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SYSTEMATIC REVIEWS

Prognostic and predictive biomarkers in metastatic colorectal cancer anti-EGFR therapy

Cristiana Lo Nigro, Vincenzo Ricci, Daniela Vivenza, Cristina Granetto, Teresa Fabozzi, Emanuela Miraglio, Marco C Merlano

Cristiana Lo Nigro, Vincenzo Ricci, Daniela Vivenza, Cristina Granetto, Emanuela Miraglio, Marco C Merlano, Department of Oncology, S. Croce and Carle Teaching Hospital, 12100 Cuneo, Italy

Teresa Fabozzi, Department of Oncology, San Raffaele Institute, 20132 Milan, Italy

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Correspondence to: Cristiana Lo Nigro, PhD, Laboratory of Cancer Genetics and Translational Oncology, Department of Oncology, S. Croce and Carle Teaching Hospital, via Carle 25, 12100 Cuneo, Italy. lonigro.c@ospedale.cuneo.it
Telephone: +39-0171-616338
Fax: +39-0171-616331

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Abstract

AIM: To reviewing genetic and epigenetic make-up of metastatic colorectal cancers (mCRCs) addicted to epidermal growth factor receptor (EGFR) signalling.

METHODS: The present study summarizes the potential value of prognostic and predictive biomarkers in selecting mCRC patients treated with anti-EGFR therapy. A meta-analysis was performed using a systematic search of PubMed, Medline and Web of Science to identify eligible papers until March 21st, 2016 using these following terms: "colorectal cancer", "predictive biomarkers", "anti-EGFR therapy", "KRAS", "NRAS", "PIK3CA", "TP53", "PTEN", "EGFR", "MET", "HER2", "epiregulin", "amphiregulin", "prognostic biomarkers", "BRAF", "miRNA" and "antibody-dependent cell-mediated cytotoxicity (ADCC) activity". Two investigators independently evaluated and extracted data from each identified studies based on selected criteria of inclusion and exclusion.

RESULTS: The introduction of agents targeting EGFR such as cetuximab and panitumumab increased overall survival of mCRCs. Nevertheless, it has firstly became evident that response rates to cetuximab regimens in unselected patient populations were typically lower than 30%. Clinical data confirmed the predictive value of RAS mutations for resistance to cetuximab and panitumumab leading to the license of these monoclonal antibodies exclusively for the management of patients with RAS-wild type colorectal cancers. So far the identification of predictive biomarkers have generated interesting, though preliminary and, at times, conflicting data on the importance of tumour mRNA levels of EGFR ligands, of activating mutations in

other genes such as *NRAS* and *PIK3CA*. The prognostic value of selected microRNAs level and ADCC activity is under investigation, while the prognostic impact of *BRAF* status remains controversial.

CONCLUSION: This review focuses on the personalized treatment of mCRC and discusses the potential of new prognostic and predictive biomarkers in selecting patients treated with anti-EGFR therapy.

Key words: Metastatic colorectal cancer; Anti-epidermal growth factor receptor therapy; *KRAS*; Biomarkers; Antibody-dependent cell-mediated cytotoxicity

Core tip: This review focuses on progress in the metastatic colorectal cancer (mCRC) personalized treatment and on the role of prognostic and predictive biomarkers available for selecting patients treated with anti-epidermal growth factor receptor (EGFR) therapy. Not only the *KRAS* mutational status but also *BRAF*, *NRAS*, *PIK3CA*, *TP53* and *PTEN* alterations might be useful in selecting patients who likely will respond to anti-EGFR treatments. In particular, we focused on the following points: (1) predictive biomarkers of response to anti-EGFR therapy; (2) prognostic biomarkers; and (3) new prognostic value of antibody-dependent cell-mediated cytotoxicity activity induced by cetuximab in mCRC.

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INTRODUCTION

Colorectal cancer represents the third most frequent neoplastic disorder worldwide and one of the main causes of tumour-related mortality[1].

Treatments of metastatic colorectal cancer (mCRC) in the last 20 years have been improved and median overall survival (OS) increased approximately from 10 to 30 mo.

This significant increase of OS is due to the introduction, in systemic treatments, of biologic drugs targeting either angiogenesis such as bevacizumab, aflibercept and regorafenib, or epidermal growth factor receptor (EGFR) such as cetuximab and panitumumab[2].

EGFR on the cancer cell surface allows to transmit signals of proliferation, angiogenesis, metastasis. Cetuximab, a chimeric IgG1 monoclonal antibody (mAb) and panitumumab, a humanised IgG2 mAb, are now approved for patients with mCRC. They are used in combination with chemotherapy, either in first or in second line, or alone in refractory disease. Identification of tumors addicted to EGFR signalling and so susceptible to anti-EGFR therapy became mandatory, since, at first, response rates to cetuximab in unselected patients were less than 30%[3].

