated an analysis of *KRAS* gene mutations to determine patient eligibility for treatment. The first of these was the COIN study mentioned above. Despite multiple divergences from the protocol, COIN still represented a prospective study involving an analysis of mutations in the *KRAS* gene. Cetuximab combined with the FOLFOX chemotherapy regimen or capecitabine plus oxaliplatin (CAPOX) in patients with wild-type *KRAS* gene increased median OS to 23 months and PFS to 8.3 months – an outcome that was impossible to achieve in patients with KRAS mutations receiving the same therapy (13.4 and 5.5 months, respectively) [10]. In two other studies, CAIRO-1 (Capecytabine, oxalipaltyn and Bevacizumab with or without Cetuximab in First-Line Advanced Colorectal Cancer) and PACCE (Panitumumab Advanced Colorectal Cancer Evaluation) [39, 40], subjects with wild-type *KRAS* gene also failed to benefit from anti-EGFR antibody treatment added to chemotherapy, if the efficacy of treatment were to be compared to chemotherapy alone.

In Poland, AOTM's explicit opinion was used as a basis for developing a drug programme under which anti-EGFR antibody treatment can be prescribed to mCRC patients provided that cancer cells are free from *KRAS* gene mutations (without further specification mutation types).

KRAS gene mutation types have been analyzed retrospectively in the context of assessing their importance for the development of refractoriness to anti-EGFR antibodies. Research conducted over the past two years has established unambiguously that a mutation in codon 12 is a negative predictive factor. On the other hand, it seems possible to achieve response to cetuximab and panitumumab therapy in the presence of mutations in codon 13 of the *KRAS* gene – regardless of treatment line or modality. In the study by Pentheroudakis *et al.*, patients with a mutation in codon 12 of the *KRAS* gene who received chemotherapy combined with cetuximab had a median overall survival of 19 months, whereas patients with other *KRAS* mutations and *KRAS*-wild type gene subjects treated with the same modality had a considerably longer median survival of nearly 30 months [41–44]. Furthermore, there are many reports on patients with the wild-type *KRAS* gene who are refractory to anti-EGFR treatment. The cause of refractoriness has been identified in ca. 15% of mCRC patients as mutations in the *BRAF* gene, primarily V600E substitution. Raf family proteins are downstream of Ras proteins in the signal transduction pathway originating at EGFR. It comes as no surprise, then, that activating mutations in the *BRAF* gene occurring in cancer cells have a similar clinical effect to *KRAS* gene mutations, making them refractory both to cetuximab and panitumumab [6, 27, 45–47]. Di Nicolantonio *et al.* reviewed patients treated with these antibodies, finding objective response in two patients with *KRAS* gene mutations (6%) and in 22 mutation-free subjects (28%). In the group of patients with wild-type KRAS gene the authors identified a total of 11 patients with *BRAF* mutations (14%). Among them, there were no objective responses to anti-EGFR treatment, and PFS and OS were reduced. In *in vitro* cultures the authors successfully overcame refractoriness of BRAF-mutated cancer cells to cetuximab using a combination of cetuximab and the multikinase inhibitor sorafenib which inhibits, among others, Raf kinase [48]. Findings on the impact of *BRAF* mutations on the efficacy of anti-AGFR antibody treatment (both in monotherapy and in combination with chemotherapy) in mCRC patients have also been corroborated by other authors. Tol *et al.* have identified mutations in the *BRAF* gene as an unfavourable prognostic factor [49]. Pentherodaukis *et al.* showed that the presence of *BRAF* mutations in patients treated with chemotherapy combined with cetuximab is even a weaker predictive factor for this type of treatment than a mutation in codon 12 of the *KRAS* gene. As previously mentioned, patients having both genes of the wild-type who are treated with anti-EGFR antibodies had a median survival close to 30 months. By contrast, subjects with a mutation in codon 12 of the *KRAS* gene had a median survival of 19 months and those with a mutation in the *BRAF* gene – only 12 months [41].