KRAS is a cytoplasmic GTP-binding protein with low GTPase activity. When GTP binds KRAS, signals of cellular proliferation and inhibition of apoptosis are released, thus KRAS acts as a classical oncogene. *KRAS* mutations were found mainly in exon 2, causing the abrogation of the GTPase activity and the lock of KRAS protein in the active form.

Those mutations, activating the *RAS/RAF/MAPK* pathway, make the targeting of EGFR therapeutically unuseful[4]. The value of *KRAS* exon 2 mutations in predicting resistance to cetuximab and panitumumab were confirmed by clinical data; thus these mAbs were licenced exclusively for in *KRAS*-wild type (WT) CRC patients[5,6].

KRAS and *NRAS* are closely related *RAS* oncogene family members. Alterations in exons 2, 3 and 4 of either gene constitutively activate RAS and are mutually exclusive, which suggests functional redundancy. So far, several retrospective, non-prespecified analyses of randomized clinical trials validated the pan-*RAS* mutations as negative predictive factors for anti-EGFR therapy[7,8].

On this base, the European regulatory authority (EMA) restricted the use of cetuximab and panitumumab to patients not having any mutation in *KRAS* or in *NRAS* codon 12, 13, 59, 61, 117 and 146 hotspots, defined as *RAS*-WT patients.

Interesting and, sometimes, conflicting preliminary data indicated new potential predictive biomarkers as the tumour mRNA levels of EGFR ligands and activating mutations in *BRAF* and *PIK3CA*[9].

BRAF activating mutations, mainly V600E, identify molecularly a subgroup (8%-10%) of CRCs. *BRAF* mutant (*BRAF*-mut) tumours have been thus defined to present specific clinical and histopathological characteristics, as typically are associated with female sex, old age, right-sided CRC, high-grade mucinous histotype, MSI, methylator phenotype and peritoneal and lymph node metastases[10].

This review focuses on progress in the mCRC personalized treatment and on the role of prognostic and predictive biomarkers available for selecting patients treated with anti-EGFR therapy.

MATERIALS AND METHODS

The selected literatures were determined via an electronic search of Medline, PubMed and Web of Science using these following terms: "colorectal cancer", "predictive biomarkers", "anti-EGFR therapy", "KRAS", "NRAS", "PIK3CA", "TP53", "PTEN", "EGFR", "MET", "HER2", "epiregulin", "amphiregulin", "prognostic

biomarkers", "BRAF", "miRNA" and "antibody-dependent cell-mediated cytotoxicity (ADCC) activity".

The last search was updated in March 21st, 2016. The search strategy used both MeSH terms and free-text words to increase the sensitivity of the search. The present study was performed in accordance with the standard guidelines for systematic reviews[11].

Inclusion and exclusion criteria

The two investigators (RV, LNC) independently assessed all the eligible studies and extracted the data. Studies were considered eligible if they met the following criteria: (1) response to anti-EGFR therapy; (2) primary and acquired resistance mechanisms to anti-EGFR treatment; (3) mutations and therapeutic modulation of EGFR; and (4) published as a full paper in English. Exclusion criteria are the following: (1) abstracts; (2) studies investigating the *RAS/RAF/MEK/ERK* pathway not involved in the response to anti-EGFR therapy; (3) studies without usable data; (4) studies published in language other than English; and (5) duplicate publications.

Data extraction

Two investigators (RV, LNC) independently evaluated and extracted data from each identified studies based on criteria of inclusion and exclusion.

RESULTS

Predictive biomarkers of response to anti-EGFR therapy

***KRAS* mutations:** The *Erb* family includes cell membrane receptors such as HER1/erbB1 (EGFR), HER2/c-neu (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4)[12]. *EGFR* gene is one of the major target of biologic drugs, and favoured the use of anti-EGFR mAbs and tyrosine kinase inhibitors (TKIs). Cetuximab (anti-IgG1 mAb) and panitumumab (anti-IgG2 mAb) bind to the extracellular ligand site, while erlotinib and gefitinib, as EGFR TKIs, compete with ATP to the TK binding domain and inhibit EGFR autophosphorylation. Both mAbs and TKIs interrupt the downstream intracellular signalling pathway. First clinical trial with anti-EGFR mAb enrolled patients with high tumoural EGFR expression; however overall response rates (ORRs) were low[13], suggesting that other unknown factors might affect response to these drugs[14].

Lièvre *et al*[15] identified a correlation between lack of response to anti-EGFR therapy and *KRAS* mutations. They analysed 30 patients receiving second- or third-line treatment with cetuximab plus irinotecan. The OS was significantly higher in *KRAS*-WT patients than in those having a *KRAS* mutation (median OS: 16.3 mo *vs* 6.9 mo, respectively, $P = 0.016$)[15].

KRAS protein is a GTPase bound to the intracellular part of the cell membrane, acting in the *EGFR/RAS/RAF/MEK/ERK* kinase signalling. It transfers extracellular signals from the EGFR to the nucleus and regulates proliferation, cell growth and apoptosis. The *KRAS* oncogene belongs to the *Erb* family and lies in the short arm of chromosome 12. Point mutations in the *KRAS* gene are usually in codon 12 (82%-87%) and 13 (13%-18%) (exon 2), in codon 61 (exon 3) and in codon 146 (exon 4)[16]. In *KRAS*-WT patients, the binding of anti-EGFR antibodies to the receptor induces conformational changes affecting its internalization and sequentially causes the direct inhibition of TK activity and the blockage of *RAS/RAF/MEK/ERK* downstream pathway. In fact, *KRAS* mutations abrogate the mAb-induced inhibition of EGFR and constitutively activate the KRAS intracellular domain. In CRC the incidence of *KRAS* mutations is 30%-45%[17]. *KRAS* mutational analysis may be done either in the primary tumour or in the metastatic sites since mutations are usually concordant (around 95%) in those two samples[18].

Two studies demonstrated a survival benefit over best supportive care only in *KRAS*-WT patients treated with cetuximab (median OS: 9.5 mo *vs* 4.8 mo) or panitumumab (median PFS: 12.3 wk *vs* 7.3 wk), respectively[5,19]. In patients with *KRAS* mutated tumours, mAbs did not improve PFS or OS as compared to the best supportive care. Moreover, the effectiveness of first- and second-line chemotherapy increases when combined with anti-EGFR mAbs in mCRC.

KRAS exon 2 mutations are extremely specific negative biomarkers of response to anti-EGFR mAbs in mCRC, although not all *KRAS* mutations are equivalent in the effect on cell proliferation and drug resistance[20].

***KRAS* p.G13D mutation:** Some preclinical evidence showed that neoplastic cells with *KRAS* codon 13 glycine (G) to aspartate (D) mutations (p.G13D), having an incidence of 10%-15%[21], respond to cetuximab similarly to WT clones[22]. Furthermore, about 10% of response to anti-EGFR mAbs in patients carrying a *KRAS* mutation in tumour tissue and a further 15% of patients obtained a long-term disease stabilization[23,24] have been reported. In responding patients, codon 13 mutation is more frequent than in the whole *KRAS*-mut tumour population.

As previously stated, not all *KRAS* mutations are equivalent in the effect on cell proliferation and drug resistance. A large retrospective analysis of 579 chemorefractory mCRC patients treated with cetuximab reported that OS and PFS were significantly longer in patients with p.G13D mutation ($n = 32$) than in patients with other *KRAS*-mutations (median OS = 7.6 mo *vs* 5.7 mo, $P = 0.005$; median PFS = 4 mo *vs* 1.9 mo, $P = 0.004$). No significant difference in terms of ORR was observed between patients with p.G13D mutations and other mutations (6.3% *vs* 1.6%, respectively, $P = 0.15$). Authors did not specify the number of patients achieving the clinical benefit in the two groups. However, the significant longer PFS observed in patients with p.G13D mutation might

suggest a difference in clinical benefit. A considerable correlation between type of *KRAS* mutation (p.G13D *vs* other *KRAS* mutations) and OS was observed in cetuximab treatment (HR = 0.30, 95%CI: 0.14-0.67, *P* = 0.003)[20].

In a pooled analysis of CRYSTAL and OPUS studies, where mCRC patients were randomized to receive FOLFIRI (CRYSTAL) or FOLFOX (OPUS) with or without cetuximab as first-line treatment, the addition of cetuximab to chemotherapy in patients with *KRAS* p.G13D mutation was found advantageous[21]. Precisely, among 83 patients with p.G13D mutation, those receiving chemotherapy plus cetuximab performed better in PFS and OS than patients treated with chemotherapy alone; on the contrary, patients with any other *KRAS* mutation, did not benefit from combined treatment[21].

The role of *KRAS* mutations in codons 12 or 13 has been studied in a pooled analysis of clinical trials in which panitumumab was added to FOLFOX4 in first-line[25], to FOLFIRI in second-line[26], or compared with best supportive care in heavily pre-treated mCRC patients[13]. In conclusion it was found that codon 13 *KRAS*-mut tumours are unlikely to benefit from panitumumab in the same way as tumours mutated in codon 12.

***NRAS* mutations:** Neuroblastoma-ras (*NRAS*) gene belongs to the *RAS* oncogene family and is located on chromosome 1. It encodes for a GTPase membrane protein that shuttles between the cell membrane and the Golgi system. *NRAS* mutations, with a 3%-5% rate in CRC[9], are associated with anti-EGFR treatment failure. Moreover *KRAS*, *BRAF* and *NRAS* mutations are mutually exclusive[27].

A retrospective analysis suggested a potential negative prognostic role of *RAS* mutations in patients with mCRC, reporting a worse median OS in *NRAS* and *KRAS* mutated patients (25.6 mo *vs* 30.2 mo, respectively) in comparison to all WT (42.7 mo)[28].