During the 2013 ASCO Annual Meeting, however, there were reports stating that the presence of mutations in the *BRAF* gene had no predictive value for anti-EGFR therapy and was a negative prognostic factor only in CRC patients. The studies showed that negative predictive factors in panitumumab therapy included not only common mutations in the *KRAS* gene but also rare mutations in genes encoding RAS proteins. The authors characterized the effects of additional mutations in codons 59, 117 and 146 of the *KRAS* gene, and mutations in codons 12, 13, 59, 61, 117 and 146 of the *NRAS* gene, on the efficacy of panitumumab combined with FOLFOX6 chemotherapy in first-line mCRC treatment (PEAK study), and the efficacy of panitumumab monotherapy (20020408 study). The retrospective analysis revealed that the occurrence of these

rare mutations (the incidence of *NRAS* mutations ranges from 5 to 8.3%, while that of mutations in codons 59, 117 and 146 of the *KRAS* gene does not exceed 10%) reduced the chance of achieving response to panitumumab therapy and shortened PFS following the introduction of this therapy. Consequently, the SPC of the drug will soon be revised to include the requirement to assess all mutations in *KRAS* and *NRAS* genes in the process of determining eligibility for panitumumab treatment. One obstacle which currently hinders compliance with the requirement is the fact that there are no methods certified for the detection of this group of mutations, since the SURVEYOR platform applied for the analysis of tissue material in PRIME and 20020408 studies is not in widespread use [50–52].

Phosphatidylinositol 3-kinase (Pi3K) consists of two subunits: one regulatory and one catalytic (subunit P110α) which is responsible for the phosphorylation of phosphatidylinositol followed by activation of the AKT/mTOR pathway. Pi3K/AKT is a pathway alternative to Ras/Raf/MAPK, transmitting signals from the activated EGFR protein to the cellular nucleus. A regulatory role in the pathway is played by the PTEN protein (phosphatase and tensin homolog) encoded by the suppressor gene *PTEN*. It can thus be concluded that activating mutations of the PI3KCA oncogene (phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit α isoform) and reduced expression of the PTEN protein may affect the efficacy of EGFR-inhibiting cancer treatments (Fig. 1) [6, 41, 46, 47, 53–56].

Activating mutations of the *PI3KCA* gene occur most commonly in exons 9 (E542K, E545K) and 20 (H1047R). The mutations are detected in 6–10% of CRC patients. Typically, they exist independently of mutations affecting the *KRAS* gene. The value of studies investigating the influence of *PI3KCA* gene mutations on the efficacy of anti-EGFR antibody treatment is limited due to their retrospective nature and small study groups. Studies by Lievre *et al.* and Perrone *et al.* found that carriers of mutations in the *PI3KCA* gene were non-responders to anti-EGFR treatment [6, 53]. By contrast, studies by Sartore-Bianchi *et al.* and Peren *et al.* demonstrated a possibility of achieving response to anti-EGFR therapy in patients with *PI3KCA* mutations (13% of patients bearing a *PI3KCA* mutation and 11% of mutation-free patients had an objective response to treatment) [54, 55]. Pentheroudakis *et al.* identified no correlations between mutations in the *PI3KCA* gene and the efficacy of chemotherapy combined with cetuximab. An attempt can be made at explaining divergences in results as attributable to differences in the extent of Pi3K activation induced by mutations in exons 9 and 20 of the *PI3KCA* gene [41]. De Roock *et al.* argue that the main factor responsible for the excessive activity of phosphatidylinositol kinase is H1047G substitution. There is, as yet, no strong evidence in favour of extending the current genetic test panel to include mutations of the *PI3KCA* gene as another element of determining eligibility of mCRC patients for anti-EGFR treatment [56].