***PIK3CA* mutations:** Phosphatidylinositol 3-kinase (PI3K) activates AKT, triggering downstream pathways and promoting proliferation and cell survival. The *PIK3CA* gene encodes the PI3K catalytic subunit; mutations in this gene result in aberrant AKT activation and cancer growth[29,30]. *PIK3CA* mutations are reported in 10%-20% of CRC and their effect on clinical outcome is not yet well know[30].

Ogino *et al*[31] showed that, in patients with *KRAS*-WT, *PIK3CA* mutation increases tumor-specific mortality (HR = 3.80, 95%CI: 1.56-9.27), but did not observe any significant effect on mortality in *KRAS*-mut patients (HR = 1.25, 95%CI: 0.585-2.96)[31]. Conversely, in mCRC, a *PIK3CA* mutation was found in 17.7% of cetuximab-treated patients, but ORR, time-to-progression and OS did not differ between mutated and WT patients[32]. In the CAIRO2 study, *PIK3CA* mutation was not linked to outcome in *KRAS*-WT tumours treated with cetuximab: 5-year survival was 90% in *PIK3CA*-WT and 82% in *PIK3CA*-mut (log-rank *P* = 0.075)[33]. Sartore-Bianchi *et al*[29] examined *PIK3CA* mutational status in exons 9 and 20 in 110 mCRC patients treated with cetuximab or panitumumab. They identified 15 patients (13.6%) with *PIK3CA* mutations, and none of them responded to anti-EGFR mAbs (*P* = 0.038). The correlation between lack of response and *PIK3CA* mutations was even more evident in *KRAS*-WT patients (*P* = 0.016), reinforcing the study by Ogino *et al*[31]. Differently Prenen *et al*[30] analyzed 200 chemorefractory mCRCs and did not find a correlation between *PIK3CA* and anti-EGFR mAb resistance: 13% of *PIK3CA*-mutated patients responded to cetuximab and 11% did not (*P* = 0.78)[30].

These conflicting results keep a high degree of uncertainty about the predictive role of *PIK3CA* mutations.

***TP53* mutations:** p53 protein is activated when DNA damage occurs but also when oncogenes are inappropriately activated, in order to induce cell apoptosis. p53 pathway alteration has been systematically observed in non-small cell lung cancer with activating *EGFR* mutations, suggesting that p53 inactivation is necessary to allow expansion of a cell with EGFR activation. Moreover, it has been proposed that p53 acts as a brake for the activated PI3K transduction cascade since PI3K signalling activates p53 mediated growth suppression. These data confirm the hypothesis that EGFR activation is oncogenic, and therefore anti-EGFR mAbs might be efficient in tumours only if p53 is inactivated[34].

PTEN: *PTEN* is a key tumour suppressor gene involved in the homeostatic maintenance of *PI3K/AKT* pathway. Loss of PTEN expression, evaluated by IHC, is reported in 20%-40% of CRCs and increases phosphatidylinositol-3,4,5-triphosphate, the major substrate of PTEN; this induces a persistent activation of PI3K effectors[35,36]. Differently from *KRAS* status, only 60% of primary tumour are concordant in PTEN expression with metastases, since *PTEN* loss is more frequent in distant metastases[37,38].

Low PTEN expression in primary tumour of mCRC patients treated with cetuximab plus irinotecan did not affect outcome, but, if PTEN was measured in the metastatic tissue, ORR and PFS were significantly longer in patients with high PTEN expression than in those with low: 26% *vs* 5% (*P* = 0.007) and 4.7 mo *vs* 3.3 mo (*P* = 0.005), respectively[18].

Sartore-Bianchi *et al*[29] reported, in 81 mCRC patients, that PTEN loss correlted with lack of response to cetuximab and panitumumab (*P* = 0.001), with a shorter PFS and OS.

***EGFR* p.S492R mutation:** The *EGFR* p.S492R mutation, caused either by 1476C>A or 1474A>C substi-

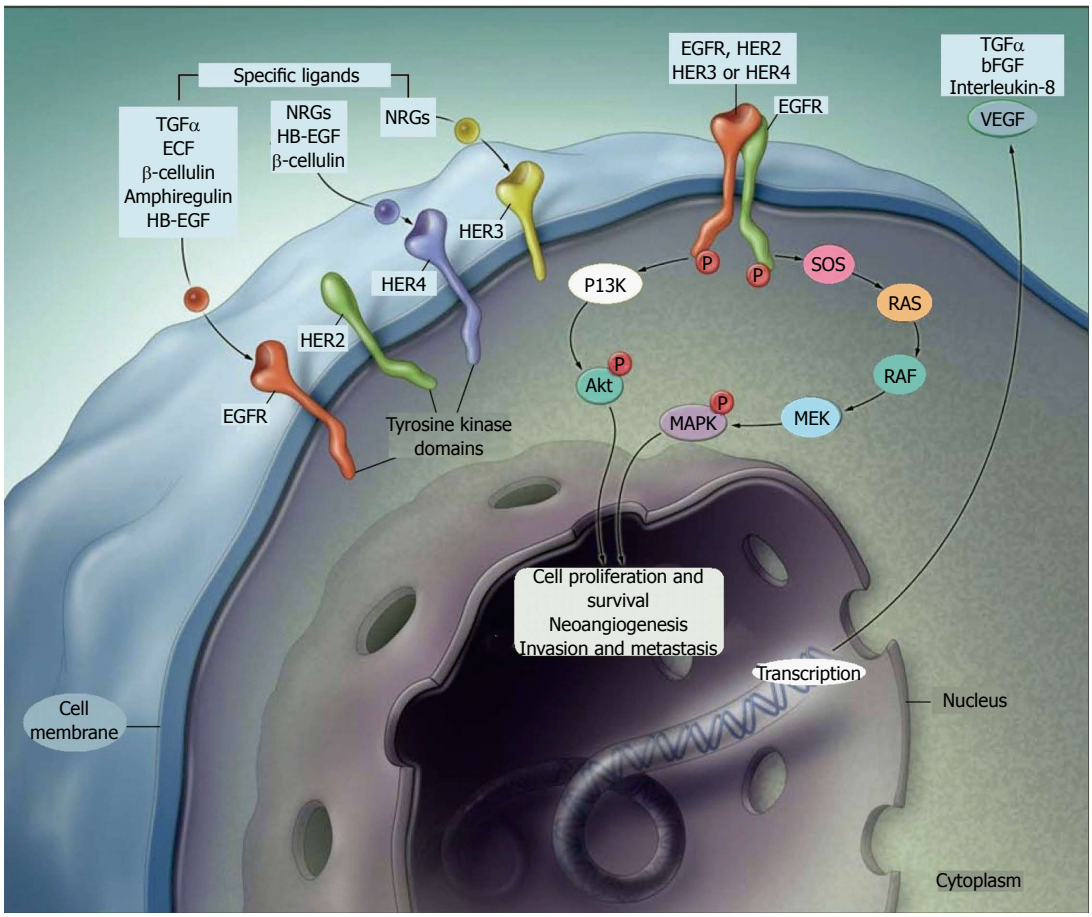

Figure 1 Signal transduction pathways controlled by the activation of epidermal growth factor receptor. Adapted from Ref. [64]. TGFα: Transforming growth factor alpha; ECF: Eosinophil chemotactic factor; HB-EGF: Heparin-binding EGF-like growth factor; NGR: Neuregulin; EGFR: Epidermal growth factor receptor; bFGF: Basic fibroblast growth factor; VEGF: Vascular endothelial growth factor; PI3K: Phosphoinositide 3-kinase; SOS: Son of sevenless homolog protein.

tution, alters binding to cetuximab but not to panitumumab and it has been described in mCRCs with acquired resistance to cetuximab. In a retrospective analysis of patients with available samples from ASPECCT, 16% of patients in the cetuximab arm and 1% of patients in the panitumumab developed *EGFR* p.S492R mutation[39]. Patients with *EGFR* p.S492R mutation in the cetuximab arm had longer treatment duration before progressive disease and appeared to have worse OS than patients with WT p.S492 in the cetuximab arm[39] (Figure 1).

Acquired resistance mechanisms

***KRAS* mutant clones:** A number of studies have identified *KRAS* somatic mutations as a biomarker of intrinsic resistance to anti-EGFR drugs in CRC patients, but the molecular basis for acquired resistance are still obscure. Alterations in *KRAS* gene, causing drug resistance, can be due either to the selection of pre-existent *KRAS* mutant and to the amplified clones or to new mutations, since the pressure of cetuximab might induce *de novo KRAS* mutation. *KRAS* mutant alleles are detected in the 0.4% to 17% of resistant tumors[40].

MET: The *MET* gene encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF) and has an oncogenic role in several solid tumors, where it is activated by gene amplification, overexpression, activating mutations or autocrine stimulation. MET and its ligand HGF are involved in acquired resistance to targeted therapies[41].

MET inhibitors (including the clinically approved drug crizotinib for patients with mutant *ALK* or *ROS-1*) are effective. Preclinical data in which CRC xenopatients carrying *MET* amplification are treated is encouraging. *MET* gene amplification is a novel mechanism of both primary and acquired resistance to cetuximab or panitumumab. The rate of *MET* amplification in untreated mCRC is around 1%; it correlated with resistance to anti-*EGFR* therapy and might be overcome by MET kinase inhibitors[41].

***HER2* amplification:** *HER2* gene amplification and protein overexpression were identified in about 3%-6% of CRC patients[42].

HER2 mutations activate intracellular signaling pathways, increase anchorage-independent growth in soft agar and produce resistance to the EGFR

monoclonal antibodies (cetuximab and panitumumab) in colon cell lines. Therefore *HER2* activating mutations may themselves be a drug target for the treatment of colorectal cancer. It was demonstrate that these *HER2* mutations cause oncogenic transformation of colon epithelial cells and produce resistance to cetuximab and panitumumab in two colorectal cancer cell lines[43].