While it is relatively easy to detect mutations present in *KRAS*, BRAF and even *PI3KCA* genes with the aid of dedicated CE/IVD-certified tests based on real-time PCR, an assessment of epigenetic phenomena is extremely difficult and subjective, and may yield contradictory results. This applies to attempts at investigating abnormalities within the *PTEN* gene and their predictive value for the efficacy of anti-EGFR treatment given to mCRC patients. PTEN is a potential site for a number of mutations – and for the formation of pseudogenes, hypermethylation of the promoter region, amplification of the entire gene, etc. As a result, the majority of authors confine themselves to an assessment of PTEN expression within cancer cells by immunohistochemistry. Laurent-Puig *et al.* found that the lack of PTEN expression in KRAS mutation-free patients (ca. 20% of CRC patients) was correlated with reduced survival. Loupakis *et al.* established that a *KRAS* mutation and absence of PTEN expression were negative predictive factors for response to treatment based on cetuximab in combination with irinotecan. Also, Razis *et al.* showed that the deletion of a fragment of the *PTEN* gene detected by FISH – unlike the absence of the PTEN protein expression – was a negative factor for such therapy [27, 46, 47, 53, 57–59].

Assessment of expression of EGFR ligands as predictive factors in cetuximab and panitumumab therapy

Ligands of the HER family receptors include EGF, amphiregulin, epiregulin and TGF-α. High concentrations of these ligands and high expression of their mRNA in the CRC tissue are frequently observed, and are essential for the proliferation of cancer cells. Higher concentrations of EGFR ligands are presumed to be correlated with faster tumour growth and metastatic ability. Moreover, patients with overexpression of EGFR ligands are less commonly identified with mutations in the *KRAS* gene because the carcinogenesis pathway is, in this case, independent of the mutation. Khambata-Ford *et al.* noted more frequent disease control and longer PFS for cetuximab monotherapy in patients whose cancers had high levels of EREG or AREG expression than in subjects with low expression levels of these ligands. Jacobs *et al.* examined *KRAS* wild-type patients treated with cetuximab and irinotecan, reporting a median survival of 65 weeks in patients with high EREG expression and just 31 weeks in patients with low ligand expression. Also in studies by Pentheroudakis *et al.* and Ohchi *et al.* high expression levels of AREG and EREG (high mRNA levels for these ligands) were favourable predictive factors (with a potential for the achievement of response to treatment and prolongation of overall survival) for cetuximab plus chemotherapy in *KRAS* wild-type mCRC patients [6, 41, 60–64].

Antibody-dependent cell-mediated cytotoxicity and activation of the complement system as a mechanism of action of cetuximab

Antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) belong to the most important processes allowing IgG1 antibodies to destroy microorganisms, parasites and cancer cells. Antibodies alone are unable to destroy the target cell: they can only bind specifically to epitopes of cancer antigens. If the antigen is a receptor, as in anti-EGFR therapy, the

intracellular transduction pathway is blocked but also cytotoxic cells become activated (if the antibody, like cetuximab, belongs to the IgG1 class). After coating the cancer cell, antibodies bind to NK cells and other immune cells which have receptors for the antibody Fc fragment on their surface. Some of them (IgM and IgG antibodies, with the exception of IgG4) can also bind to the C1q molecule. C1q-associated proteases then induce enzymatic conversion of C1r and C1s cells, thus initiating the classical complement activation pathway. NK cells bound to the target cell become degranulated releasing perforins, granulysins and granzymes which induce apoptosis of cancer cells. Similarly, the membrane of the target cell can become lysed as a result of activation of components of the complement system [65–68].