Epiregulin and amphiregulin: The EGFR ligands epiregulin (EREG) and amphiregulin (AREG) are commonly overexpressed in CRC. In a prospectively planned retrospective biomarker study from the PICCOLO trial, high ligand expression AREG/EREG is a predictive marker for panitumumab therapy benefit on PFS in *RAS*-WT patients; conversely, patients with low ligand expression gained no benefit[44].

Furthermore, patients with *EREG* and *AREG* mRNA expression had longer survival than those with low-expression tumors[45].

Prognostic biomarkers

***BRAF* mutations:** The cytoplasmic serine-threonine kinase BRAF is immediately downstream of KRAS, acting as one of its main effectors, and needs be phosphorylated by KRAS for its activation.

BRAF activating mutations, mainly V600E, define a molecularly specific subset (8%-10%) of CRCs. The V600E is caused by a CTG to CAG point mutation at codon 600 and results in the *RAS/RAF/MEK/ERK* pathway constitutive activation, as *KRAS* mutations do. In CRC mutations in *KRAS* and *BRAF* are mutually exclusive[46].

BRAF V600E mutation correlated with a very aggressive phenotype and poor prognosis which usually led to a median OS < 1 year[46].

In addition to this prognostic value in CRC, retrospective studies also suggested that *BRAF* mutations might predict primary resistance to anti-EGFR mAbs[9,46].

The *BRAF* V600E mutation was retrospectively analysed in a 113 patients treated with cetuximab or panitumumab, with or without chemotherapy, and was detected in 11 (13.9%) of 79 *KRAS*-WT patients, None of them reported an objective tumour response[46].

Nine percent of patients in the CRYSTAL study had *BRAF* mutations and experienced a shorter median OS in both the FOLFIRI (10.3 mo) and FOLFIRI/cetuximab (14.1 mo) arms in respect to *KRAS*-WT/*BRAF*-WT patients, where survival was 21.6 and 25.1 mo, respectively. On the other hand, *BRAF* mutations were not related to cetuximab efficacy[6]. Even in the pooled analysis of the CRYSTAL and OPUS data, *BRAF* mutation was identified as a negative prognostic marker, with lower PFS and OS in the BRAF-mut patients independently of the treatment[47]. De Roock *et al*[9] reported a mutation rate of 4.7% in 761 chemorefractory patients treated with cetuximab plus chemotherapy. In comparison to *BRAF*-WT, *BRAF*-mut patients had a significantly lower ORR and a worse PFS and OS[9].

The negative prognostic value of *BRAF* mutation emerged also from the PRIME trial[7], where patients with *RAS*-WT but *BRAF*-mut tumours had a shorter PFS and OS compared to the *RAS*-WT and *BRAF*-WT ones. In the *RAS*-WT/*BRAF*-mut patients, panitumumab added to chemotherapy a small benefit in DFS and OS (P = 0.12 and 0.76, respectively), that was not significant[7]. The PICCOLO phase Ⅲ prospective trial investigated in 1198 *KRAS*-WT mCRCs the addition of panitumumab to single-agent irinotecan, in second or subsequent-line[48]. *BRAF*-mut patients (13.6%) showed a shorter OS compared to *BRAF*-WT, and panitumumab produced a detrimental effect on survival (HR = 1.84, 95%CI: 1.10-3.08, P = 0.029)[48].

BRAF mutations at codon 594 and 596, identify mCRCs with different pathological, clinical, and prognostic characteristics when compared to *BRAF* V600E mutated ones. In *BRAF* V600E mutated tumors, the frequency of microsatellite instability is relatively high, even in the metastatic stage (about 20%); on the contrary, all the codons 594 or 596 *BRAF* mutated tumors were considered as microsatellite stable.

Cremolini *et al*[49] found that patients mutated in *BRAF* codons 594 or 596 showed a trend in longer OS if compared to *BRAF*-WT [(62.0 mo *vs* 35.9 mo) HR = 0.55 (95%CI: 0.29-1.05), P = 0.081] and a significant longer OS than *BRAF* V600E mutated [62.0 mo *vs* 12.6 mo; HR = 0.36 (95%CI: 0.20-0.64), P = 0.002].

Pietrantonio *et al*[50], in a meta-analysis of *BRAF*-mut CRCs, demonstrated that the addition of anti-EGFR mAbs not increased OS, PFS nor ORR in first- and subsequent-line treatments.

In the meta-analysis of Rowland *et al*[51], there was no sufficient evidence to definitively state a treatment benefit of anti-EGFR mAbs in *RAS*-WT/*BRAF*-mut mCRCs compared to *RAS*-WT/*BRAF*-WT.

Ultimately *BRAF* mutations act as a negative prognostic factor more than a predictive marker of resistance to anti-EGFR mAb. *BRAF*-mut patients presented a shorter survival than the *BRAF*-WT ones, independently from treatment. They might have a minimal benefit from anti-EGFR therapy than *BRAF*-WT patients.

microRNAs high level: microRNA (miRNAs) are endogenous, short (17-25 bases), non-coding single-stranded RNAs involved in the post-transcriptional regulation of target gene expression.

Experimental and clinical data, addressing the clinical role of Let-7a level in relation to the SNP in the Let-7a *KRAS* mRNA binding site and the type of *KRAS* mutation, concluded that high Let-7 miRNA level might correlate with a relevant antitumor activity from anti-EGFR therapy in the presence of *KRAS* mutations[52].

Cappuzzo *et al*[53] showed that *KRAS*-WT patients with high miR-99a/Let-7c/miR-125b cluster expression showed longer OS and PFS than patients with low levels.

Table 1 Novel molecular biomarkers

Biomarker	Incidence	Prognostic value	Predictive value	Ref
K-RAS mutations	40%	Controversial	Predictor of resistance	[20-22,24]
K-RAS G13D mutation	15%-20%	Controversial	Faint resistance	[21]
N-RAS mutations	3%-5%	Controversial	Predictor of resistance	[9,27,28]
PI3KCA mutations	10%-20%	Controversial	Controversial	[29-31]
TP53	15%-50%	-	Controversial	[34]
PTEN expression	20%-40%	Controversial	Controversial	[18,29,36]
S492R mutation	16%	-	Controversial	[39]
KRAS mutant clones	0.4%-17%	-	Controversial	[40]
MET amplification	1%	-	Controversial	[41]
HER2 amplification	3%-6%	Controversial	-	[42,43]
Epiregulin and amphiregulin	-	Controversial	-	[44,45]
B-RAF mutations	4%-15%	Poor prognosis	Controversial	[7,46-51]
miRNAs high level	-	Controversial	-	[52,53]
Cetuximab basal ADCC activity	-	Controversial	-	[62]

ADCC: Antibody-dependent cell-mediated.

Antibody-dependent cell-mediated cytotoxicity activity: ADCC has been proposed as a parallel mechanism of cetuximab activity[54].

ADCC is a response of innate immune cells that exerts antitumor cytotoxicity and is activated when the Fc fragment of the antibody interacts with the Fc receptor on the immune cells. Some polymorphisms regulating Fc:FcR interactions has been reported as relevant in the level of ADCC induced by cetuximab[55]. In particular, response to therapeutic mAbs has been correlated with specific SNPs in *FCGR2A* (H131R) and *FCGR3A* (V158F) genes[56]. However, data up to now are conflicting, since came mostly from low-powered studies with small sample sizes[57].

Drugs that target Natural Killer (NK) cells, γδ T cells, macrophages and dendritic cells might augment the immune response and enhance the antitumor activity of the mAbs[58].

Invariant CD1d-restricted natural killer T (iNKT) cells are T lymphocytes with an invariant T-cell antigen receptor-α-chain rearrangement that co-express NK markers[59].

Molling *et al*[60] observed that a circulating iNKT cells deficit is linked to poor clinical outcome in HNSCC, suggesting a critical role in immune response against tumor. Moreover, determination of iNKT cells level might help in determining which patients can benefit from immunotherapeutic adjuvant therapies which aim to reconstitute the circulating iNKT cells reservoir[60].

The question if ADCC is associated with EGFR expression and/or *RAS* and *BRAF* mutations remains not yet clear in CRC. Seo *et al*[61] observed that the ADCC is significantly related to EGFR levels, but not with mutations in *KRAS* and/or *BRAF*.

Lo Nigro *et al*[62] investigated the prognostic role of iNKT and ADCC in 41 *KRAS*-WT mCRC patients treated with cetuximab in Ⅱ and Ⅲ lines. Authors demonstrated that patients with basal level of ADCC above the median (71%) presented a longer OS in comparison to those with ADCC below (16 mo *vs* 8 mo, $P = 0.026$). No significant correlation of iNKT cells with OS ($P = 0.19$) was seen, but a tendency of a better OS after 10 mo in patients with high iNKT cells basal level (above median of 0.382 cells/mL). However, patients with both high ADCC activity and high circulating iNKT cells showed a beneficial effect compared to low ADCC and low iNKT. This benefit seems superior to the role of ADCC alone supporting the hypothesis of a positive interplay between iNKT and ADCC effector cells. Correlation of key SNPs involved in ADCC ability and OS and PFS revealed not to be significant, in line with other reports[63]. Patients having both alleles with A in *FCGR2A* and TT in *FCGR3A* showed a longer, although not significant, PFS (9 mo *vs* 5 mo, $P = 0.064$) (Table 1).

DISCUSSION

The anti-EGFR mAbs cetuximab and panitumumab, by blocking MAPK pathway, are important in the treatment of mCRC.

KRAS and *NRAS* mutations in exons 2, 3 and 4 (overall found in around 50% of mCRCs) are predictive of anti-EGFR mAbs resistance. Thus, guidelines limit the use of cetuximab and panitumumab to *RAS*-WT patients. However, some *RAS*-WT tumours do not respond to anti-EGFR therapy. Since the cost of mAbs is high and treatment-related toxicity might be severe, the need of identifying additional predictive markers in anti-EGFR therapy beside *KRAS* is still compelling.