Evidence for the impact of ADCC on the efficacy of cetuximab and absence of any influence on the efficacy of panitumumab therapy is found in *in vitro* studies (cell cultures). Other evidence was to be derived from observations into the relationship between the occurrence of polymorphic forms of genes coding receptors for the antibody Fc region and the effect of cetuximab treatment in mCRC patients. Unfortunately, results of these studies are often contradictory. Two polymorphisms with a major role for the receptor function (i.e. the degree of their affinity to IgG1) have been identified: H131R substitution in the *FcγRIIa* gene and F158V substitution in the *FcγRIIIa* gene. The first studies by Zheng *et al.* and Bibeau *et al.* showed the H allele in codon 131 in the *FcγRIIa* gene to be correlated with long time to progression in cetuximab-treated patients. Results of both studies indicate that individuals with HH homozygous genotypes in codon 131 of the *FcγRIIa* gene benefit significantly from cetuximab treatment. Identical conclusions were reached by Rodriguez *et al.* The studies, however, provided conflicting results on the association between the presence of the F or V alleles in the *FcγRIIIa* gene and the efficacy of cetuximab. Bibeau *et al.* report, however, that the time to progression in cetuximab-treated mCRC patients with wild-type *KRAS* gene is prolonged to 9.6 months in the subgroup of subjects with HH homozygous genotypes in codon 131 of the *FcγRIIa* gene or VV homozygous genotypes in codon 158 of the *FcγRIIIa* gene – compared to 4.6 months in subjects with other genotypes of receptor genes for the Fc fragment of immunoglobulins. Just one year later, however, studies by Pander *et al.* contradicted the finding that greater benefits of cetuximab therapy are achieved in patients with VV homozygous genotypes in codon 158 of the *FcγRIIIa* gene. The effect, the authors claimed, is observed rather in carriers of the FF genotype in codon 158 of the *FcγRIIIa* gene. Lurje *et al.* studied a larger patient group (n = 130) without detecting any associations between the efficacy of cetuximab and the genotype of genes for receptors for the Fc fragment of the antibodies. Pander *et al.*, investigating a group of 246 patients with CRC to determine polymorphism V176F (818A>C) and the efficacy of cetuximab, found that the C allele was an unfavourable predictive factor for cetuximab treatment. Carriers of the allele had a median PFS of just 8.2 months, whilst AA homozygous individuals survived without signs of progression for 12.8 months. In view of the fact that different authors have obtained divergent study results, the role of ADCC in the mechanism of action of cetuximab continues to be an object of debate. The study by Lopez-Albeitero *et al.*, conducted among 170 patients with head and neck cancer seems to point to the major role of ADCC in cetuximab's mechanism of action and to the modulation of its efficacy by polymorphisms within the *FcγRIIIa* gene. In addition, as there is no possibility of ADCC induction by panitumumab, the majority of studies have found no evidence for any relationship between the efficacy of the drug and the occurrence of various polymorphic forms of the *FcγRIIIa* gene [65–76].

Summary

The efficacy of monoclonal anti-EGFR antibodies has been proven in mCRC patients and, for cetuximab, also in individuals with squamous cell carcinoma of the head and neck. Advanced clinical trials (LUCAS and FLEX) with cetuximab have also been performed in patients with non-small-cell lung carcinoma (NSCLC). They were not successful, though, and did not result in the approval of cetuximab for treatment of this cancer type. It has been established clearly that response to anti-EGFR antibody treatment is only possible in selected patient groups with various cancer types. Predictive factors for the efficacy of anti-EGFR therapy have been best elucidated in CRC patients. A factor identified in multiple studies as essential for appropriate assessment of eligibility for cetuximab or panitumumab treatment is the absence of KRAS gene mutations. EGFR expression on the surface of cancer cells does not seem to have a decisive influence on the efficacy of the therapy. There are ongoing studies assessing the predictive value of the number of copies of the *EGFR* gene, mutations in the *NRAS*, *PI3KCA*, *P53* and *PTEN* genes, concentration of EGFR ligands and polymorphisms in the *EGF* and *EGFR*, and the *FcγRIIa* and *FcγRIIIa*, genes. These factors, however, have not as of yet been examined in large randomized prospective studies and hence should not be used as a basis for mCRC patient eligibility for cetuximab or panitumumab treatment. Merck provided a medical writing grant to support the manuscript development, however, Merck made no contributions to the content of the manuscript.

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