Mutations in *KRAS* exons 3 and 4, *NRAS* and not functional PTEN should be searched in mCRCs to improve clinical benefit of anti-EGFR mAbs. Uncertain is the predictive role of *PIK3CA* mutations.

Controversial remains the prognostic impact of *BRAF* mutations in CRC. A recent meta-analysis, suggested that anti-EGFR mAb therapy do not provide benefit in *BRAF* mutated mCRCs[49]. Conversely, another meta-analysis, concluded that there is not sufficient evidence to consider *BRAF*-WT as a definitive negative predictive biomarker in mCRC patients

treated with anti-EGFR mAbs. The gain in OS and PFS for *BRAF*-WT tumours may be small or less evident, but further studies are necessary to shed light to this point[50].

Cell membrane EGFR expression does not seem to influence therapy efficacy. Researchers are now ongoing to assess the predictive value of the number of *EGFR* and *HER2* copies, mutations in the *NRAS*, *PI3KCA*, *TP53* and *PTEN* genes, concentration of EGFR ligands, expression of epiregulin and amphiregulin and SNPs in the *EGF* and *EGFR*, and in the *FCGR2A* and *FCGR3A* genes.

The definition of predictive and prognostic role of *PIK3CA* mutation, *PTEN* deletion and *TP53* mutation is of interest, but there is still no consensus among clinicians on their use in clinical practice and in decision-making. Beyond a doubt, in the future, *NRAS*, *PIK3CA* and *PTEN*, in addition with *KRAS* and *BRAF* mutation analysis, will be useful in selecting mCRC patients that might benefit from anti-EGFR therapy.

NK cells are considered as major mediators of the therapeutic effect of cetuximab due to ADCC. iNKT cells number and ADCC level, exerted by NKs in the presence of cetuximab, might be useful prognostic or predictive markers of response[62].

In the next future, a robust analysis of many genes and different mutations, is likely to help in selecting patients and predicting the efficacy of anti-EGFR treatment. This approach will hopefully identify a mCRC subset with specific biological behaviour and treatment response. This will be an important step forward the "personalized medicine" of CRC patients and will inform the correct use of anti-EGFR antibodies.

COMMENTS

Background

The new therapeutic approach integrates novel molecular biomarkers with the pathologic features of a tumour to improve the prediction of prognosis and treatment efficacy. In recent years we have tried to study the molecular mechanisms underlying resistance to epidermal growth factor receptor (EGFR) inhibitors in order to obtain a better selection of patients for these treatments and improve the clinical outcome of patients treated with anti-EGFR mAbs.

Research frontiers

The metastatic colorectal cancer (mCRC) treatment has been linked to molecular progresses, which led to the discovery of prognostic and predictive biomarkers of response to anti-*EGFR* therapy. In the present study we summarize the potential value of prognostic and predictive biomarkers in selecting mCRC patients treated with anti-EGFR therapy.

Innovations and breakthroughs

It is clear that the evaluation of not only the *KRAS* mutational status but also *NRAS*, *PIK3CA*, *TP53*, *PTEN*, *EGFR*, *MET*, *HER2*, *epiregulin* and *amphiregulin* alterations might be beneficial to the selection of patients who are likely to respond to anti-EGFR therapies. Controversial remain the prognostic role of *BRAF* in addition to new potential prognostic factors such as iNKT cells and basal antibody-dependent cell-mediated cytotoxicity activity.

Applications

In summary, this review might be identify a subgroup of mCRC patients with distinct biological behaviour and response to treatments, including anti-EGFR antibodies. All of this will be a step forward in the "personalized medicine" treatment of CRC patients.

Terminology

EGFR on the cancer cell surface allows to transmit signals of proliferation, angiogenesis, metastasis. Cetuximab, a chimeric IgG1 monoclonal antibody (mAb) and panitumumab, a humanised IgG2 mAb, are currently licensed for the treatment of patients with mCRC. Markers able to select tumours addicted to EGFR signalling and so susceptible to anti-EGFR therapeutic modulation have been so far identified as *KRAS* (V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog), *NRAS* (neuroblastoma RAS viral oncogene homolog), *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha), *TP53* (tumour protein p53), *PTEN* (phosphatise and tensin homolog), *MET* (proto-oncogene receptor tyrosine kinase) and *HER2* (erb-b2 receptor tyrosine kinase 2).

Peer-review

This manuscript focuses on progress in the personalized treatment of mCRC and discusses the potential of new prognostic and predictive biomarkers in selecting patients treated with anti-EGFR therapy. The authors evaluated not only the *KRAS* mutational status but also *BRAF*, *NRAS*, *PIK3CA* and *PTEN* alterations which might be beneficial to the selection of patients who are likely to respond to anti-EGFR therapies.

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